Edited by
Karl Esser

THE MYCOTA

A Comprehensive Treatise on Fungi
as Experimental Systems for Basic and Applied Research

Systematics and Evolution
Part B

VII

Second Edition

David J. McLaughlin
Joseph W. Spatafora
Volume Editors

The Mycota

Edited by
K. Esser

The Mycota

I *Growth, Differentiation and Sexuality*
1st edition ed. by J.G.H. Wessels and F. Meinhardt
2nd edition ed. by U. Kües and R. Fischer
3rd edition ed. by J. Wendland

II *Genetics and Biotechnology*
1st and 2nd edition ed. by U. Kück

III *Biochemistry and Molecular Biology*
1st and 2nd edition ed. by R. Brambl and G. Marzluf
3rd edition ed. by D. Hoffmeister

IV *Environmental and Microbial Relationships*
1st edition ed. by D. Wicklow and B. Söderström
2nd edition ed. by C.P. Kubicek and I.S. Druzhinina
3rd edition ed. by I.S. Druzhinina and C.P. Kubicek

V *Plant Relationships*
1st edition ed. by G. Carroll and P. Tudzynski
2nd edition ed. by H.B. Deising

VI *Human and Animal Relationships*
1st edition ed. by D.H. Howard and J.D. Miller
2nd edition ed. by A.A. Brakhage and P.F. Zipfel

VII *Systematics and Evolution*
1st edition ed. by D.J. McLaughlin, E.G. McLaughlin, and P.A. Lemke†
2nd edition ed. by D.J. McLaughlin and J.W. Spatafora

VIII *Biology of the Fungal Cell*
1st and 2nd edition ed. by R.J. Howard and N.A.R. Gow

IX *Fungal Associations*
1st and 2nd edition ed. by B. Hock

X *Industrial Applications*
1st edition ed. by H.D. Osiewacz
2nd edition ed. by M. Hofrichter

XI *Agricultural Applications*
1st and 2nd edition ed. by F. Kempken

XII *Human Fungal Pathogens*
1st edition ed. by J.E. Domer and G.S. Kobayashi
2nd edition ed. by O. Kurzai

XIII *Fungal Genomics*
1st edition ed. by A.J.P. Brown
2nd edition ed. by M. Nowrousian

XIV *Evolution of Fungi and Fungal-Like Organisms*
Ed. by S. Pöggeler and J. Wöstemeyer

XV *Physiology and Genetics: Selected Basic and Applied Aspects*
Ed. by T. Anke and D. Weber

The Mycota

A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research

Edited by K. Esser

VII

Systematics and Evolution
Part B
2nd Edition

Volume Editors:
D.J. McLaughlin and J.W. Spatafora

Springer

Series Editor

Professor Dr. Dr. h.c. mult. Karl Esser
Allgemeine Botanik
Ruhr-Universität
44780 Bochum, Germany

Tel.: +49 (234)32-22211
Fax.: +49 (234)32-14211
e-mail: Karl.Esser@rub.de

Volume Editors

Professor Dr. David J. McLaughlin
Department of Plant Biology
University of Minnesota
1445 Gortner Avenue
St. Paul, MN 55108-1095, USA

Tel.: +1(612)625-5736
Fax: +1(612)625-1738
e-mail: davem@umn.edu

Professor Dr. Joseph W. Spatafora
Department of Botany and Plant Pathology
Oregon State University
Corvallis, OR 97331, USA

Tel.: +1(541)737-5304/5305
e-mail: spatafoj@science.oregonstate.edu

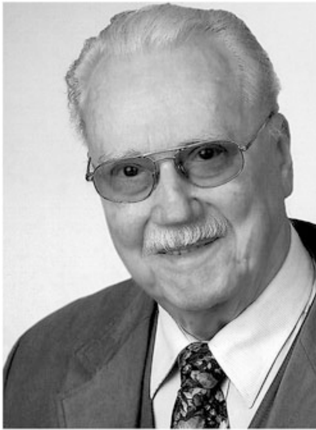
ISBN 978-3-662-46010-8 ISBN 978-3-662-46011-5 (eBook)
DOI 10.1007/978-3-662-46011-5

Library of Congress Control Number: 2015936344

Springer Heidelberg New York Dordrecht London
© Springer-Verlag Berlin Heidelberg 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer-Verlag GmbH Berlin Heidelberg is part of Springer Science+Business Media (www.springer.com)



Karl Esser
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

David J. McLaughlin
(born 1940) is retired Professor of Plant Biology and Curator of Fungi of the Bell Museum and cofounder of the Mycological Culture Collection at the University of Minnesota, St. Paul (USA). His scientific work has focused on the evolution of subcellular structure in Fungi, and phylogeny and systematics of basidomycetes, ectomycorrhizal community structure, and the Minnesota macrofungal flora with a view to establishing baseline data for mid continental macrofungi. He was formerly Editor-in-chief of Mycologia, and has been President of the Mycological Society of America and is a Fellow and Distinguished Mycologist of that Society.

Joseph S. Spatafora
(born 1964) is Professor of Botany and Plant Pathology and Curator of Oregon State University Mycological Collection at Oregon State University, Corvallis, OR (USA). His scientific work has focused on evolution of the Fungi, with emphases on phylogenetics of Ascomycota, insect pathogenic fungi and comparative genomics. He is a Fellow of, and has served as President of, the Mycological Society of America, and he is currently a Senior Editor of the journal Fungal Biology.

Series Preface

Mycology, the study of fungi, originated as a sub discipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for self incompatibility, termed "heterothallism", and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with *Neurospora crassa*, led to the one gene: one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to *Saccharomyces cerevisiae*, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title *The Mycota*. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this Series of Volumes the names adopted for major groups of fungi (representative genera in parentheses) areas follows:

Pseudomycota

Division: Oomycota (*Achlya, Phytophthora, Pythium*)
Division: Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (*Allomyces*)
Division:	Zygomycota (*Mucor, Phycomyces, Blakeslea*)
Division:	Dikaryomycota
Subdivision:	Ascomycotina
Class:	Saccharomycetes (*Saccharomyces, Schizosaccharomyces*)
Class:	Ascomycetes (*Neurospora, Podospora, Aspergillus*)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (*Ustilago, Tremella*)
Class:	Homobasidiomycetes (*Schizophyllum, Coprinus*)

We have made the decision to exclude from *The Mycota* the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The Series throughout will address three basic questions: what are the fungi, what do they do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this Series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather than ingest, nutrients and they have rigid cell walls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is a multitude of spore forms, fungal spores are basically only of two types: (i) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (ii) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy.The metabolic capacities of fungi have attracted much interest in natural products chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insect associated fungi and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important. There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this Series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these Volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission or inconsistency in this Series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine Volumes.

Bochum, Germany — Karl Esser
Auburn, AL, USA — Paul A. Lemke
April 1994 — *Series Editors*

Volume Preface to the Second Edition

There have been major changes in our knowledge of the systematics and evolution of fungi since the first edition of the Mycota, Vol. VII. These changes have been driven by an outpouring of molecular phylogenetic analyses at first based on one or a few genes but now by multiple conserved genes. The Assembling the Fungal Tree of Life projects have been a major contributor to the data needed to construct the molecular phylogenies along with work from many additional labs. The resulting phylogenies have made possible a new taxonomic outline for the Fungi (Hibbett D.S. et al., 2007, Mycol. Res. 111: 509–547), which has provided a more stable systematic treatment for this kingdom, although some of the basal groups of Fungi remain incompletely resolved (Table 1). Agreement among many mycologists on nomenclature is providing a stable framework for Fungi that has been incorporated into reference works and online databases (McLaughlin D. J. et al., 2009, Trends Microbiol. 17: 488–497), and has provided an escape from the conflicting phenetic classifications of the past. These nomenclatural changes are incorporated into these volumes along with much new information on the evolution and ecology of these organisms made possible by a variety of methods, including environmental sequencing and reevaluation of character evolution using molecular phylogenies.

While there is agreement on nomenclature within Kingdom Fungi, there is less agreement on the names for groups of fungus-like organisms, although these organisms remain a major interest of those who study fungi. Some of the confusion arises from the treatment of fungus-like organisms under two nomenclatural codes (Table 1). Of special concern has been the treatment of the oomycetes and their relatives with variant spellings of the kingdom and common name. The solution adopted by Beakes (Chap. 3, Vol. VII, Part A) reserves Straminipila for the kingdom and uses the widely cited stramenopiles for the common name.

Chapters in this edition of the Mycota, Vol. VII, vary from updates of chapters published in the first edition to new chapters. All systematic chapters treat monophyletic groups; clearly polyphyletic groups, such as those based on yeasts or asexual stages (anamorphs), have been omitted. While authors have been encouraged to provide illustrations of the diversity within each group, the results are somewhat uneven. Some authors have extensively illustrated the organisms, while others for reasons of time or access have provided limited illustrations. In the interest of getting these chapters to press in a not too tardy manner, the authors have not been unduly pressed to add illustrations. The reader's understanding is requested for the omissions, which is caused in part by the difficulty of getting all of the chapters needed to cover a wide spectrum of organisms.

Table 1 Taxonomic outline for Fungi and fungus-like organisms[a]

Fungus-like organisms
- Supergroup: Amoebozoa
  - Phylum: Dictyosteliomycota
  - Phylum: Myxomycota
- Supergroup: Excavata
  - Phylum: Acrasiomycota
- Supergroup: Sar[b]
  - Subgroup: Rhizaria
    - Phylum: Phytomyxea
  - Kingdom: Straminipila[c]
    - Phylum: Labyrinthulomycota
    - Phylum: Hyphochytriomycota
    - Phylum: Oomycota

Fungi
- Supergroup: Opisthokonta
  - Kingdom: Fungi
    - *Basal fungi*
      - Phylum: Cryptomycota[d]
      - Phylum: Microsporidia
    - *Traditional Chytridomycota*
      - Phylum: Chytridiomycota
      - Phylum: Monoblepharidomycota
      - Phylum: Neocallimastigomycota
      - Phylum: Blastocladiomycota
    - *Zygomycotan (Zygomycetous) Fungi*
      - Phylum: Entomophthoromycota
      - Phylum/a incertae sedis:
        - Subphylum: Kickxellomycotina
        - Subphylum: Mortierellomycotina
        - Subphylum: Mucoromycotina
        - Subphylum: Zoopagomycotina
      - Phylum: Glomeromycota
    - Subkingdom Dikarya
      - Phylum: Basidiomycota
        - Subphylum: Pucciniomycotina
        - Subphylum: Ustilaginomycotina
        - Subphylum: Agaricomycotina
      - Phylum: Ascomycota
        - Subphylum: Taphrinomycotina
        - Subphylum: Saccharomycotina
        - Subphylum: Pezizomycotina

[a]Names for Fungi and fungus-like organisms traditionally studied by botanists are governed by the *International Code for Nomenclature of algae, fungi and plants (Melbourne Code)* (McNeil J. et al., 2012, Regnum Vegetabile 154, Koeltz Scientific Books). Multiple names exist for eukaryotic microorganisms that are treated under both the Melbourne Code and the International Code of Zoological Nomenclature, except for Microsporidia, which are classified under the zoological code

[b]Sar (Stramenopiles, Alveolata, and Rhizaria)

[c]Also known as Stramenopila or Stramenopiles. The latter is used by Adl et al. (2012, J. Eukaryot. Microbiol. 59: 429–493) and as a common name, stramenopiles, for Straminipila

[d]Also known as Rozellida and Rozellomycota

The Mycota, Vol. VII, includes treatments of the systematics and related topics for Fungi and fungus-like organisms in four eukaryotic supergroups (Table 1) as well as specialized chapters on nomenclature, techniques, and evolution. Most Fungi and fungus-like organisms are covered, including the Microsporidia. Chapter 1, Vol. VII, Part A, provides an overview of fungal origins and evolution.

Chapters 2–4, Vol. VII, Part A, cover the fungus-like organisms, and Chaps. 5 to 14, Vol. VII, Part A, and Chaps. 1–6, Vol. VII, Part B, cover the Fungi. Each of these chapters covers approximately the following topics: occurrence and distribution, economic importance, morphology and ultrastructure, development of the taxonomic theory, classification, and maintenance and culture. The fungus-like organisms are distributed in three distantly related supergroups (Table 1). The basal fungi and traditional Chytridiomycota are treated as six phyla and covered in four chapters, including Chap. 1, Vol. VII, Part A. The zygomycetous fungi, whose deeper relationships remain unresolved, and Glomeromycota are covered in two chapters. The Basidiomycota and Ascomycota, the largest groups of fungi, are treated in five or six chapters each. In the Basidiomycota two chapters cover Pucciniomycotina and Ustilaginomycotina, respectively, while three chapters are devoted to classes of the Agaricomycotina. In the Ascomycota a single chapter covers Taphrinomycotina and Saccharomycotina, while eight classes of the Pezizomycotina are covered in five chapters.

The following topics are treated in Chaps. 7–11 in Vol. VII, Part B: Chap. 7 deals with the nomenclatural changes necessitated by the recent changes to the International Code for Nomenclature of algae, fungi, and plants (Table 1), including the elimination of separate names for anamorphic fungi. Chapter 8 deals with methods for preservation of cultures and specimens, while Chap. 9 reviews the phylogenetic implications of subcellular and biochemical characters and methods for ultrastructural study. Chapter 10 deals with the fungal fossil record and Chap. 11 with the impact of the availability of whole genomes on studies of Fungi.

We are entering a new era in the study of fungi with whole genomes becoming available for an increasing number of species across all the known clades of Fungi. This genome-enabled mycology will utilize large numbers of genes in phylogenomic analyses to resolve difficult to determine relationships in fungi and to provide insights into fungal biology (Hibbett D.S. et al., 2013, Mycologia 106: 1339–1349). Initial studies are already having a significant impact on our understanding of biochemical processes and their ecological impacts. In time genomic studies may shed light on the genetic processes and the genes that control the great morphological diversity in Fungi from the subcellular to the macroscopic level. Thus, there is much new information on the systematics and evolution of fungi to be expected in the future.

We thank Meredith Blackwell for sharing unpublished manuscripts and discussions on the classification system, Esther G. McLaughlin for advice throughout the work, and the U.S. National Science Foundation for support to many labs for the AFTOL 1 and AFTOL 2 projects (including DEB-0732550 to DJM, and DEB-0732993 to JWS), and numerous scientists who have contributed to the work which has made the advances in these volumes possible.

St. Paul, MN — David J. McLaughlin
Corvallis, OR — Joseph W. Spatafora
22 May 2014

Volume Preface to the First Edition

This is an exciting time to produce an overview of the systematics and evolution of the fungi. Homoplasy is evident in all lineages, e.g., those based on the gross morphology of the chytrid zoospore, the perithecium and apothecium, the smut teliospore and the agaric fruiting body, and some classifications based on light microscope morphology have been shown to be unsound. Molecular and subcellular characters, aided by new methods of phylogenetic analysis, have allowed us to see through the conflicts between various phenetic classification schemes and have given us some confidence that we are beginning to achieve a true phylogeny of the fungi. Molecular data have both supported ultrastructural characters that first began to unravel the homoplasies unrecognized at the light microscopic level, and have also revealed the relationships of fungi to other eukaryotes. They continue to enlarge the scope of the fungi, e.g., with the recent addition of the Microsporidia (see Cavalier-Smith, Chap. 1, Vol. VII, Part A), and they have shown the need for more detailed chemical, subcellular, and developmental studies for a fuller understanding of these organisms and their relationships.

This volume is a mixture of phylogenetic and more classical systematics. Progress in knowledge of species and development of taxonomic characters is mixed. Groups with few species have been studied in great detail, while in groups with large numbers of species much effort is still needed to find and determine the taxa. Classical systematics groups organisms on a phenetic basis, then sets up a classification; phylogeny is a secondary consideration. Phylogenetic systematics first determines organism relationships, then constructs a systematic classification that reflects the phylogeny. Molecular characters have made possible the establishment of a monophyletic and, hopefully, more permanent classification for the fungi. Thus, Volume VII of *The Mycota* contains both classical and phylogenetic classifications, reflecting the available data and the orientation of different authors. The incompleteness of some classifications, e.g., those for the Urediniomycetes (Swann, Frieders, and McLaughlin, Chap. 2, Vol. VII, Part B) and Homobasidiomycetes (Hibbett and Thorn, Chap. 5, Vol. VII, Part B), demonstrates that we are in the early stages of a phylogenetic systematics for these groups.

The taxonomic outline used in *The Mycota*, Vol. VII, differs somewhat from that of other volumes in the series (Table 1), reflecting current mycological systematics. There is a lack of agreement on the naming of higher taxa, and the rules of nomenclature permit more than one name for these taxa. Cavalier-Smith (Chap. 1, Vol. VII, Part A) presents an alternative view to the taxonomic outline used for the remainder of the volume (Table 2). Some of the nomenclatural problems stem from a lack of resolution of deep branches in molecular evolutionary trees, a problem that appears likely to be resolved only with additional

Table 1 Taxonomic outline at the kingdom, phylum, and class levels as used in other volumes in the series and in this volume. The classification in this volume is necessarily confusing at this time because authors are using their own classifications rather than an imposed classification

Mycota, Vol. I	Mycota, Vol. VII
PSEUDOMYCOTA	PSEUDOMYCOTA[a,b]
Oomycota	Oomycota[c]
	Peronosporomycetes
Hyphochytriomycota	Hyphochytriomycota
	Hyphochytriomycetes
	Plasmodiophoromycota
	Plasmodiophoromycetes
EUMYCOTA	EUMYCOTA
Chytridiomycota	Chytridiomycota[d]
	Chytridiomycetes
Zygomycota	Zygomycota[d]
	Zygomycetes
	Trichomycetes
Dikaryomycota	
Ascomycotina	Ascomycota[e]
Saccharomycetes	Saccharomycetes
Ascomycetes	Plectomycetes
	Hymenoascomycetes[a]
	Loculoascomycetes[a]
Basidiomycotina	Basidiomycota
Heterobasidiomycetes	Urediniomycetes
	Ustilaginomycetes
	Heterobasidiomycetes[a,f]
Homobasidiomycetes	Homobasidiomycetes[a,f]

[a]Artificial taxon

[b]For a natural classification for Oomycota and Hyphochytriomycota, kingdom Stramenopila (Stramenipila, Dick, Chap. 2, Vol. VII, Part A) or Chromista have been proposed, and for Plasmodiophoromycota, kingdom Protozoa (see Cavalier-Smith, Chap. 1, Vol. VII, Part A)

[c]Or Heterokonta (see Cavalier-Smith, Chap. 1, and Dick, Chap. 2, Vol. VII, Part A)

[d]Probably paraphyletic (see Cavalier-Smith, Chap. 1, Vol. VII, Part A, and Berbee and Taylor, Chap. 10, Vol. VII, Part B)

[e]A phylogenetic classification for Ascomycota is not available. Current thinking among ascomycete scholars is that three classes should be recognized, as follows: "Archiascomycetes", which may not be monophyletic, Hemiascomycetes (see Kurtzman and Sugiyama, Chap. 9, Vol. VII, Part A), and a filamentous group, Euascomycetes, that eventually will be subdividable, perhaps at the subclass level [M.E. Berbee and J.W. Taylor, 1995, Can J Bot 73 (Suppl. 1):S677, and Chap. 10, Vol. VII, Part B; J.W. Spatafora, 1995, Can J Bot 73 (Suppl. 1):S811]. Saccharomycetes as used here (see Barr, Chap. 8, Vol. VII, Part A) includes "Archiascomycetes" and Hemiascomycetes. See the relevant chapters for further speculation on the ultimate disposition of these groups

[f]Heterobasidiomycetes as used in Vol. VIIB cannot be separated from Homobasidiomycetes. Hymenomycetes [E.C. Swann and J.W. Taylor, 1995, Can J Bot 73 (Suppl. 1):S862] has been proposed as a class for these groups (see Berbee and Taylor, Chap. 10, Vol. VII, Part B)

data from multiple genes and the addition of missing taxa to the analysis. Problems also arise from a difference of opinion among authors. The term *fungi* has assumed an ecological meaning for all organisms with a similar nutritional mode, and therefore, Eumycota, rather than Fungi, is less confusing for the members of the phylum that encompasses a monophyletic group of these organisms. *Pseudofungi* (Cavalier-Smith, Chap. 1, Vol. VII, Part A) implies that organisms that lie outside the Eumycota but possess the fungal lifestyle are not fungi, but in an ecological sense they are fungi. P*seudomycota* is therefore used in this series for these fungal organisms that lie outside the Eumycota.

Table 2 Taxonomic outline at the kingdom, phylum, and class levels as used in the rest of this volume compared with that of Cavalier-Smith, Chap. 1, Vol. VII, Part A

Mycota, Vol. VII	Chapter 1, Vol. VII, Part A
PSEUDOMYCOTA[a]	CHROMISTA
Oomycota	Bigyra
Peronosporomycetes	Oomycetes
Hyphochytriomycota	
Hyphochytriomycetes	Hyphochytrea
	PROTOZOA
Plasmodiophoromycota	Cercozoa
Plasmodiophoromycetes	Phytomyxea
EUMYCOTA	FUNGI
Chytridiomycota	Archemycota
Chytridiomycetes	Chytridiomycetes
	Allomycetes
Zygomycota	
Zygomycetes	Zygomycetes
	Bolomycetes
	Glomomycetes[b]
Trichomycetes	Enteromycetes
	Zoomycetes[c]
	Microsporidia
	Minisporea
	Microsporea
Ascomycota	Ascomycota
Saccharomycetes	Taphrinomycetes
	Geomycetes
	Endomycetes
Plectomycetes	Plectomycetes
Hymenoascomycetes	Discomycetes
	Pyrenomycetes
Loculoascomycetes	Loculomycetes
Basidiomycota	Basidiomycota
Urediniomycetes	Septomycetes
Ustilaginomycetes	Ustomycetes
Heterobasidiomycetes	Gelimycetes[b]
Homobasidiomycetes	Homobasidiomycetes

[a]Artificial taxon
[b]Probably paraphyletic
[c]Includes Zygomycetes, Ascomycetes, and Trichomycetes

The Mycota, Vol. VII, includes treatments of the systematics and related topics of the Eumycota and Pseudomycota as well as specialized chapters on nomenclature, techniques, and evolution. Certain groups are not treated in this volume: the Labyrinthulomycetes (Pseudomycota) and the slime molds. The evolutionary position of the slime molds has been controversial. Recent evidence suggests that most slime molds are more closely related to the Eumycota than previously believed (S.L. Baldauf and W.F. Doolittle, 1997, Proc Natl Acad Sci USA 94:12007), and they should continue to be of interest to those who study fungi for both ecological and phylogenetic reasons.

Chapters 2 to 4, Vol. VII, Part A, cover the Pseudomycota, and Chaps. 5–14, Vol. VII A, and Chaps. 1–5, Vol. VII, Part B, the Eumycota. The Pseudomycota contains distantly related groups of fungi (Table 1). The Chytridiomycota and

Zygomycota are treated in one and two chapters, respectively, while the Ascomycota and Basidiomycota are treated in five or six chapters each, with separate chapters for yeasts in each phylum, although the yeasts are not monophyletic groups. Chapter 14, Vol. VII, Part A, discusses the special problems of anamorphic genera and their relationships to the teleomorphic genera and describes the attempts being made to incorporate anamorphs into modern phylogenetic systematics. In Chap. 6, Vol. VII, Part B, Hawksworth discusses the development of a unified system of biological nomenclature. Chapters 7 and 8, Vol. VII, Part B, deal with techniques for cultivation and data analysis, respectively. The final two chapters in Vol. VII, Part B, consider speciation and molecular evolution.

The Mycota, Vol. VII, was originally intended to have been Vol. I in the series. Several changes in editors and the unfortunate death of Paul Lemke delayed its production. Added to these difficulties was the fact that these are tumultuous times in systematics because of the rapid development of molecular and phylogenetic analysis techniques and the explosive accumulation of data. As these techniques and new data are more broadly incorporated into systematics, a more stable and useful classification of the fungi will result.

We thank Heather J. Olson for her substantial efforts in compiling the indices.

St. Paul, MN — David J. McLaughlin
Minneapolis, MN — Esther G. McLaughlin
April 2000 — *Volume Editors*

Contents

Fungi

1 Saccharomycotina and Taphrinomycotina: The Yeasts and Yeastlike Fungi of the Ascomycota 3
CLETUS P. KURTZMAN, JUNTA SUGIYAMA

2 Pezizomycotina: Pezizomycetes, Orbiliomycetes 35
DONALD H. PFISTER

3 Pezizomycotina: Sordariomycetes and Leotiomycetes 57
NING ZHANG, ZHENG WANG

4 Pezizomycotina: Lecanoromycetes 89
CÉCILE GUEIDAN, DAVID J. HILL, JOLANTA MIADLIKOWSKA, FRANCOIS LUTZONI

5 Pezizomycotina: Eurotiomycetes 121
DAVID M. GEISER, KATHERINE F. LOBUGLIO, CÉCILE GUEIDAN

6 Pezizomycotina: Dothideomycetes and Arthoniomycetes 143
CONRAD SCHOCH, MARTIN GRUBE

Nomenclature and Documentation

7 The Shifting Sands of Fungal Naming Under the ICN and the One Name Era for Fungi 179
ANDREW M. MINNIS

8 The Role of Herbaria and Culture Collections 205
GERARD J.M. VERKLEY, AMY ROSSMAN, JO ANNE CROUCH

Evolution

9 Subcellular Structure and Biochemical Characters in Fungal Phylogeny 229
DAVID J. MCLAUGHLIN, T.K. ARUN KUMAR, MEREDITH BLACKWELL, PETER M. LETCHER, ROBERT W. ROBERSON

10 Fungal Diversity in the Fossil Record 259
Thomas N. Taylor, Michael Krings, Edith L. Taylor

11 Phylogenomics Enabling Genome-Based Mycology 279
Jason E. Stajich

Biosystematic Index 295

Subject Index 305

List of Contributors

MEREDITH BLACKWELL
Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

JO ANNE CROUCH
Systematic Mycology & Microbiology Laboratory, USDA-ARS, Rm. 246, B010A 10300 Baltimore Ave., Beltsville, MD 20705, USA

DAVID M. GEISER
Department of Plant Pathology and Environmental Microbiology, 121 Buckhout Laboratory, The Pennsylvania State University, University Park, PA 16802, USA

MARTIN GRUBE
Institute of Plant Sciences, Karl-Franzens-University, Holteigasse 6, 8010 Graz, Austria

CÉCILE GUEIDAN
Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

CSIRO – National Research Collections Australia, Australia National Herbarium, Clunies Ross Street, Canberra, ACT 2601, Australia

DAVID J. HILL
School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

MICHAEL KRINGS
Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA

Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA

Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, Richard-Wagner-Straße 10, Munich 80333, Germany

Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, Munich 80333, Germany

T.K. Arun Kumar
Department of Botany, The Zamorin's Guruvayurappan College, Calicut, Kerala 673 014, India

Cletus P. Kurtzman
Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, IL 61604, USA

Peter M. Letcher
Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA

Katherine F. LoBuglio
Farlow Herbarium, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA

Francois Lutzoni
Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA

David J. McLaughlin
Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA

Jolanta Miadlikowska
Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA

Andrew M. Minnis
USDA-US Forest Service, Center for Forest Mycology Research, One Gifford Pinchot Dr., Madison, WI 53726, USA

Donald H. Pfister
Farlow Herbarium and Library of Cryptogamic Botany, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

Robert W. Roberson
School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Amy Rossman
Systematic Mycology & Microbiology Laboratory, USDA-ARS, Rm. 246, B010A 10300 Baltimore Ave., Beltsville, MD 20705, USA

Conrad Schoch
NCBI/NLM/NIH, 45 Center Drive, Bethesda, MD 20892, USA

Jason E. Stajich
Department of Plant Pathology & Microbiology and Institute for Integrative Genome Biology, University of California-Riverside, Riverside, CA 92521, USA

Junta Sugiyama
Chiba Branch Office & Laboratory, TechnoSuruga Laboratory Co., Ltd., 3-1532-13 Hasama-cho, Funabashi-shi, Chiba 274-0822, Japan

Thomas N. Taylor
Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA

Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA

Edith L. Taylor
Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA

Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA

Gerard J.M. Verkley
CBS Biodiversity Center, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Zheng Wang
Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT 06520, USA

Ning Zhang
Department of Plant Biology & Pathology, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901, USA

Fungi

1 Saccharomycotina and Taphrinomycotina: The Yeasts and Yeastlike Fungi of the Ascomycota

Cletus P. Kurtzman[1], Junta Sugiyama[2]

CONTENTS

I. Introduction 3
II. Occurrence, Distribution, and Ecology 3
III. Importance 4
A. Food, Beverage, and Industrial Uses 4
B. Agriculturally Important Yeasts 5
1. Plant Pathogens 5
2. Biocontrol Yeasts 5
C. Food and Beverage Spoilage 6
D. Human and Animal Pathogens 6
IV. Reproduction 7
A. Asexual 7
1. Budding, Fission, Endospores, Chlamydospores 7
2. Pseudohyphae and True (Septate) Hyphae 8
B. Sexual Reproduction 9
V. Taxonomic Methods 11
A. Phenotypic Characterization 11
B. Genotypic Characterization 12
VI. Phylogeny and Classification 13
A. Phylogeny 13
B. Classification 17
1. Saccharomycotina 20
2. Taphrinomycotina 20
VII. Isolation, Maintenance, and Culture Availability 23
A. Isolation 23
B. Maintenance 25
C. Culture Availability and Distribution... 26
VIII. Future Directions 26
References 27

[1]Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, IL 61604, USA; e-mail: cletus.kurtzman@ars.usda.gov

[2]Chiba Branch Office & Laboratory, TechnoSuruga Laboratory Co., Ltd., 3-1532-13 Hasama-cho, Funabashi-shi, Chiba 274-0822, Japan; e-mail: jsugiyam@tecsrg.co.jp

I. Introduction

Yeasts are found in nearly all regions of the Earth, including hot deserts, polar areas, in freshwater, in salt water, and in the atmosphere, where they are commonly transported by prevailing winds. Though yeast growth is mainly saprotrophic, some yeasts are important pathogens of animals and plants. **The term yeast** has come to mean those fungi that divide by budding or fission and that have sexual states unenclosed in a fruiting body. Consequently, yeasts occur among the Ascomycota and the Basidiomycota. The focus of this chapter are those taxa assigned to Saccharomycotina and Taphrinomycotina of Ascomycota. As we will discuss, some members of these subphyla are among the economically most important fungi known.

II. Occurrence, Distribution, and Ecology

Although yeasts occur worldwide, some have restricted habitats, whereas others are found in many different environments. The key to understanding yeast ecology and the extent of habitat specificity is the accurate identification of species, which is now possible through DNA-based methods. Prior to the application of molecular methods, the identification of species from phenotype often resulted in misclassification, which rendered results from many ecological studies uncertain or misleading.

Yeasts are often associated with insects, and numerous studies have detailed these interactions (Phaff et al. 1956; van der Walt and Scott

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

1971; Vega and Blackwell 2005; Wickerham 1969). Contemporary studies have expanded on this earlier work and refined it through molecular-based species identification. Notable has been the work of H. J. Phaff and W. T. Starmer and colleagues (Phaff et al. 1987; Starmer et al. 1992), who examined yeast–*Drosophila* interactions among various species of cacti. In one of these studies, *Pichia kluyveri* was recognized to be comprised of three closely related and partially interfertile species that were found to show significant habitat differences.

Other yeasts are strongly associated with plants, including the well-known interaction of *Saccharomyces cerevisiae* with grapes (e.g., Mortimer and Polsinelli 1999). Ripe apples have significant surface populations of *Saccharomyces*, *Torulaspora*, *Zygosaccharomyces*, and other yeasts that are attributed to the transfer of soluble sugars onto the surface of the fruit, and many species of the genus *Ogataea* are found on leaves and decaying wood. Species of *Ogataea* utilize methanol as their sole source of carbon, which is present in the environment as a degradation product of lignin (de Koning and Harder 1992) and is formed in leaf respiratory processes (Fall and Benson 1996).

Some yeasts, such as *Debaryomyces hansenii* and *Meyerozyma guilliermondii* (anamorph: *Candida guilliermondii*), occur widely in nature and are common in water, plant debris, and soil. Both of the aforementioned species also represent opportunistic human pathogens. Although yeasts are commonly isolated from soil, few are believed to have soil as a primary habitat. Many *Lipomyces* species are an exception and have been isolated only from soil.

III. Importance

A. Food, Beverage, and Industrial Uses

Since ancient times, human societies worldwide have used beverages and foods fermented by yeasts (Legras et al. 2007). Archaeologists have found evidence that fermented beverages were consumed in Neolithic times (8500–4000 B.C.) in China, Iran, Egypt, and other areas of the world (Legras et al. 2007; McGovern et al. 2004), and the production of fermented beverages and foods seems to have paralleled the beginning of agriculture. Louis Pasteur provided the insight that fermentation was the result of microorganisms and noted that yeasts occurred on grapes, thereby providing a ready source of inoculum for wine (Dubos 1960; Mortimer and Polsinelli 1999). The commonplace **processes of baking, brewing, and wine making** are often taken for granted but represent major industries with a combined worldwide annual value that may exceed US$1 trillion (Hansen 2004; Verstrepen et al. 2006). Other food-related yeast processes include the natural fermentation of cocoa beans, coffee beans, pickles, olives, and similar products.

The industrial importance of yeasts has vastly expanded over the past few decades to include much more than bread making and brewing. *Yarrowia lipolytica* was initially used for the production of single-cell protein from hydrocarbons, but the species is now recognized as a major producer of citric acid, an important acidulant for industrial and food and beverage uses. Other industrially significant metabolites from ascomycetous yeasts include riboflavin from *Eremothecium gossypii* (≡*Ashbya gossypii*) (Wickerham et al. 1946), lactase from *Kluyveromyces marxianus* (Rubio-Texeira 2006), biosurfactants, such as sophorolipids, from members of the *Starmerella* clade (Kurtzman et al. 2010), and lipases from *Y. lipolytica* and *Candida cylindracea* (Gellissen et al. 2005). *S. cerevisiae* is widely used in the production of **recombinant proteins** for medical and other uses, but the methanol-assimilating yeasts, such as *Komagataella pastoris* (≡*Pichia pastoris*) and *Ogataea polymorpha* (≡*Hansenula polymorpha*), are also important in this role (Cregg and Madden 1988; Veenhuis et al. 1983).

The conversion of plant **biomass to biofuels** is of major interest, and the yeasts *Pachysolen tannophilus*, *Scheffersomyces stipitis* (≡*Pichia stipitis*), and *Candida shehatae*, which were discovered to ferment the D-xylose of biomass to ethanol, have been the mainstays in this effort (Slininger et al. 1987, and references therein). More recently, *Spathaspora passali-*

darum and *Candida jeffriesii* were found to ferment D-xylose to ethanol (Nguyen et al. 2006).

B. Agriculturally Important Yeasts

1. Plant Pathogens

In this section, we discuss two important agricultural aspects of yeasts, those that are plant pathogens and those that are antagonists of pathogens. *Taphrina* and *Protomyces*, both members of Taphrinomycotina, are perhaps the best known of the yeastlike taxa that cause plant diseases. *T. deformans*, the cause of **peach leaf curl**, is worldwide in its distribution and the most economically devastating of the diseases caused by species of *Taphrina* (Fonseca and Rodrigues 2011; Mix 1949). Young leaves, stems, and fruit are often severely distorted when infected by *T. deformans*. Early application of fungicides usually controls peach leaf curl (Daughtrey et al. 2003), but failure to do so can result in significant crop losses. Other tree crops, such as almonds and pears, may be severely affected by *Taphrina* infections. Trees, such as alders and poplars, are also susceptible to various *Taphrina* species (Fonseca and Rodrigues 2011; Mix 1949). The effect of these infections on tree health is generally limited, but the appearance of infected leaves and flowers can be distressing when the trees are used in ornamental plantings.

Species of *Protomyces* cause symptoms similar to those seen from *Taphrina* infections. All known ***Protomyces* species are plant parasitic** and cause galls on stems, leaves, and fruits of Compositiae, Umbelliferae, and certain other plants (Tubaki 1957). Economic losses are seldom great, but *P. macrosporus* infection of coriander (*Coriandrum sativum*) was reported to have damaged up to 11 % of the crop during one growing season (Tripathi et al. 2003).

Several genera of Saccharomycotina also cause plant diseases. Most notable are species of *Eremothecium*, some of which were previously classified in the genera *Ashbya*, *Nematospora*, and *Holleya*, all of which are plant pathogens. *E. ashbyi* has a long history of causing **cotton boll rot** in various species of *Gossypium* and cankers on citrus fruit (Batra 1973). Similarly, *E. gossypii* causes staining and rot of cotton bolls and is pathogenic to coffee (*Coffea* spp.), soybean (*Glycine max*), and other crops. *E. coryli* can infect cotton, but it is also a pathogen of hazelnuts, tomatoes, and beans. Symptoms are generally disfigurement and disruption of the infected plant tissue. *E. sinecaudum* was discovered by Holley et al. (1984) to cause seed infection in oriental and yellow mustard in Saskatchewan, Canada. The remaining known species of *Eremothecium*, *E. cymbalariae*, appears uncommon but has been isolated as a pathogen of flax and other plants (Arnaud 1913). Other known pathogenic yeasts are *Galactomyces candidus* (anamorph *Geotrichum candidum*) and *Galactomyces citri-aurantii* (anamorph: *Geotrichum citri-aurantii*), which commonly cause sour rot of citrus, tomatoes, cantaloupes, peaches, lychee, and carrots (Butler et al. 1965; Wells 1977). Losses are sufficiently great to require treatment of the produce by a fungicide.

2. Biocontrol Yeasts

Yeasts assigned to Saccharomycotina as well to Basidiomycota are used in the biocontrol of plant diseases (Andrews 1992; Chalutz et al. 1991). Our focus will be on the ascomycetous yeasts used for this purpose. Yeasts are common on leaf surfaces and on fruits, the latter containing abundant, easily utilizable carbon sources. The finding that naturally occurring yeasts on apples can protect fruit against postharvest diseases (Janisiewicz 1987) markedly stimulated work on the utilization of yeasts as an alternative to chemical pesticides for the protection of fruits against storage diseases.

M. guilliermondii (anamorph: *Candida guilliermondii*) has been successfully used in a number of studies to control fruit rots. Guetsky et al. (2002) reported a 50 % reduction in *Botrytis cinerea* rot of strawberries following application of *M. guilliermondii* and suggested that the control mechanism is competition for nutrients. Similarly, *M. guilliermondii* was

effective at controlling fungi that cause rot of citrus (Droby et al. 1993). *M. guilliermondii* is widespread in nature, but it is also an opportunistic human pathogen, raising concerns about its safety as a biocontrol agent on fruit and other produce that will be consumed without cooking. *Candida oleophila*, which is not a clinical yeast, shows good control of fruit storage rots and has been commercialized under the trade name Aspire for the protection of citrus from rots caused by species of *Penicillium* (Droby et al. 1998). *Metschnikowia fructicola* and *Metschnikowia pulcherrima* have also been tested extensively for the biocontrol of fruit rots (Janisiewicz et al. 2001; Karabulut et al. 2004). Additionally, *Wickerhamomyces anomalus* (≡*Pichia anomala*) has been effective in the biocontrol of mold-induced spoilage of ensiled maize (Passoth et al. 2006). A concern is that *W. anomalus* has been implicated in some human infections, and its suitability as a biocontrol agent is uncertain.

C. Food and Beverage Spoilage

The spoilage of foods and beverages by contaminating yeasts results in major economic losses worldwide (e.g., Fleet 1990). Yeasts responsible for food spoilage are not known to cause infection or food poisoning in humans, as do certain bacteria. The composition of foods and beverages often determines the species of yeasts that are likely to be found. Products with high sugar content (40–70 %) are commonly spoiled by *Zygosaccharomyces* spp., *Torulaspora delbrueckii*, *Schizosaccharomyces octosporus*, and *Wickerhamomyces subpelliculosus* (≡*Pichia subpelliculosa*). If salt (NaCl) is used as a preservative, other species can predominate. During the fermentation of cucumbers (10–16 % NaCl) and other products, such as soy sauce, *Candida etchellsii* and *Candida versatilis* are common. Species of *Debaryomyces*, especially *D. hansenii*, often overgrow aged cheeses, salami, and other salted meat products, imparting flavor and the potential for spoilage. Spoilage of fresh fruits may be caused by *Candida stellata*, *P. kluyveri*, *Pichia fermentans*, *M. pulcherrima*, and species of *Hanseniaspora* and its anamorph *Kloeckera*. Species of *Brettanomyces* and its teleomorph *Dekkera* are often responsible for turbidity and off-flavors in wine, beer, and soft drinks.

D. Human and Animal Pathogens

Candida albicans is the most common cause of **candidiasis** and is the species most often isolated from cases of oral infections and vaginitis (Bialkova and Subik 2006; Kaur et al. 2005). DNA sequence analysis of *C. albicans* strains resulted in the discovery of the closely related and phenotypically nearly identical species *Candida dubliniensis* (Sullivan et al. 2005). *C. dubliniensis* is mainly isolated from oral candidiasis in HIV-infected patients, but it is also isolated from healthy individuals, as is the case for *C. albicans*. Following *C. albicans*, the second most common cause of blood stream infections is *Candida glabrata*, and the increased frequency of infections by this species has been attributed to the larger population of immunocompromised individuals and the widespread use of antimycotics. DNA sequence analysis resulted in the discovery of the clinically important species *Candida bracarensis* and *Candida nivariensis*, both of which are phenotypically similar to *C. glabrata* (Alcoba-Flórez et al. 2005; Correia et al. 2006).

Numerous other ascomycetous yeasts are obtained as clinical isolates, but most are widespread in the environment and regarded as opportunistic pathogens. Among them are *M. guilliermondii*, *Pichia kudriavzevii* (anamorph: *Candida krusei*), *Candida parapsilosis*, *Candida tropicalis*, and even *S. cerevisiae*. Both animals and humans can develop yeast infections. *Kazachstania pintolopesii* has devastated colonies of laboratory mice (Kurtzman et al. 2005), and *Macrorhabdus ornithogaster* appears to be a cause of decline in birds such as budgerigars (Tomaszewski et al. 2003).

IV. Reproduction

A. Asexual

1. Budding, Fission, Endospores, Chlamydospores

Asexual reproduction, sometimes termed **vegetative reproduction**, occurs in ascomycetous yeasts by budding or by fission. The formation of pseudohyphae and septate (true) hyphae constitutes other forms of asexual reproduction that are discussed subsequently. Buds may arise either on yeast cells or on hyphal cells, and budding is initiated by the formation of a small evagination or outgrowth at some point on the surface of the cell. The parent (mother) cell remains more or less constant in size as the bud (blastoconidium) increases in size to form a new cell and then usually separates from the parent cell. Budding is termed **holoblastic** or **enteroblastic**, depending on how the bud is formed. All layers of the wall of the parent cell are involved in holoblastic budding and the bud separates, usually on a narrow base, leaving a scar through which no further budding occurs. Von Arx and Weijman (1979) considered holoblastic budding to be characteristic of Saccharomycotina. When budding is enteroblastic, the first bud arises through a rupture in the wall of the parent cell through which the innermost layer evaginates and ultimately grows out to form the outermost layer of the bud. The site of budding is eventually surrounded by a collarette owing to the recurrent formation and abscission of a succession of buds arising from the inner layer of the wall of the cell. Enteroblastic budding is characteristic of basidiomycetous yeasts and some members of Taphrinomycotina (Kurtzman and Sugiyama 2001; Sugiyama and Nishida 1995).

Budding is also classified on the basis of the position of the site where it occurs. Budding that is restricted to one pole of a cell is termed **monopolar**, and budding that occurs at both poles of a cell is termed **bipolar**. When the buds are abstricted on a rather broad base by the formation of a cross wall, the process is referred to as "budding on a broad base" and "bud fission" (Fig. 1.1). Recurrent budding leads to the formation of multiple scars or annellations at the poles of the cell (Streiblová 1971). Bipolar budding is characteristic of apiculate yeasts. Budding from various sites on a cell is termed **multilateral** or **multipolar** and is the most common type of budding among ascomycetous yeasts (Fig. 1.1). Budding is also described in terms of the way successive buds are produced. **Sympodial** budding occurs on a conidiophore that extends in growth by a succession of apices. A blastoconidium is produced at each apex, and the growth continues to the side of the apex; the result is a zigzag appearance, for example, *Blastobotrys*. Acropetal budding entails the formation of successive buds in a chain with the youngest at the apex. In basipetal budding, successive buds are formed, with the oldest at the apex.

Reproduction by **fission** is the division of an asexual cell by means of a septum growing inward from the cell wall to bisect the long axis of the cell. The newly formed fission cells, which are termed **arthroconidia** (arthrospores), elongate, and the process is repeated. Recurrent fission by a cell may give rise to transverse multiple scars or annellations (Streiblová 1971). This manner of reproduction is characteristic of *Schizosaccharomyces* and *Dipodascus* (Fig. 1.1).

Asexual cells may be globose, subglobose, ellipsoid, ovoid, obovoid, cylindrical, botuliform, bacilliform, elongate, apiculate, ogival, lunate, or triangular. Definitions and illustrations of the various possibilities can be found in *Ainsworth & Bisby's Dictionary of the Fungi* (Kirk et al. 2008). The shape of the cell may reflect the mode of reproduction, and in some cases, it is characteristic of particular genera or species. Some examples include the lemon-shaped cells of the apiculate yeasts *Hanseniaspora* and *Wickerhamia*, the lunate cells of *Metschnikowia lunata*, and the triangular cells of *Trigonopsis variabilis* (Kurtzman and Robnett 2007).

Endospores are asexual cells that are formed within single cells and in hyphal cells and seem to arise by budding. Endospores are not commonly formed, but they have been observed in strains of *Candida* and a few other genera. No special media have been

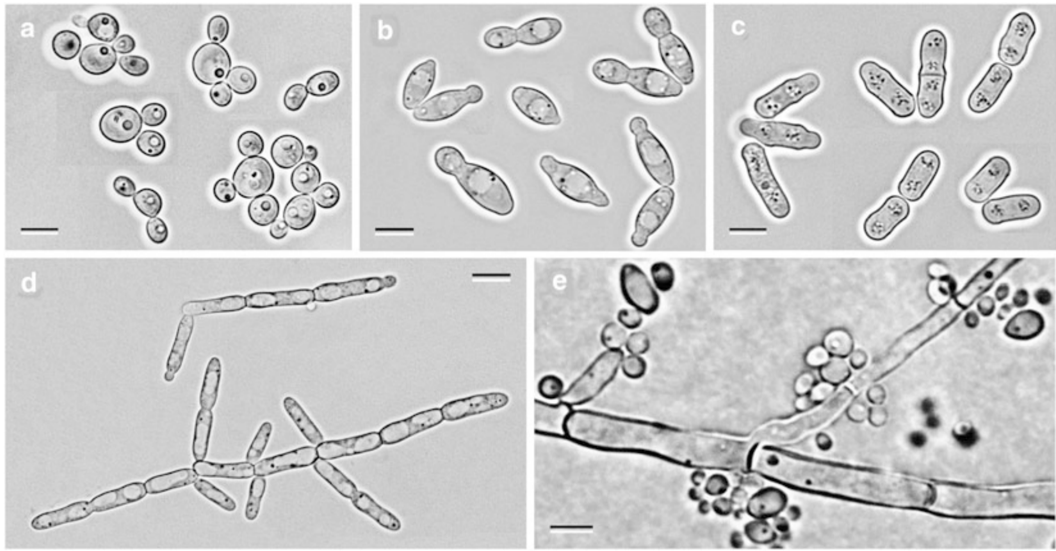


Fig. 1.1 Various forms of asexual reproduction. (**a**) Multilateral budding (*Pichia nakasei*). (**b**) Bipolar budding on a wide base (*Hanseniaspora osmophila*). (**c**) Fission (*Schizosaccharomyces pombe*). (**d**) Pseudohyphae (*Metschnikowia gruessii*). (**e**) True (septate) hypha with side branches bearing blastoconidia (*Candida ontarioensis*). (Panels **a**–**d**, T. van Beest and T. Boekhout, CBS Web site; panel **e**, C.P. Kurtzman). Bars = 5 μm

devised to stimulate the development of endospores (do Carmo-Sousa 1969).

Chlamydospores have been defined as thick-walled, nondeciduous, intercalary or terminal asexual spores formed by the rounding of a cell or cells (Ainsworth 1971; Hughes 1985; Stalpers 1987). The asexual nature of the chlamydospore distinguishes it from the teliospore of basidiomycetous yeasts from which the basidium is produced. Chlamydospores are generally rich in lipids and well adapted to maintain viability through periods of dormancy. In older cultures, chlamydospores shed their outer layers just before or during germination. Chlamydospores are characteristic of *C. albicans*, *C. dubliniensis*, and *Metschnikowia* species. Chlamydospores fulfill a dual function in *Metschnikowia* and either germinate by budding or are transformed into asci.

2. Pseudohyphae and True (Septate) Hyphae

Mature buds can either become detached as individual cells or remain attached to the parent cell and give rise to chains or clusters of cells. The tendency of some yeasts to form chains of cells results in the formation of pseudohyphae. A **pseudohypha** is defined as a filament composed of a chain of cells that has been formed by budding (Fig. 1.1). Pseudohyphae may be either rudimentary, in which case they consist of cells of similar size and shape, or they may be differentiated into elongated cells, each of which may produce blastoconidia. The form of a pseudohypha can be markedly affected by cultural conditions (van der Walt 1970). Some species of *Dekkera* form an unusual type of pseudohypha called a blastese, which is a slender, aseptate hypha that develops from germinating blastospores (Langeron and Guerra 1940).

Some yeasts produce **true septate branching hyphae**, which elongate by continuous growth of the hyphal tip followed by the formation of septa (Fig. 1.1). The fine structure of hyphal septa varies among taxa, but light microscopy does not reveal much detail except for the presence of large septal pore bodies in

Ambrosiozyma. Hyphae may proliferate by simple branching, or they may produce blastoconidia on differentiated conidiogenous cells. The presence of blastoconidia on denticles is a characteristic of species of *Trichomonascus* (anamorph *Blastobotrys*), *Hyphopichia*, and certain other genera. Hyphae are sometimes joined by a process in which there is the fusion of branches of the same or different hyphae, and this is called **anastomosis**. The media most commonly used for detecting pseudohyphae and true hyphae are corn meal (maize) agar, morphology agar, 5 % malt extract agar, and potato-dextrose agar, but some clinical laboratories use rice agar, which will also promote the formation of chlamydospores by *C. albicans*.

B. Sexual Reproduction

Many yeasts reproduce sexually, resulting in an alternation of generations with the formation of characteristic cells in which reduction division takes place. In ascogenous yeasts, the site of meiosis is the **ascus** where the haploid generation of ascospores is formed by so-called free-cell formation, i.e., the process by which the cytoplasm surrounding the meiotic nuclei becomes enveloped by a wall. Ascogenous yeasts may be homothallic or heterothallic, and the asexual phase may be diploid or haploid, but sometimes both haploid and diploid cells are present in the same culture. Higher degrees of ploidy have also been reported for some species, such as *S. cerevisiae* and *Lachancea kluyveri* (≡*Saccharomyces kluyveri*) (Wickerham 1958).

For haploid **homothallic yeasts**, plasmogamy, karyogamy, and meiosis occur within the zygote, which is often formed by the conjugation of two separate budding cells or by conjugation between a cell and its bud (mother–daughter cell conjugation or bud meiosis). The diplophase is usually restricted to the diploid zygote within which the ascospores are formed. In the case of conjugation between a cell and its bud, the bud remains attached to the parent cell, which is converted into an ascus in which usually one to four ascospores are formed. Asci bearing such vestigial buds are found in *Debaryomyces*, *Torulaspora, Pichia*, and certain other genera. A process comparable to cell–bud conjugation appears to operate in the genus *Nadsonia*, where karyogamy is initiated by the fusion of the nuclei of a bud and its parent. The contents of the zygote then move into a bud at the opposite pole, which is abstricted by a septum and becomes the ascus. When diploidization occurs by the fusion of two independent haploid cells, the cells may form elongated conjugation tubes, which fuse to give a dumbbell shape, as is characteristic for *Zygosaccharomyces*, *Kodamaea* and certain other taxa (Fig. 1.2).

Heterothallic species may occur as haploid mating types or as diploid strains, which are normally heterozygous for the mating-type genes and are sometimes termed bisexual. Unisexual diploid strains are known (Wickerham 1958); asci from these strains are unconjugated, and unisexual haploid ascospores of both mating types are formed. Ascospores of opposite mating types either conjugate within the ascus, giving rise to the diplophase, as in *Saccharomycodes*, or the ascospores are released and germinate to give haploid asexual cells of opposite mating types. Normally, the diplophase can only be restored if conjugation of haploid cultures of opposite mating types occurs. Active cultures of mating types are not invariably stable and may revert to sporulating cultures as a result of mutation of the mating-type alleles (Takano and Oshima 1970). Some species, such as *Pichia membranifaciens*, seem to have both heterothallic and homothallic strains. Heterothallic strains of some species may show sexual agglutination, as in *Wickerhamomyces canadensis* (=*Hansenula wingei*) and *Lachancea kluyveri* (≡*Saccharomyces kluyveri*) (Wickerham 1958), in which cells of opposite mating types agglutinate when mixed. Agglutination in *W. canadensis* is mediated by complementary glycoproteins present on the surface of cells of the opposite mating types (Crandall and Brock 1968).

Ascospores vary in the number present in asci. Asci with either one or two ascospores

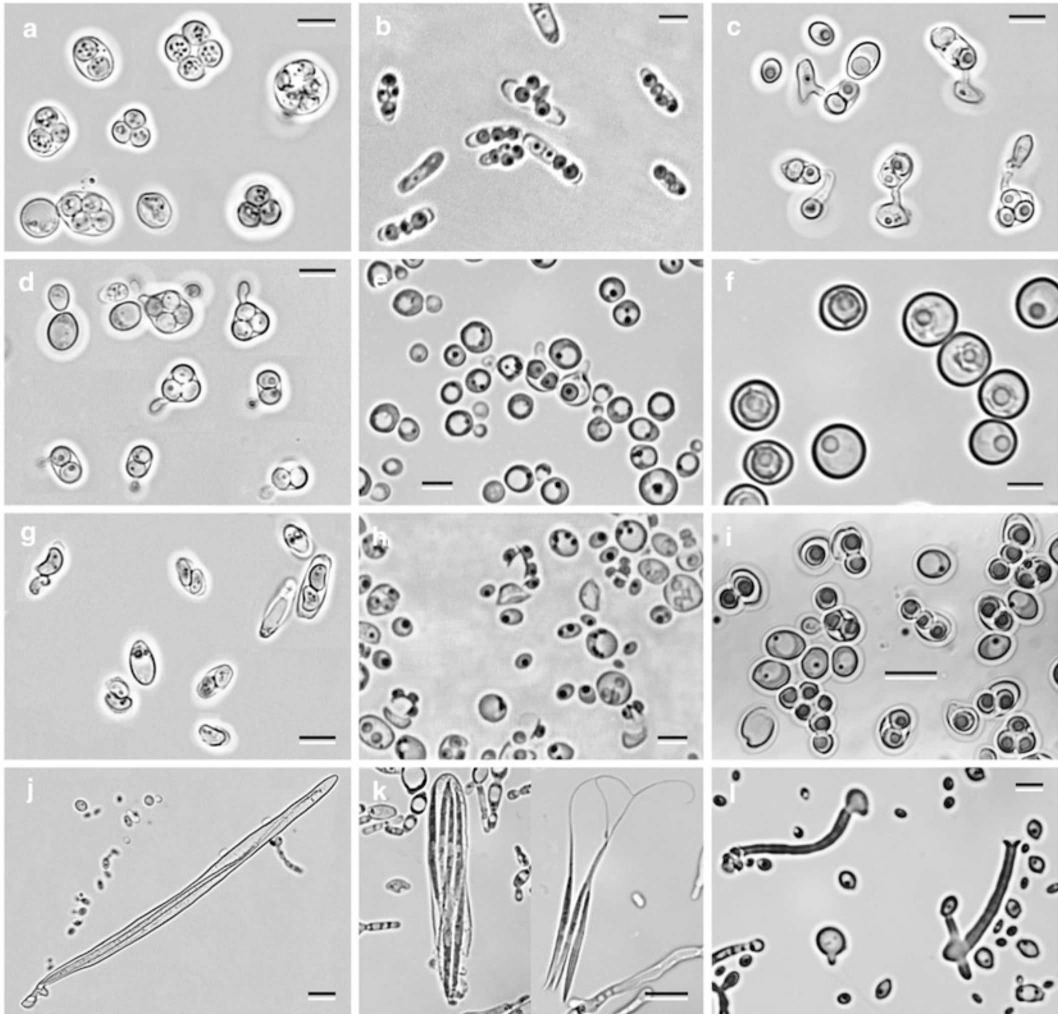


Fig. 1.2 Various forms of ascospore formation. (**a**) Persistent, unconjugated asci with globose ascospores (*Saccharomyces paradoxus*). (**b**) Deliquescent, unconjugated asci with globose and hat-shaped ascospores (*Pichia membranifaciens*). (**c**) Asci with globose ascospores formed from conjugation of complementary mating types (*Kodamaea ohmeri*). (**d**) Persistent asci with globose ascospores formed by conjugation between cells and their buds (*Schwanniomyces pseudopolymorphus*). (**e**) Persistent asci with tapered bud-conjugants and globose ascospores (*Torulaspora delbrueckii*). (**f**) Persistent, unconjugated asci, each with a roughened, spherical ascospore (*Citeromyces siamensis*). (**g**) Deliquescent asci with bean-shaped ascospores (*K. marxianus*). (**h**) Hat-shaped ascospores released from deliquescent asci (*Cyberlindnera veronae*). (**i**) Saturn-shaped ascospores formed in deliquescent asci (*Saturnispora ahearnii*). (**j**) Elongated, needle-shaped ascospores in a persistent ascus (*Metschnikowia hawaiiensis*). (**k**) Elongated ascospores with a whiplike tail released from a deliquescent ascus (*Eremothecium coryli*). (**l**) Hat-shaped ascospores released from an ascus formed at the tip of an ascophore (*Pachysolen tannophilus*). (Panels **a**, **c**, **d**, **g**, **j**, T. van Beest and T. Boekhout, CBS Web site; panels **b**, **e**, **f**, **h**, **i**, **k**, **l**, C.P. Kurtzman). Bars = 5 μm

are typical for *Lodderomyces*, *Metschnikowia*, and some species of *Debaryomyces*, whereas four spores are characteristic of some species of *Pichia*, *Saccharomyces*, and certain other genera. In contrast, species of *Ascoidea* and *Vanderwaltozyma* may form in excess of 100 ascospores in each ascus. The shape of ascospores varies widely and includes globose,

ellipsoidal, cylindrical, reniform, crescentic, clavate, hat-shaped (galeate), cap-shaped, saturnoid, walnut-shaped, falcate, needle-shaped, and spindle-shaped with a whiplike appendage (Fig. 1.2). The surface may be smooth or rough, but surface ornamentation, including brims and ledges, may be reduced to such an extent that this morphology cannot be detected by light microscopy. Phylogenetic analyses have shown that ascospore shape is of uncertain value for assessing genus assignments and sometimes even varies within strains of a species (Kurtzman et al. 2008). An example of the latter is *Kodamaea ohmeri*, where both hat-shaped and globose ascospores have been found depending on the mating types paired (Wickerham and Burton 1954).

Ascosporulation may be induced under conditions that restrict asexual growth, but many strains sporulate without any special preparation. Some genera sporulate best on a particular medium. Acetate agar has been recommended for *Saccharomyces* (e.g., McClary et al. 1959) and dilute V8 agar for *Metschnikowia* (Pitt and Miller 1968). Many strains of *Pichia* and related genera sporulate on YM or malt agars. What follows represents a suggested start for ascospore detection. Strains placed on slant cultures of YM, 5 % malt extract, dilute V8, and RG agars should be incubated at 15 and 25 °C and examined weekly for 2 months [see Kurtzman et al. (2011) for the composition of culture media given in this chapter]. Some yeasts sporulate rapidly, i.e., within 24–48 h, especially when first isolated; others may require much longer, up to 6 weeks or more. If ascosporulation is not detected, then other media, such as Gorodkowa and acetate agars, can be tried. Strains that do not form ascospores may represent mating types and should be mixed to determine whether conjugation and ascosporulation occur. Conjugation often occurs within 24–48 h, but occasionally 1–2 weeks may be required. Ascospores can be stained with malachite green (Wickerham 1951), but visualization of unstained cells with either bright field or phase contrast microscopy is favored by many observers over staining.

V. Taxonomic Methods

A. Phenotypic Characterization

In addition to differentiation by cellular morphology, responses on fermentation and growth (assimilation) tests are still used in some laboratories to identify taxa. The **physiological tests** commonly used are fermentation of seven to eight carbohydrates, growth on various carbon and nitrogen sources, requirements for vitamins, growth at various temperatures, growth on media with a high content of sugar or sodium chloride, hydrolysis of urea, and resistance to antibiotics.

There is no known exception to the rule that when a yeast strain ferments a carbohydrate, it is also able to grow on it. However, the reverse does not hold true; many yeasts grow aerobically on sugars they cannot ferment. Yeasts vary in their ability to ferment sugars as measured by the production of carbon dioxide.

Various tests have been devised to detect the production of carbon dioxide from carbohydrates, but Durham tubes are the most useful method for the routine detection of carbohydrate fermentation (Kurtzman et al. 2011; Wickerham 1951). Durham tubes are test tubes with a small inverted tube inserted to collect any gas that may be produced. The fermentation of D-glucose, D-galactose, sucrose, maltose, lactose, raffinose, and trehalose is generally tested for routine identification; other compounds, such as inulin, starch, melibiose, cellobiose, and D-xylose, are sometimes used. The sugars are tested as 2 % (w/v) solutions, except for raffinose, where 4 % is usually used because some strains cleave and ferment only part of the molecule of this trisaccharide.

Assimilation tests determine the ability of a yeast to grow aerobically on a particular carbon compound supplied as the sole source of energy. The tests can be done either on solid media or in liquid media, but liquid media are believed by some taxonomists to give more reproducible results.

The size of growth tubes and the amount of medium used can vary widely among laboratories, but the method employing tubes of liquid media as described by Wickerham (1951) is commonly used. The results

are improved by gently shaking the tubes during incubation. Some laboratories incubate the tests for a period of 3 weeks, others for 4 weeks. These long incubations allow the yeasts to adapt to utilize some compounds. Tests on solid media can be done in two ways. The first is the auxanographic method of Beijerinck (1889), in which the yeast is suspended in agar in pour plates and the test sugars are spotted at intervals around the circumference. The second method is to incorporate the test compound into a nutrient agar basal medium in petri dishes and inoculate the test yeast as either a streak or a point on the surface.

B. Genotypic Characterization

During the past 10–15 years, gene sequence analyses have been used for yeast identification, usually replacing the phenotypic methods described earlier. Nonetheless, the collection of phenotypic data provides important information about the biology of the strains under study and their potential biotechnological applications. DNA comparisons of yeasts have paralleled the increasing sophistication of methods for nucleic acid characterization. Initial studies were restricted to determining the mol% guanine+cytosine (G+C) content of DNA. From this work it was seen that ascomycetous yeasts had a nuclear DNA content of ca. 28–50 mol%, whereas basidiomycetous yeasts had a noticeably higher range of 50–70 mol% (Nakase and Komagata 1968; Price et al. 1978). These studies suggested that strains differing by 1–2 mol% were likely to represent different species, thereby providing a means for excluding strains incorrectly assigned to a particular species. Quantitation of gene sequence similarity between strains became possible with the development of DNA reassociation techniques that measure the extent of pairing of nucleotide sequences when DNA is made single-stranded and allowed to re-pair as a double strand. An interpretation of DNA reassociation data was provided by Martini and Phaff (1973) and Price et al. (1978), who suggested that, on the basis of shared phenotype, strains that showed 80 % or greater nuclear DNA relatedness are members of the same species. A limitation of DNA reassociation experiments has been that genetic resolution extends no further than to closely related species. In contrast, gene sequence comparisons offer the opportunity to resolve closely related species, as well as more distantly related taxa, and a database of sequences can be developed and expanded for further use.

The variable domain 2 (D2) from nuclear large subunit ribosomal RNA (LSU rRNA) was initially examined and found to resolve closely related species (Peterson and Kurtzman 1991). This work was expanded to include domains 1 and 2 (D1/D2) and applied to all described species of ascomycetous yeasts, resulting in a diagnostic database (barcode) for rapid species identification (Kurtzman and Robnett 1998). A comparison of nuclear DNA reassociation values suggested that conspecific strains differed by no more than 3 nucleotides among the 500–600 nucleotides of the **D1/D2 domains**, whereas differences of 6 or more nucleotides (1 %) indicated that the strains were different species. The preceding estimates of divergence were treated as a prediction (Kurtzman and Robnett 1998) because exceptions were known. Among them are hybrid species (Groth et al. 1999; Peterson and Kurtzman 1991; Vaughan-Martini and Kurtzman 1985) and certain DNA polymorphisms (Lachance et al. 2003).

A significant advantage to using **rRNA gene sequences** is that ribosomes have a common evolutionary history, and within the sequences are highly conserved regions between the variable regions that serve for pan-specific primer attachment for PCR amplification and sequencing. In contrast, protein coding genes tend to be variable across the entire gene, often making primer design difficult. Nonetheless, the gene sequences encoding several proteins have been examined for phylogenetically divergent groups of species. Daniel et al. (2001) compared *Candida* spp. from several clades and showed that phylogenetic trees generated from actin sequences were congruent with rRNA gene trees. Daniel and Meyer (2003) compared the resolution of closely related species from actin sequences and from D1/D2 LSU. As with D1/D2, actin did not always provide a clear separation of species, but in general, actin sequences had a greater number of substitutions, providing easier recognition of closely related species. Similar resolution was reported for the translation elongation factor-1α gene

(Kurtzman et al. 2008) and the cytochrome oxidase II (COX II) gene (Belloch et al. 2000; Kurtzman and Robnett 2003).

In the examples presented, determination of whether strains are conspecific or members of separate species can be confused by hybridization events, by unexplained sequence polymorphisms, and by differences in nucleotide substitution rates. Multigene analyses offer a means for detecting these changes, which would be signalled by lack of congruence for a particular gene tree.

The multigene approach was recommended by Goodman (1976) for vertebrates, for bacteria by Dykhuizen and Green (1991), and for fungi by Taylor et al. (2000). The paper by Taylor et al. (2000) provides an inclusive review of species concepts, and the term genealogical concordance phylogenetic species recognition (GCPSR) was introduced to describe the concept of multigene analysis for species recognition (see Taylor and Berbee, Chap. 1, Vol. VII, Part A).

Comparison of strains from single gene sequences, such as D1/D2 LSU rRNA and the sequences of ITS, has provided a rapid means for species identification. However, the preceding discussion makes clear that some closely related species are not resolved by these sequences and that the occurrence of hybrids further complicates identification. Consequently, critical species identification requires a comparison of multiple genes, hence the increasingly widespread application of multilocus sequence typing (MLST) in strain identification.

VI. Phylogeny and Classification

A. Phylogeny

The relationship of ascomycetous yeasts with other members of the Ascomycota has been controversial for over 100 years. Because yeasts are morphologically simple, it was proposed that either they represent primitive forms of Ascomycota (e.g., Guilliermond 1912) or that they represent morphologically reduced forms of more evolved taxa (Cain 1972; Redhead and Malloch 1977; von Arx and van der Walt 1987).

The issue of relationships within the Ascomycota remained uncertain until the use of gene sequence analysis to estimate phylogeny. Walker (1985) sequenced 5S rRNA for selected ascomycetes, and the analysis of this data set divided the Ascomycota into three groups: (1) *Schizosaccharomyces* and *Protomyces*, (2) budding yeasts, and (3) so-called filamentous fungi. Berbee and Taylor (1993) analyzed a larger group of species from nuclear SSU rRNA gene sequences, which showed the same three major ascomycete lineages and that yeasts and filamentous fungi are sister taxa, whereas *Schizosaccharomyces* and relatives diverged prior to these two clades. Kurtzman and Robnett (1994) showed from partial LSU and SSU rRNA sequences that all currently accepted ascomycetous yeast genera were members of a single clade, which was separate from *Schizosaccharomyces* and members of the filamentous fungi. The finding from single-gene analyses that Ascomycota is comprised of three separate lineages was supported by multigene sequence analyses (Fitzpatrick et al. 2006; James et al. 2006; Kuramae et al. 2006). The phylogenetic trees generated from sequence analyses have significantly changed classification within the fungi, and these changes will be discussed. The outline of ascomycetous yeast classification is given in Table 1.1 and is based on the classification of the kingdom Fungi presented by Hibbett et al. (2007) and from multigene analyses reported by Kurtzman (2003), Kurtzman and Robnett (2003, 2007, 2010, 2013), Kurtzman and Suzuki (2010), and Kurtzman et al. (2007, 2008). Figure 1.3 shows the phylogenetic relationship among taxa of **Saccharomycotina, Taphrinomycotina, and Pezizomycotina** and was determined from multigene sequence analysis (Sugiyama et al. 2006). Support for early diverging lineages in many multigene trees is often weak, and assignment of genera to

Table 1.1 Subphyla, classes, orders, families, and genera of yeasts and yeastlike taxa of phylum Ascomycota[a,b,c]

Class	Order	Family	Genus
Subphylum Taphrinomycotina			
Archaeorhizomycetes	Archaeorhizomycetales Rosling & T. James	Archaeorhizomycetaceae Rosling & T. James	*Archaeorhizomyces* Rosling & T. James (A)
Neolectomycetes	Neolectales Landvik, O.E. Eriksson, Gargas & P. Gustafsson	Neolectaceae Redhead	*Neolecta* Spegazzini (T)
Pneumocystidomycetes	Pneumocystidales O.E. Eriksson	Pneumocystidaceae O.E. Eriksson	*Pneumocystis* P. Delanoë & Delanoë (T)
Schizosaccharomycetes	Schizosaccharomycetales O.E. Eriksson, Svedskog & Landvik	Schizosaccharomycetaceae Beijerinck ex Klöcker	*Schizosaccharomyces* Lindner (T)
Taphrinomycetes	Taphrinales Gäumann & C.W. Dodge	Protomycetaceae Gray	*Burenia* M.S. Reddy & C.L. Kramer (T)
			Protomyces Unger (T)
			Protomycopsis Magnus (T)
			Taphridium Lagerheim & Juel ex Juel (T)
			Volkartia Maire (T)
		Taphrinaceae Gäumann & C.W. Dodge	*Lalaria* R.T. Moore emend. Á. Fonseca (A)
			Saitoella S. Goto, Sugiyama, Hamamoto & Komagata (A)
			Taphrina Fries (T)
Subphylum Saccharomycotina			
Saccharomycetes	Saccharomycetales Kudryavtsev	Ascoideaceae J. Schröter	*Ascoidea* Brefeld & Lindau (T)
		Cephaloascaceae L.R. Batra	*Cephaloascus* Hanawa (T)
		Debaryomycetaceae Kurtzman & M. Suzuki	*Debaryomyces* Lodder & Kreger-van Rij (T)
			Hyphopichia von Arx & van der Walt[d] (T)
			Kurtzmaniella Lachance & Starmer[d] (T)
			Lodderomyces van der Walt[d] (T)
			Meyerozyma Kurtzman & M. Suzuki[d] (T)
			Millerozyma Kurtzman & M. Suzuki[d] (T)
			Priceomyces M. Suzuki & Kurtzman[d] (T)
			Scheffersomyces Kurtzman & M. Suzuki[d] (T)
			Schwanniomyces Klöcker emend. M. Suzuki & Kurtzman (T)
			Spathaspora Nguyen, Suh & Blackwell[d] (T)
			Wickerhamia Soneda[d] (T)
			Yamadazyma Billon-Grand[d] (T)
		Dipodascaceae Engler & E. Gilg	*Dipodascus* Lagerheim (T)
			Galactomyces Redhead & Malloch (T)
			Geotrichum Link:Fries (A)
			Magnusiomyces Zender (T)
			Saprochaete Coker & Shanor ex D.T.S. Wagner & Dawes (A)
		Endomycetaceae J. Schröter	*Endomyces* Reess (T)
			Helicogonium W.L. White (T)
			Phialoascus Redhead & Malloch (T)

Lipomycetaceae E. K. Novak & Zsolt	*Dipodascopsis* Batra & P. Millner emend. Kurtzman, Albertyn & Basehoar-Powers (T)
	Lipomyces Lodder & Kreger-van Rij (T)
	Myxozyma van der Walt, Weijman & von Arx (A)
Metschnikowiaceae T. Kamienski	*Aciculoconidium* King & Jong (A)
	Clavispora Rodrigues de Miranda (T)
	Kodamaea Y. Yamada, T. Suzuki, Matsuda & Mikata emend. Rosa, Lachance, Starmer, Barker, Bowles & Schlag-Edler (T)
	Metschnikowia Kamienski (T)
Phaffomycetaceae Y. Yamada, Kawasaki, Nagatsuka, Mikata & Seki	*Barnettozyma* Kurtzman, Robnett & Basehoar-Powers (T)
	Cyberlindnera Minter (T)
	Phaffomyces Y. Yamada, (T)
	Starmera Y. Yamada, Higashi, S. Ando & Mikata (T)
	Wickerhamomyces Kurtzman, Robnett & Basehoar-Powers (T)
Pichiaceae Zender	*Ambrosiozyma* van der Walt (T)
	Brettanomyces Kufferath & van Laer[e] (A)
	Dekkera van der Walt[e] (T)
	Kregervanrija Kurtzman (T)
	Ogataea Y. Yamada, Maeda & Mikata (T)
	Pichia E.C. Hansen (T)
	Saturnispora Liu & Kurtzman (T)
Saccharomycetaceae G. Winter	*Cyniclomyces* van der Walt & D.B. Scott (T)
	Eremothecium Borzi emend. Kurtzman (T)
	Kazachstania Zubkova (T)
	Kluyveromyces van der Walt (T)
	Lachancea Kurtzman (T)
	Nakaseomyces Kurtzman (T)
	Naumovozyma Kurtzman (T)
	Saccharomyces Meyen ex Reess (T)
	Tetrapisispora Ueda-Nishimura & Mikata emend. Kurtzman (T)
	Torulaspora Lindner (T)
	Vanderwaltozyma Kurtzman (T)
	Zygosaccharomyces Barker (T)
	Zygotorulaspora Kurtzman (T)
Saccharomycodaceae Kudryavtsev	*Hanseniaspora* Zikes (T)
	Kloeckera Janke (A)
	Saccharomycodes E.C. Hansen (T)

(continued)

Table 1.1 (continued)

Class	Order	Family	Genus
		Saccharomycopsidaceae von Arx & van der Walt	*Saccharomycopsis* Schiönning (T)
		Trichomonascaceae Kurtzman & Robnett	*Blastobotrys* von Klopotek (A)
			Diddensiella Péter, Dlauchy & Kurtzman (T)
			Spencermartinsiella Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki & Kurtzman (T)
			Starmerella Rosa & Lachance (T)
			Sugiyamaella Kurtzman & Robnett (T)
			Trichomonascus H.S. Jackson emend. Kurtzman & Robnett (T)
			Wickerhamiella van der Walt (T)
			Yarrowia van der Walt & von Arx (T)
			Zygoascus M.Th. Smith (T)
	Saccharomycetales *incertae sedis*		*Ascobotryozyma* J. Kerrigan, M.Th. Smith & J.D. Rogers (T)
			Babjeviella Kurtzman & M. Suzuki (T)
			Botryozyma Shann & M.Th. Smith (A)
			Candida Berkhout (A) *pro parte*
			Citeromyces Santa María (T)
			Coccidiascus Chatton emend. Lushbaugh, Rowton & McGhee (T)
			Komagataella Y. Yamada, Matsuda, Maeda & Mikata (T)
			Kuraishia Y. Yamada, Maeda & Mikata (T)
			Macrorhabdus Tomaszewski, Logan, Snowden, Kurtzman & Phalen (A)
			Nadsonia Sydow (T)
			Nakazawaea Y. Yamada, Maeda & Mikata (T)
			Pachysolen Boidin & Adzet (T)
			Peterozyma Kurtzman & Robnett (T)
			Schizoblastosporion Ciferri (A)
			Sporopachydermia Rodrigues de Miranda (T)
			Trigonopsis Schachner emend. Kurtzman & Robnett (A)

[a](A) = anamorphic genus, (T) = teleomorphic genus

[b]Anamorphic and teleomorphic genera are placed together in the same family when relationships are known. For many anamorphic and teleomorphic genera, phylogenetic relationships are unclear and the genera are placed in Saccharomycetales *incertae sedis* until family relationships become known. The polyphyletic anamorphic family Candidaceae is not listed in this table

[c]Classification is based on the following publications: Hibbett et al. (2007), Kurtzman (2003), Kurtzman and Robnett (2003, 2007, 2010, 2013), Kurtzman and Suzuki (2010), and Kurtzman et al. (2007, 2008)

[d]Placement in the family Debaryomycetaceae is tentative

[e]Placement of the genera *Brettanomyces* and *Dekkera* in the family Pichiaceae is tentative

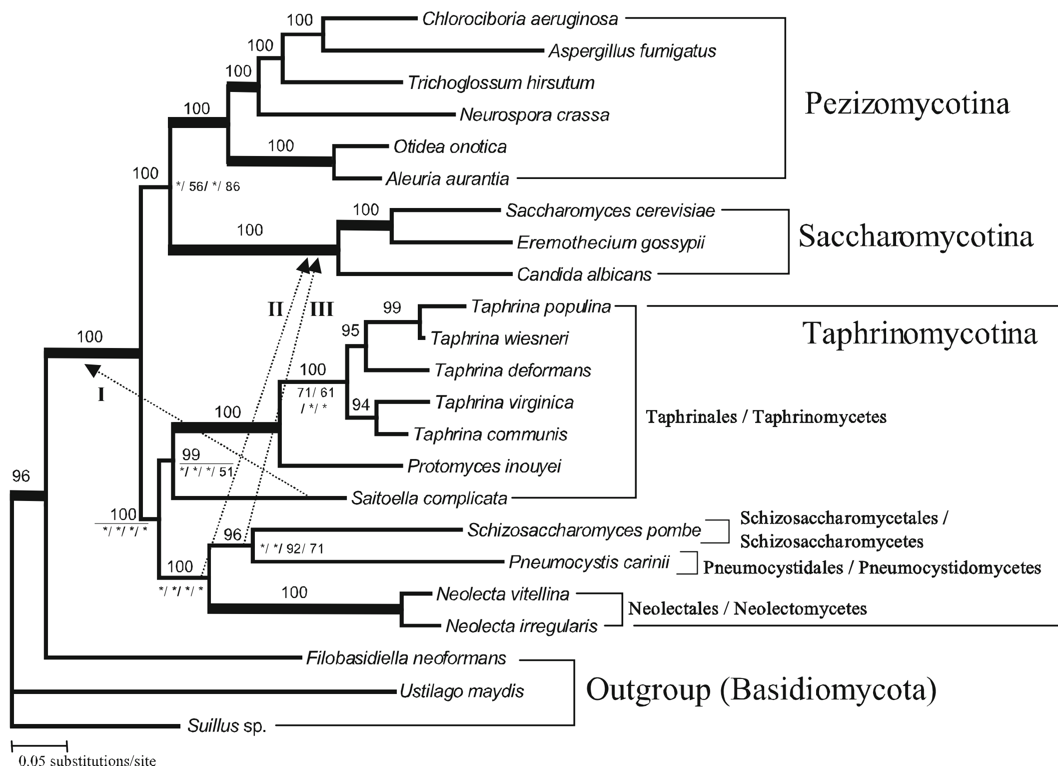


Fig. 1.3 Phylogenetic resolution of members of the Ascomycota into three major clades (Pezizomycotina, Saccharomycotina, Taphrinomycotina) from a multigene analysis based on the 50 % majority rule consensus of 18,000 Bayesian MCMCMC generated trees. In this analysis, the alternate positions for *Saitoella complicata* provide an example of the effect that weak basal lineages have on taxon placement. Numbers on branches are Bayesian posterior probability. Numbers separated by a *slash* (/) indicate bootstrap values based on parsimony analysis with third codon position/parsimony analysis without third codon position/neighbor joining (NJ) analysis with third codon position/NJ analysis without third codon position. An *asterisk* (*) indicates lack of bootstrap support. Branches supported by ≥95 % posterior probability and ≥70 % bootstrap value in all analyses (parsimony vs. NJ, third codon included vs. excluded) are indicated by *thick lines*. *Dotted arrows* indicate alternative groupings found with ≥70 % bootstrap support: I = parsimony analysis with third codon position; II = both parsimony and NJ analyses with third codon position; III = NJ analysis without third codon position, which demonstrates the impact of data set composition on the outcome of the analysis. For details and GenBank accession numbers of the four genes (SSU rRNA, D1/D2 LSU rRNA, RNA polymerase 2, and beta-tubulin) used in this analysis, see Sugiyama et al. (2006). Additionally, see Assembling the Fungal Tree of Life Web site (http://aftol.org/data.php) and the GenBank homepage (http://www.ncbi.nih.gov/Genbank/index.html). Modified from Sugiyama et al. (2006); reproduced with permission from Mycologia. @The Mycological Society of America

families is tentative for many of the taxa. The analyses presented in Figs. 1.3 and 1.4a, b are examples of this issue, and the uncertain placement of the anamorphic genus *Saitoella* has been indicated in Fig. 1.3, whereas Fig. 1.4b suggests that Taphrinomycotina may consist of two clades. However, other multigene analyses have supported the monophyly of Taphrinomycotina (Liu et al. 2009; Schoch et al. 2009).

B. Classification

Two events are having a profound impact on the classification of fungi. The first of these, as discussed earlier, is gene sequence analyses, which have changed our understanding of relationships among members of Ascomycota. The genera accepted in Saccharomycotina and Taphrinomycotina are listed in Table 1.1, and most have now been

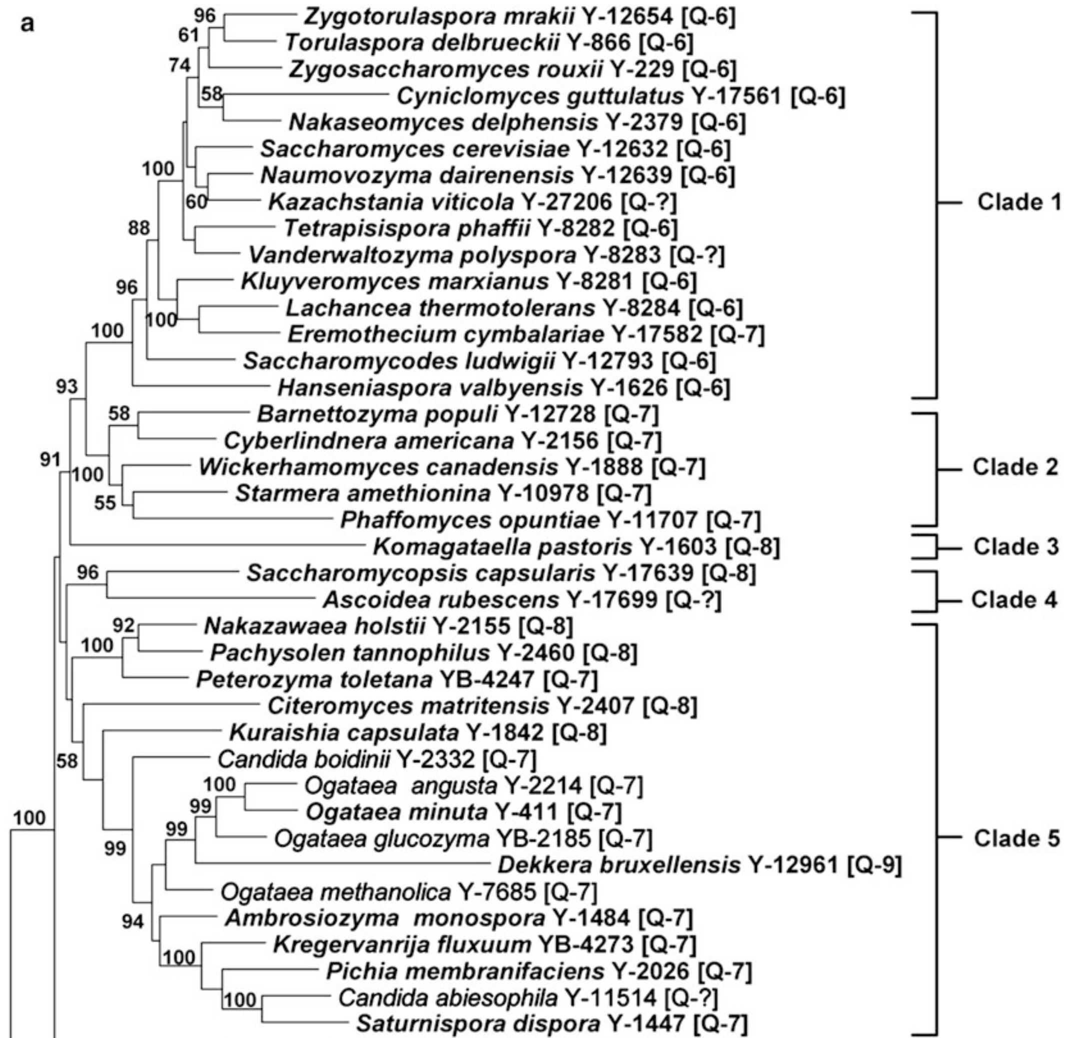


Fig. 1.4 (continued)

circumscribed from multigene phylogenetic analyses. Phylogenetic relationships among the genera are shown in Fig. 1.4a, b. This analysis is based on five gene sequences, but bootstrap support for placement of some genera in the phylogenetic tree is weak and their assignment to families is tentative.

The second event that is having a major impact on the classification of fungi is the result of recent changes in the *International Code of Nomenclature for algae, fungi and plants* (Melbourne Code, e.g., Hawksworth 2012; Knapp et al. 2011; Norvell 2011). The genera of fungi may now include species with known sexual states (teleomorphs) as well as species for which sexual states have not been discovered (anamorphs). With this change, genera will be phylogenetically circumscribed to include related species whether or not sexual states are known, and the terms anamorph and teleomorph will have no status in genus descriptions. Because this change in rules for classification is so new, few yeast genera have been recircumscribed to include both sexual and asexual species. For this reason, the terms anamorph and teleomorph are being used in

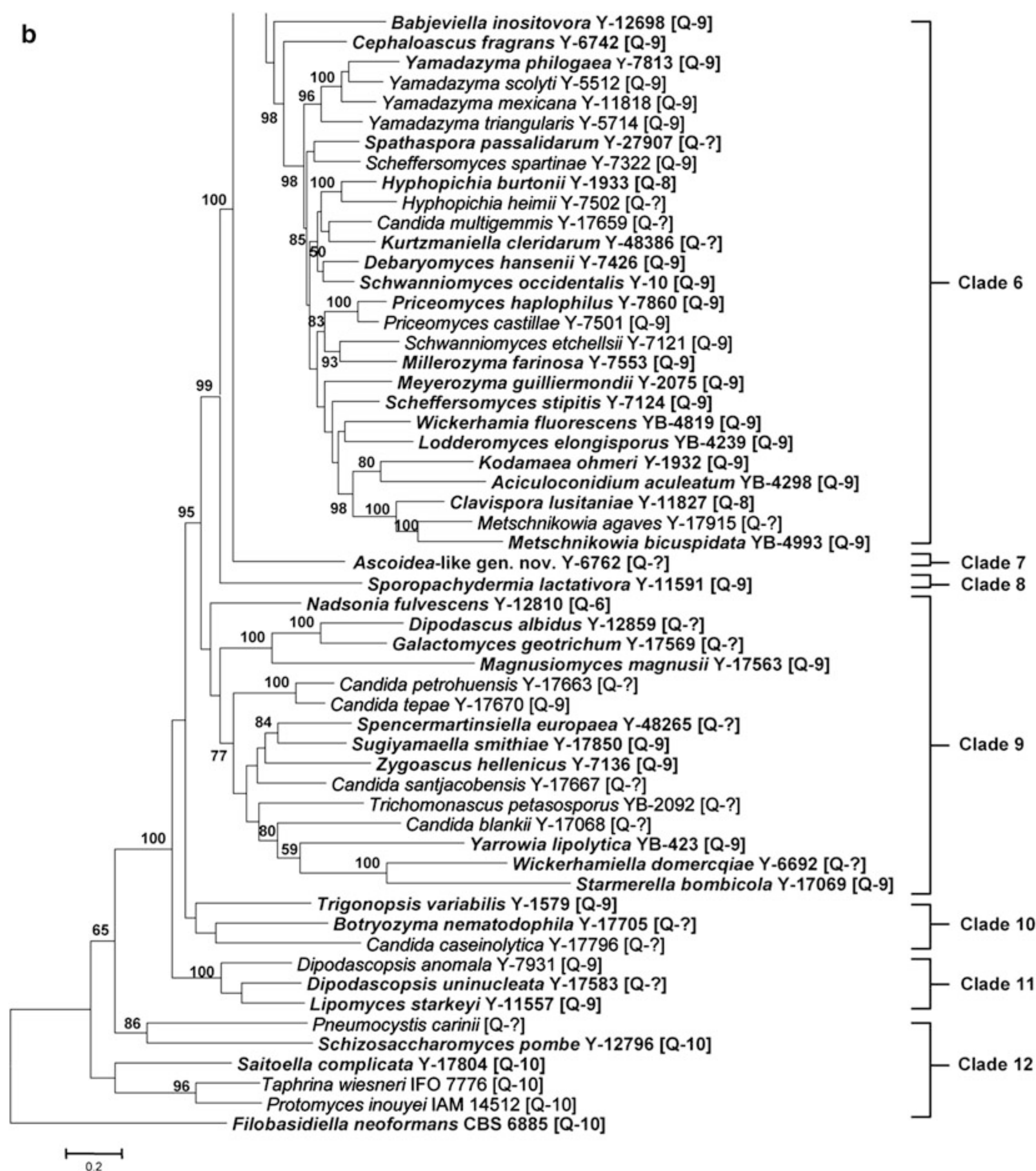


Fig. 1.4 (**a**) Phylogenetic relationships among type species of ascomycetous yeast genera and reference taxa determined from maximum-likelihood analysis using concatenated gene sequences for LSU rRNA, SSU rRNA, EF-1α, RPB1, and RPB2. *Filobasidiella neoformans* was the designated outgroup species in the analysis. Names in *bold font* are type species of currently recognized genera, whereas names in standard font are not type species. *Pneumocystis* is represented by the type species, but not the type strain. *Protomyces* and *Taphrina* are not represented by type species. Bootstrap values (1,000 replicates) >50 % are given at branch nodes. Strain accession numbers are NRRL unless otherwise indicated. Designations in *brackets* indicate the coenzyme *Q* value for each species. Y and YB prefixes are NRRL strain numbers. Type species of the genera *Ascobotryozyma* (anamorph, *Botryozyma*), *Coccidiascus*, *Endomyces*, *Helicogonium*, *Phialoascus*, *Macrorhabdus*, and *Schizoblastosporon* (teleomorph, *Nadsonia*), several of which are not known from culture, were not included in the analysis (Kurtzman and Robnett 2013). Basal lineages of some taxa are not well resolved, which demonstrates the need to include additional gene sequences in analyses. Because of this, many family assignments are tentative. Clade 1, Saccharomycetaceae and Saccharomycodaceae (*Saccharomycodes* and *Hanseniaspora*, which appear more closely related in some analyses); Clade 2, Phaffomyce-

this chapter to provide a reference to the classification of yeasts presented in the recently published fifth edition of *The Yeasts, A Taxonomic Study* (Kurtzman et al. 2011), which was prepared before the new code was adopted.

1. Saccharomycotina

Many of the teleomorphic clades of Saccchar-omycotina include species of *Candida*. Under the old code, the genus *Candida* was a "dumping ground" for budding yeasts that do not form ascospores, and it represents a polyphyletic group of species. With the introduction of sequence analysis, many species of *Candida* are seen to be members of teleomorphic clades and, because of the aforementioned changes in the code, will be transferred to those genera. However, other *Candida* species are in clades with no known ascosporic state and will be classified in new genera. What has become apparent from recent research is that much of the future classification of yeasts will rest on the phylogenetic analysis of gene sequences rather than on the phenotypic characters that we observe on a petri dish or under the microscope.

2. Taphrinomycotina

On the basis of phylogenetic analyses of various gene sequences, members of the subphylum Taphrinomycotina are composed of the early diverging and biologically diverse members of Ascomycota (e.g., Kurtzman and Robnett 1998; Kurtzman and Sugiyama 2001; Sugiyama et al. 2006). The number of known taxa assigned to Taphrinomycotina is surprisingly small considering that the more recently evolved Saccharomycotina and Pezizomycotina are much more species rich. Whether this reflects an absence of species diversity or ineffective isolation strategies is unknown, but the recent description of *Archaeorhizomyces* (the new class Archaeorhizomycetes) (Rosling et al. 2011), an early diverging and widely distributed genus, suggests that more effective isolation methods will reveal Taphrinomycotina to be a larger group of species than previously thought.

Taphrinomycotina are characterized by ascosporic states that lack ascogenous hyphae. Asexual reproduction is by budding or fission. With the exception of *Neolecta*, an apothecial ascomycete genus (Landvik 1996; Landvik et al. 1993), neither ascomata nor conidiomata are formed. Saccharomycotina have cell walls that show two layers and undergo holoblastic conidiogenesis, in contrast to basidiomycetous yeasts, which characteristically have multilayered cell walls and enteroblastic, repetitive percurrent conidiogenesis (Moore 1987). However, *Saitoella complicata* and *Taphrina wiesneri* are exceptional because, although they have two-layer cell walls, they show enteroblastic budding (Goto et al. 1987; Sjamsuridzal et al. 1997).

Originally, it was thought that *Taphrina* and *Schizosaccharomyces* lacked chitin in their cell walls (e.g., Cavalier-Smith 1987), thereby separating them from other fungi, but Sietsma and Wessels (1990) reported glucosaminoglycan in *Schizosaccharomyces pombe*. Bowen et al. (1992) determined the DNA sequence of a chitin synthase fragment from *S. pombe*, and Nishida and Sugiyama (1994a) demonstrated DNA sequences for chitin synthase fragments in *Taphrina wiesneri*, *Protomyces inouyii*, and *Saitoella complicata*. Furthermore, Garner et al. (1991) reported that chitin is an integral part of the cell wall of *Pneumocystis carinii* trophozoites and cysts. The carbohydrate composition of cell walls from *Taphrina*, *Protomyces*, and *Saitoella* is characterized by rhamnose, glucose, and mannose (Prillinger et al. 1990; Sugiyama et al. 1985), but *Schizosaccharomyces* does not contain rhamnose (Prillinger et al. 1990). Members of Taphrinomycotina show a negative diazonium blue B (DBB) reaction, as is typical

Fig. 1.4 (continued) taceae; Clade 3, *Komagataella*; Clade 4, *Saccharomycopsis* (Saccharomycopsidaceae) and *Ascoidea* (Ascoideaceae); Clade 5, Pichiaceae and closely related genera. (**b**) Phylogenetic relationships among type species of ascomycetous yeast genera and reference taxa determined from maximum likelihood analysis using concatenated gene sequences for LSU rRNA, SSU rRNA, EF-1α, RPB1, and RPB2. Clade 6, Debaryomycetaceae, Metschnikowiaceae, and other related taxa; Clade 7, *Alloascoidea*, an *Ascoidea*-like new genus; Clade 8, *Sporopachydermia*; Clade 9, Dipodascaceae and Trichomonascaceae; Clade 10, *Trigonopsis* and *Botryozyma*; Clade 11, Lipomycetaceae; Clade 12, Taphrinomycotina, which in this analysis shows a dichotomy but in some other analyses all genera share a common lineage

of Saccharomycotina (Kurtzman and Sugiyama 2001; Kurtzman et al. 2011; Sugiyama and Nishida 1995).

Order Pneumocystidales. *Pneumocystis carinii* is a principal causal agent of pneumonia in patients with HIV/AIDS, and for many years this organism was considered to be a protozoan. Edman et al. (1988) showed *P. carinii* to be a fungus based on 18S sequence comparisons. Watanabe et al. (1989) suggested from 5S rRNA analysis that *Pneumocystis* is closely related to Zygomycotina, but ultrastructural studies showed cell division by fission (Yoshida 1989). Taylor et al. (1994) and Sugiyama and Nishida (1995) suggested that *P. carinii* and *S. pombe* have a similar life cycle, and Nishida and Sugiyama (1994b) placed *Pneumocystis* in Taphrinomycotina. Subsequently, Hibbett et al. (2007) accommodated the genus in the class Pneumocystidiomycetes in the subphylum Taphrinomycotina.

Pneumocystis was included for the first time in *The Yeasts, A Taxonomic Study*, 5th Edition, in which Cushion and Keely (2011) fully redescribed and accepted five species, i.e., the type species *P. carinii* and four other species.

According to the genus diagnosis (Cushion and Keely 2011), "no species of *Pneumocystis* has been continuously cultivated outside the mammalian lung," asexual reproduction is by binary fission, and eight ascospores are produced within an ascus as a result of meiosis. The correct name for the taxon called *Pneumocystis carinii* from human lungs is *Pneumocystis jirovecii*, whereas the name *P. carinii* is only found in the lungs of immunosuppressed rats (Cushion and Keely 2011).

Order Schizosaccharomycetales. *Schizosaccharomyces* is saprotrophic and undergoes asexual reproduction by fission. The genus is comprised of four species: *S. japonicus*, *S. octosporus*, *S. pombe*, and *S. cryophilus* (Vaughan-Martini and Martini 2011). Yamada and Banno (1987) reassigned *S. octosporus* and *S. japonicus* to other genera on the basis of differences in ascospore morphology, ubiquinone type, and cellular linoleic acid content, but this separation was not supported by rRNA gene sequence analyses (Kurtzman and Robnett 1991, 1998; Naehring et al. 1995). Strains of *Schizosaccharomyces* are isolated from high sugar substrates, such as fruit juices, honey, dried fruits, and tree fluxes. *Schizosaccharomyces* is widely used in studies of molecular biology.

Order Neolectales. The genus *Neolecta* is characterized by clavate, stalked apothecia and cylindrical aparaphysate eight-spored asci. Korf (1973) placed the genus in Helotiales of the Discomycetes, whereas Redhead (1977) created the new family Neolectaceae and tentatively placed it in Lecanorales. Landvik et al. (1993) and Landvik (1996) proposed the new order Neolectales because their SSU rRNA gene sequence analyses placed *Neolecta* outside of Pezizomycotina. Because of these and other comparisons, Eriksson and Hawksworth (1995) and Sjamsuridzal et al. (1997) placed the genus *Neolecta* in what is now Taphrinomycotina. According to Redhead's (1977) description and illustrations, asci of *Neolecta vitellina* occasionally are filled with numerous conidia, which has also been observed in *Taphrina* (cf. Sugiyama 1998).

Order Taphrinales. Included in this order are two families, **Protomycetaceae** and **Taphrinaceae** (Table 1.1). Protomycetaceae includes six genera, *Burenia*, *Protomyces*, *Protomycopsis*, *Saitoella*, *Taphridium*, and *Volkartia*. Some of these genera are well studied, others are not, and we will discuss three of the better studied genera of the order. *Protomyces* is parasitic on plants mainly of Apiaceae (Umbelliferae) and Asteraceae (Compositae). *Protomyces inouyei*, for example, attacks the composite *Youngia japonica* in Japan, causing a gall on stems (Tubaki 1957). The thick-walled resting spores that are formed in infected plant tissue germinate, giving an asculike tube in which spores develop (Fig. 8 in Kurtzman and Sugiyama 2001). *Protomyces* species generally grow at lower temperatures (15–25 °C) and produce pigmented colonies, much as is seen for *Taphrina* and *Saitoella*, which also tend to grow at lower temperatures.

The SSU rRNA gene sequences from most species of *Protomyces* contain one or two group I introns (Nishida and Sugiyama 1995; Nishida et al. 1993, 1998, 2000),

whereas introns have not been detected in the rRNA genes of *Taphrina* species.

The genus *Taphrina*, which may include as many as 95 species, is parasitic on a wide variety of vascular plants, primarily ferns, the Rosales, and the Fagales (Kramer 1973; Mix 1949). The morphology and life cycles of *Taphrina* spp. are unique. Species are dimorphic with a saprotrophic haploid, uninucleate state, and a parasitic ascogenous binucleate mycelial state that develops in the host tissue. Moore (1990) described the genus *Lalaria* for the anamorphic states of *Taphrina*, some of which are occasionally isolated from the environment as a yeast state. *Taphrina deformans*, which causes peach leaf curl, is especially well studied (Kramer 1960; Martin 1940; Syrop and Beckett 1976). Another well-studied species is *T. wiesneri* (=*T. cerasi*), which attacks the Japanese cherry tree (*Cerasus yedoensis*), causing witches' broom (Fig. 9 in Kurtzman and Sugiyama 2001; Tubaki 1978). Ascospores of *T. wiesneri* bud within asci on the leaves of the host plant (Fig. 10 in Kurtzman and Sugiyama 2001). Characteristic of most other *Taphrina* spp., the budding haploid phase of *T. wiesneri* appears as pigmented yeastlike colonies on agar media. The color is due to the formation of carotenoid pigments.

The yeast states of *Taphrina* (Fonseca and Rodrigues 2011), *Protomyces* (Kurtzman 2011), and *Saitoella* (Sugiyama and Hamamoto 2011) are similar culturally, biochemically, and chemotaxonomically. Especially noteworthy is the fact that the **cell walls of the three genera are two-layered** and typical of ascomycetous yeasts, whereas conidiogenesis (budding) is enteroblastic and typical of basidiomycetous yeasts (Goto et al. 1987; Sjamsuridzal et al. 1997).

Phylogenetic analysis of SSU rRNA gene sequences from 14 species of *Taphrina* and 4 species of *Protomyces* verified the presence of two genera and demonstrated that they are monophyletic (Sjamsuridzal et al. 1997). On the basis of rRNA sequence analyses (Kurtzman 1993), Protomycetales appear to be synonymous with Taphrinales, hence placement of the preceding genera in a single order, Taphrinales. This treatment has been supported by the multigene phylogenetic analysis of Hibbett et al. (2007).

The history of studies on the anamorphic genus *Saitoella* was fully described by Sugiyama et al. (1993). The first known species of this genus, *S. complicata*, was initially identified as *Rhodotorula glutinis* (Goto and Sugiyama 1970), but later Goto et al. (1987) proposed that *S. complicata* was closely related to Taphrinales. This supposition, based on phenotypic comparisons, was verified from analyses of SSU rRNA gene sequences (Nishida and Sugiyama 1993, 1994b; Nishida et al. 1993; Sugiyama et al. 1993). On the basis of multigene phylogenetic analysis, *Saitoella* appears to be a member of Taphrinomycotina (Fig. 2 in Sugiyama et al. 2006). A second species of *Saitoella* was reported and described as *S. coloradoensis* (Kurtzman and Robnett 2012). This new species was isolated from insect frass occurring in an Engelmann spruce (*Picea engelmannii*) growing in the state of Colorado in the USA, and multigene analysis showed that *S. complicata* and *S. coloradoensis* are closely related. The teleomorph of *Saitoella* is unknown, and the evolutionary relationships between *Saitoella* and other members within Taphrinomycotina remain uncertain. At present, the genus *Saitoella* has two assigned species described from three strains (Kurtzman and Robnett 2012; Sugiyama and Hamamoto 2011), but the genus may be larger. Allison et al. (2010) cloned DNA from soil sampled in an Alaskan (USA) boreal forest. Of the 433 fungal sequences obtained, 3 (GU212336, GQ892426, GQ892425) were identical to the D1/D2 LSU rRNA gene sequence of *S. complicata*.

New major lineages within Ascomycota. Several studies on fungal diversity in soils using a total environmental DNA sampling have revealed potentially new early diverging ascomycete lineages independent of Taphrino-

mycotina (Blackwell 2011; Jumpponen and Johnson 2005; Schadt et al. 2003; Sugiyama et al. 2006; Vandenkoornhuyse et al. 2002). Subsequently, Porter et al. (2008) suggested a major new clade as Soil Clone Group I (SCGI) of Ascomycota equivalent to a subphylum. Currently the SCGI clade is known only from sequence data. As discussed earlier, the genus *Archaeorhizomyces* (Rosling et al. 2011), which was assigned to Archeorhizomycetes, appears to be a member of the Taphrinomycotina. Both molecular- and cultivation-based methods for isolation of novel ascomycete taxa from environments are needed to elucidate the whole phylogenetic picture of Ascomycota.

VII. Isolation, Maintenance, and Culture Availability

A. Isolation

Yeasts are recovered from a wide range of aquatic, marine, and terrestrial habitats, as well as from the atmosphere. Many yeasts are widely distributed, whereas others appear to be confined to specific habitats. Yeasts seldom occur in the absence of either molds or bacteria. Consequently, selective techniques are often used for the recovery of yeasts. The composition of selective media is determined by the fact that yeasts are generally capable of developing at pH levels and water activities that reduce or inhibit the growth of bacteria. Antibiotics may also be used to suppress bacteria. Fungistatic agents for the suppression of molds can be used, but these compounds may also inhibit yeasts. In addition to the methods discussed in this chapter, the publications of Beech and Davenport (1971), Deak (2003), and Kurtzman et al. (2011) discuss the **isolation of yeasts from natural habitats**, and the publications of Buckley (1971) and Staib et al. (1989) provide methods for isolating **clinical yeasts.** When yeasts are present in high numbers, they may be isolated by directly plating the material, or suspensions of the material, on acidified agar media that may also contain antibiotics or have other selective formulations. Dilution plate techniques can be used for quantitative studies.

Temperatures for isolation and growth. Cultures are usually incubated at 20–25 °C because most yeasts are mesophilic; however, temperatures between 4 and 15 °C are essential for psychrophilic taxa. Higher temperatures, in the range of 30–37 °C, are often required for yeasts that are strictly associated with warm-blooded sources. Among these species are human and animal pathogens assigned to *Candida*, *Kazachstania*, *Macrorhabdus*, and *Cyniclomyces*. The latter two genera have exceptional nutritional requirements (Kurtzman et al. 2011). Incubation temperatures can also serve to selectively isolate particular groups of species.

Acidified media (pH 3.5–5). Acidified media provide selective isolation, and hydrochloric and phosphoric acids are often used to acidify the media. Organic acids, such as acetic acid, are not recommended because they are only slightly dissociated at pH 3.5–5, and high concentrations of undissociated acids have an inhibitory effect on many yeasts. Exceptions include *Zygosaccharomyces bailii*, *Z. bisporus*, and some strains of *Pichia membranifaciens* (Yarrow 1998).

Agar in media with a low pH is hydrolyzed when autoclaved. To resolve this problem, sterilized molten agar is cooled to approximately 45 °C, and a predetermined volume of acid is added. The medium and acid are quickly but gently mixed to avoid air bubbles and immediately poured into petri dishes. The addition of approximately 0.7 % (v/v) 1 N hydrochloric acid to YM agar or glucose–peptone–yeast extract agar usually gives the desired pH of 3.7–3.8. Many yeasts can be recovered at pH 3.7, but some species, such as those of the genus *Schizosaccharomyces*, are inhibited by high acid media, and moderately acidic media with a pH in the range 4.5–5.0 will give a higher recovery.

When yeasts are present in low numbers, population size can be increased by incubating the sample in a liquid medium at a pH of 3.7–3.8. The use of antibiotics or high or low temperatures can provide further selection. The development of molds can be restricted by

excluding air from the culture by pouring sterile paraffin oil over the surface of the medium to form a layer about 1 cm deep. This procedure favors the development of fermentative strains but may fail to recover aerobic strains. Another method for restricting mold development involves incubating flasks of isolation media on a rotary shaker (Wickerham 1951). Molds in shaken flasks often do not conidiate and tend to grow as pellets that are outgrown by yeasts. The yeasts may be separated from the molds either by allowing the pellets of mold to settle for a few minutes and then streaking the suspension of yeasts onto agar in petri dishes or by removing suspended pellets by filtering through sterile glass wool. These pregrowth methods cannot be used for the quantitation of the initial population size in the sample tested.

Osmotic media. Yeasts can often grow on media with concentrations of sugar that are high enough to inhibit the development of many bacteria. A medium such as glucose–peptone–yeast extract agar or YM agar containing glucose at a concentration of 30–50 % is suitable for recovering osmophilic and osmotolerant yeasts from foodstuffs and juice concentrates of low water activity. The selective action of these media can be enhanced by lowering the pH to around 4.5. Osmotolerant yeasts recovered in this way can usually be successfully subcultured on media containing successively decreasing amounts of sugar, for example, 30, 10, 4, and 2 %.

Antibiotics and other selective compounds. Several media containing antibiotics have been described (Bills and Foster 2004) that can be used to suppress co-occurring microorganisms. Antibacterial antibiotics, which are often used, include tetracycline at 50 mg/L or a combination of penicillin G and streptomycin sulfate, each at a concentration of 150–500 mg/L. Many of these antibiotics are heat labile and must be added after the medium has been autoclaved and is cool to the touch. In contrast, chloramphenicol (actidione) is heat stable and can be added to the medium prior to sterilization. **Antifungal antibiotics**, which suppress filamentous fungi, may also suppress yeasts. Commonly used antifungals include cycloheximide (100–500 mg/L), cyclosporin A (4–10 mg/L), and pimaricin (5–100 mg/L).

Selective media can be used to isolate genera, species, or groups of species with a particular property. Van der Walt and van Kerken (1961) isolated species of *Dekkera* using media containing cycloheximide and sorbic acid at pH 4.8. Van Dijken and Harder (1974) used a medium containing methanol, cycloserine, and penicillin G for the isolation of methanol-assimilating yeasts of biotechnological importance. Various species of the Lipomycetaceae can be recovered from soil and insect frass by a procedure that depends on the utilization of thymine as a nitrogen source and resistance to cycloheximide.

Isolation using membrane filters. Yeasts can be recovered from liquid substrates by passing the liquid through a 0.45 μm membrane filter (e.g., Mulvany 1969). Solid substrates, such as soils, can be washed to suspend the yeast cells prior to passing the wash solution through the membrane. The filters are placed face up on the surface of a selective agar medium, followed by incubation at a temperature appropriate for the target organisms. Plates should be inspected daily. This technique is particularly useful for recovering yeasts when they are present in low concentrations, and the method can serve as a means to quantitate the densities of yeast communities.

Purification of cultures. Isolates are obtained in pure culture from natural materials or from enriched cultures by streaking on a suitable medium, such as glucose–peptone–yeast extract agar or YM agar (Kurtzman et al. 2011). Persistent bacterial contamination can often be eliminated by acidifying the media or by adding antibiotics. Single, well-separated colonies of each form are selected and streaked again. Twice is generally sufficient to obtain pure cultures. When two or more morphologically distinct colonies persistently appear after replating of a single colony, these may represent morphological or sexual variants of a single species. Because of this, it is useful to initially save multiple colonies from isolations because some may represent mating types.

B. Maintenance

The maintenance of yeast cultures on a medium that contains glucose as the only carbon source reduces the risk of changes in growth and fermentative patterns due to the selection of mutants (Scheda 1966). The majority of yeasts may be stored at temperatures between 4 and 12 °C and subcultured at intervals of 6–8 months. Some yeasts, such as certain *Kazachstania* spp., need to be subcultured every month. Strains of *Dekkera* and *Brettanomyces* produce excessive amounts of acetic acid, and inclusion of 1–2 % calcium carbonate in the medium prolongs viability. Nevertheless, these yeasts still need to be subcultured every 2 months, or sometimes more frequently.

Some yeasts lose their ability to produce ascospores when maintained by serial cultivation on laboratory media, whereas other isolates still sporulate after 50 or more years in cultivation. However, for many strains, the ability to sporulate is either impaired or lost within a period that varies from a few weeks to several years. For ascosporogenous heterothallic species, one mating type may selectively predominate in laboratory culture, which results in the loss of the sexual state. For these reasons, it is important to preserve nomenclatural types and reference strains with one of the more permanent **conservation techniques** as soon as possible after acquisition. Suitable techniques are lyophilization (Kirsop and Kurtzman 1988), L-drying (Mikata and Banno 1989), and freezing in either liquid nitrogen vapor or a mechanical freezer at temperatures between −60 and −135 °C, although liquid nitrogen is preferred.

The procedure of **lyophilization** freeze-dries cultures to an inactive, low-moisture state. The process starts with suspending actively growing cultures in a cryoprotectant, such as sterile bovine serum or skim milk. The volume of cryoprotectant depends on the size of the ampoule used, and ampoules used in the ARS Culture Collection (NRRL) are made from 6 mm outside diameter pyrex glass tubing. Lyophilized strains generally survive for many decades, though there are exceptions, and the cultures should be checked periodically for viability. Lyophilized cultures are usually stored in a refrigerator at 4–5 °C.

In the ARS process, ca. 0.1 mL of suspension containing cells and bovine serum is added aseptically to the sterile, labeled lyophil tubes, which have a small cotton plug (Kurtzman et al. 2011). After filling, tubes are inserted into rubber connectors on the manifold of the lyophil machine. The manifold is lowered so that the ends of the tubes are immersed in a 50 % ethylene glycol bath cooled to ca. −30 °C with dry ice. Once the contents of the tubes are frozen, the bath temperature is raised to ca. −20 to −25 °C with fresh ethylene glycol, and the vacuum pump is started. Drying takes ca. 2 h and is finalized by raising the manifold and allowing further drying at room temperature. Ampoules are sealed with a gas-oxygen torch. Some lyophil machines use larger ampoules that may contain 1 mL of cell suspension. These larger tubes may be initially frozen but do not need immersion in a freezing bath because evaporation of this larger volume is adequate to keep the preparations frozen.

A process termed **L-drying** has been successfully used by several large culture collections in Japan (Mikata and Banno 1989). The process is similar to lyophilization, with the exception that cells and cryoprotectant are dried more slowly under vacuum and do not freeze. L-dried ampoules are sealed in the same manner as lyophilized preparations.

Frozen storage at low temperatures is a common alternative for preserving microorganisms that do not survive lyophilization or L-drying. Storage in −80 and −125 °C mechanical freezers is often satisfactory, but storage at still lower temperatures is preferable. The choice is the vapor phase of liquid nitrogen, which is ca. −180 °C. Liquid nitrogen at atmospheric pressure has a slightly lower temperature of −196 °C (−320 °F), but a concern with immersion storage is that ampoules occasionally leak, and when thawed, the trapped liquid nitrogen will expand to gas so rapidly that the ampoules explode.

The general procedure for liquid nitrogen storage of microorganisms is to suspend cells from an actively growing culture in a cryoprotectant such as 10–15 % glycerol. Usually 1 mL of suspension is placed in a 2 mL cryoampoule. Ordinarily, the ampoules are transferred to a storage box in the liquid nitrogen freezer and

Table 1.2 Some large international culture collections that maintain a diversity of yeast cultures[a]

Agricultural Research Service Culture Collection (NRRL) Peoria, Illinois, USA http://nrrl.ncaur.usda.gov
All-Russian Culture Collection (VKM) Moscow Region, Pushchino, Russia http://www.vkm.ru
American Type Culture Collection (ATCC) Manassas, Virginia, USA http://www.atcc.org
Belgian Coordinated Collections of Micro-Organisms (BCCM) Various cities, Belgium http://bccm.belspo.be/index/php
Centraalbureau voor Schimmelcultures (CBS) Utrecht, The Netherlands http://www.cbs.knaw.nl
Japan Collection of Microorganisms (JCM)[b] Tsukuba, Japan http://www.jcm.riken.jp
National Collection of Yeast Cultures (NCYC) Norwich, United Kingdom http://www.NCYC.co.uk
National Institute of Technology Evaluation – Biological Resource Center (NBRC)[c] Chiba, Japan http://www.nbrc.nite.go.jp
Phaff Yeast Culture Collection, University of California, Davis, California, USA http://www.phaffcollection.org
Portuguese Yeast Culture Collection Caparica, Portugal http://www.sec-biot@fct.unl.pt

[a]Other culture collections that maintain yeasts can be located at the Web site of the World Federation for Culture Collections (WFCC) (http://www.wfcc.info/datacenters.html)
[b]Cultures maintained at IAM Culture Collection (IAM) were transferred to JCM in 2007
[c]Cultures maintained at Institute for Fermentation, Osaka (IFO), were transferred to NITE-NBRC in July 2002

allowed to undergo uncontrolled freezing. Occasionally, the freeze rate needs to be controlled to ensure high cell viability, which can be done using commercially available devices that have a programmable freezing rate (see Verkley et al., Chap. 8, this volume).

C. Culture Availability and Distribution

The shipment of cultures is governed by a variety of **national and international laws and regulations**, and some of these regulations have developed in recent years because of the threat of global bioterrorism. Within the USA, shipment of plant and animal pathogens requires a permit from the USDA Animal and Plant Health Inspection Service (APHIS) (APHIS 526 for plant pathogens, APHIS VS 16-3 for animal pathogens). A permit is required from the U.S. Public Health Service when a pathogen is imported into the USA or when a foreign pathogen is redistributed within the USA. Other countries often have their own specific regulations. Failure to comply with requirements can result in the closure of laboratories, large fines, or even incarceration. The shipment of cultures may be governed by the Convention on Biodiversity, also known as the Rio Treaty. Here the concern is that part of a country's national heritage, i.e., unique microbial germplasm, will be exploited by outside parties without due compensation. Consequently, before novel or other strains are shipped between countries, the requirements of the Rio Treaty must be known. Culture collections that maintain a wide diversity of yeasts are listed in Table 1.2.

VIII. Future Directions

Yeasts are central to many present-day agricultural, medical, and industrial processes, and with the development of recombinant DNA technologies, as well as new societal needs, the role of yeasts in human advancement can be expected to increase. Rapid DNA sequencing technologies have made whole genome sequencing relatively inexpensive, thereby permitting large-scale comparative genomics for yeasts, which will lead to enormous advances in understanding the genetics of metabolic pathways, reproductive processes, and bioengineering. Molecular systematics will play a key role in future uses. The application of molecular methods for strain identification will markedly assist medical diagnostics as well as define industrially and agriculturally important species, including those important in food spoilage and the biocontrol of pests and pathogens. Molecular identification methods will

lead to the discovery of numerous new species, which will give us a far better understanding of biodiversity.

References

Ainsworth GC (1971) Ainsworth & Bisby's dictionary of the fungi, 6th edn. Commonwealth Mycological Institute, Kew

Alcoba-Flórez J, Méndez-Álvarez S, Cano J, Guarro J, Pérez-Roth E, Arévalo M (2005) Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. J Clin Microbiol 43:4107–4111

Allison SD, McGuire KL, Treseder KK (2010) Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol Biochem 42:1872–1878

Arnaud G (1913) Sur le genre *Eremothecium* Borzi. Bull Trim Soc Mycol Fr 29:572–576

Andrews JH (1992) Biological control in the phyllosphere. Annu Rev Phytopathol 30:603–635

Batra LR (1973) Nematosporaceae (Hemiascomycetidiae): taxonomy, pathogenicity, distribution and vector relations. Tech Bull No 1469, US Dept of Agriculture, Washington, DC

Beech FW, Davenport RR (1971) Isolation, purification and maintenance of yeasts. In: Morris JR, Ribbons DW (eds) Methods in microbiology, vol 4. Academic, New York, pp 153–182

Beijerinck MW (1889) L'auxanographie, ou la méthode de l'hydrodiffusion dans la gélatine appliquée aux recherchesmicrobiologiques. Arch Néerl Sci Exactes Nat 23:367–372

Belloch C, Querol A, Garcia MD, Barrio E (2000) Phylogeny of the genus *Kluyveromyces* inferred from the mitochondrial cytochrome-*c* oxidase II gene. Int J Syst Evol Microbiol 50:405–416

Berbee ML, Taylor JW (1993) Ascomycete relationships: dating the origin of asexual lineages with 18S ribosomal RNA gene sequence data. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 67–78

Bialkova A, Subik J (2006) Biology of the pathogenic yeast *Candida glabrata*. Folia Microbiol (Praha) 51:3–20

Bills GF, Foster MS (2004) Formulae for selected materials used to isolate and study fungi and fungalales. In: Bills GF, Fostser MS, Mueller GM (eds) Biodiversity of fungi. Inventory and monitoring methods. Elsevier, Amsterdam, pp 595–618

Blackwell M (2011) The fungus: 1, 2, 3…5.1 million species? Am J Bot 98:426–438

Bowen AR, Chen-Wu JL, Momany M, Young R, Szaniszlo PJ, Robbins PW (1992) Classification of fungal chitin synthases. Proc Natl Acad Sci U S A 89:519–523

Buckley HR (1971) Fungi pathogenic for man and animals: 2. The subcutaneous and deep-seated mycoses. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 4. Academic, New York, pp 461–478

Butler EE, Webster RK, Eckert JW (1965) Taxonomy, pathogenicity and physiological properties of the fungus causing sour rot of citrus. Phytopathology 55:1262–1268

Cain RF (1972) Evolution of the fungi. Mycologia 64:1–14

Cavalier-Smith T (1987) The origin of fungi and pseudofungi. In: Rayner ADM, Brasier CM, Moore D (eds) Evolutionary biology of the fungi. Cambridge University Press, Cambridge, pp 339–353

Chalutz E, Droby S, Cohen L, Weiss B, Barkai-Golan R, Daus A, Fuchs Y, Wilson CL (1991) Biological control of *Botrytis*, *Rhizopus*, and *Alternaria* rots of tomato fruit by *Pichia guilliermondii*. In: Wilson CL, Chalutz E (eds) Biological control of postharvest diseases of fruits and vegetables. Workshop Proc. US GPO, Washington, DC, pp 71–85

Correia A, Sampaio P, James S, Pais C (2006) *Candida bracarensis* sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. Int J Syst Evol Microbiol 56:313–317

Crandall MA, Brock TD (1968) Molecular basis of mating in the yeast *Hansenula wingei*. Bacteriol Rev 32:139–163

Cregg JM, Madden KR (1988) Development of the methylotrophic yeast, *Pichia pastoris*, as a host system for the production of foreign proteins. Dev Ind Microbiol 29:33–41

Cushion MT, Keely SP (2011) Pneumocystis Delanoë & Delanoë. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 709–717

Daniel H-M, Meyer W (2003) Evaluation of ribosomal RNA and actin gene sequences for the identification of ascomycetous yeasts. Int J Food Microbiol 86:71–78

Daniel H-M, Sorrell TC, Meyer W (2001) Partial sequence analysis of the actin gene and its potential for studying the phylogeny of *Candida* species and their teleomorphs. Int J Syst Evol Microbiol 51:1593–1606

Daughtrey ML, Hodge KT, Shishkoff N (2003) Archiascomycete and Hemiascomycete pathogens. In: Trigiano RN, Windham MT, Windham AS (eds) Plant pathology: concepts and laboratory exercises. CRC, Boca Raton, FL, pp 111–116

Deak T (2003) Detection, enumeration and isolation of yeasts. In: Boekhout T, Robert V (eds) Yeasts in food: beneficial and detrimental aspects. Behr's Verlag, Hamburg, pp 39–68

de Koning W, Harder W (1992) Methanol-utilizing yeasts. In: Murell JC, Dalton H (eds) Methane

and methanol utilizers. Plenum, New York, pp 207–244

do Carmo-Sousa L (1969) Endospore formation in the genus *Trichosporon*. In: Kocková-Kratochvílová A (ed) Proc 2nd symp on yeasts, 1966, Bratislava, pp 87–92

Droby S, Hofstein R, Wilson CL, Wisniewski M, Fridlender B, Cohen L, Weiss B, Daus A, Timar D, Chalutz E (1993) Pilot testing of *Pichia guilliermondii*: a biocontrol agent of postharvest diseases of citrus fruit. Biol Control 3:47–52

Droby S, Cohen L, Daus A, Weiss B, Horev B, Chalutz E, Katz H, Keren-Tzur M, Shachnai A (1998) Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. Biol Control 12:97–101

Dubos RJ (1960) Louis Pasteur, free lance of science. De Capo, New York

Dykhuizen DE, Green L (1991) Recombination in *Escherichia coli* and the definition of biological species. J Bacteriol 173:7257–7268

Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML (1988) Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature 334:519–522

Eriksson OE, Hawksworth DL (1995) Notes on ascomycete systematics—nos. 1885–2023. Syst Ascomyc 14:41–77

Fall R, Benson A (1996) Leaf methanol—the simplest natural product from plants. Trends Plant Sci 1:296–301

Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol Biol 6:99–113

Fleet GH (1990) Food spoilage yeasts. In: Spencer JFT, Spencer DM (eds) Yeast technology. Springer, Berlin, pp 124–166

Fonseca Á, Rodrigues MG (2011) *Taphrina* Fries (1832). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 823–858

Garner R, Walker AN, Horst MN (1991) Morphologic and biochemical studies of chitin expression in *Pneumocystis carinii*. J Protozool 38:125–145

Gellissen G, Kunze G, Gaillardin C, Cregg JM, Berardi E, Veenhuis EM, van der Klei I (2005) New yeast expression platforms based on methylotrophic *Hansenula polymorpha* and *Pichia pastoris* and on dimorphic *Arxula adeninivorans* and *Yarrowia lipolytica*—a comparison. FEMS Yeast Res 5:1079–1096

Goodman M (1976) Protein sequences in phylogeny. In: Ayala FJ (ed) Molecular evolution. Sinauer Associates, Sunderland, MA, pp 141–159

Goto S, Sugiyama J (1970) Studies on Himalayan yeasts and molds (IV). Several asporogenous yeasts, including two new taxa of *Cryptococcus*. Can J Bot 48:2097–2101

Goto S, Sugiyama J, Hamamoto M, Komagata K (1987) *Saitoella*, a new anamorphic genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formerly identified as *Rhodotorula glutinis*. J Gen Appl Microbiol 33:75–85

Groth C, Hansen J, Piskur J (1999) A natural chimeric yeast containing genetic material from three species. Int J Syst Bacteriol 49:1933–1938

Guetsky R, Shtienberg D, Elad Y, Fischer E, Dinoor A (2002) Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology 92:976–985

Guilliermond A (1912) Les Levures. Encyclopédie scientifique. O Doin et Fils, Paris

Hansen EB (2004) Microorganisms. In: Hui YH, Meunier-Goddik L, Hansen AS, Josephsen J, Nip W-K, Stanfield PS, Toldrá F (eds) Handbook of food and beverage fermentation technology. Marcel Dekker, New York, pp 9–22

Hawksworth DL (2012) Managing and coping with name of pleomorphic fungi in a period of transition. IMA Fungus 3:15–24

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Holley RA, Allan-Wojtas P, Phipps-Todd BE (1984) *Nematospora sinecauda* sp. nov., a yeast pathogen of mustard seeds. Antonie Van Leeuwenhoek 50:305–320

Hughes SJ (1985) The term chlamydospore. In: Arai T, Kuga T, Terao K, Yamazaki M, Miyaji M, Unemoto T (eds) Filamentous microorganisms: biomedical aspects. Japan Scientific Societies Press, Tokyo, pp 1–20

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüssler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA,

Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822

Janisiewicz WJ (1987) Postharvest biological control of blue mold on apples. Phytopathology 77: 481–485

Janisiewicz WJ, Tworkoski TJ, Kurtzman CP (2001) Biocontrol potential of *Metschnikowia pulcherrima* strains against blue mold of apple. Phytopathology 91:1098–1108

Jumpponen A, Johnson LC (2005) Can rDNA analyses of diverse fungal communities in soil and roots detect effects of environmental manipulations—a case study from tallgrass prairie. Mycologia 97:1177–1194

Karabulut OA, Tezcan H, Daus A, Cohen L, Wiess B, Droby S (2004) Control of preharvest and postharvest fruit rot in Strawberry by *Metschnikowia fructicola*. Biocontrol Sci Technol 14:513–521

Kaur R, Domergue R, Zupancic ML, Cormack BP (2005) A yeast by any other name: *Candida glabrata* and its interaction with the host. Curr Opin Microbiol 8:378–384

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Ainsworth & Bisby's dictionary of the fungi, 10th edn. CAB International, Wallingford

Kirsop BE, Kurtzman CP (eds) (1988) Living resources for biotechnology. Yeasts. Cambridge University Press, Cambridge

Knapp S, McNeill J, Turland NJ (2011) Changes to publication requirements made at the XVIII International Botanical Congress in Melbourne—what does e-publication mean for you? BMC Evol Biol 11:251–254

Korf RP (1973) Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The Fungi, an advanced treatise, vol IV A. Academic, New York, pp 249–319

Kramer CL (1960) Morphological development and nuclear behavior in the genus *Taphrina*. Mycologia 52:295–320

Kramer CL (1973) Protomycetales and taphrinales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The fungi—an advanced treatise, vol. IVA. Academic, New York, pp 33–41

Kuramae EE, Robert V, Snel B, Weiss M, Boekhout T (2006) Phylogenomics reveal a robust fungal tree of life. FEMS Yeast Res 6:1213–1220

Kurtzman CP (1993) Systematics of the ascomycetous yeasts assessed from ribosomal RNA sequence divergence. Antonie Van Leeuwenhoek 63:165–174

Kurtzman CP (2003) Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*. *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*. FEMS Yeast Res 4:233–245

Kurtzman CP (2011) *Protomyces* Unger (1833). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 725–731

Kurtzman CP, Robnett CJ (1991) Phylogenetic relationships among species of *Saccharomyces, Schizosaccharomyces, Debaryomyces* and *Schwanniomyces* determined from partial ribosomal RNA sequences. Yeast 7:61–72

Kurtzman CP, Robnett CJ (1994) Orders and families of ascosporogenous yeasts and yeast-like taxa compared from ribosomal RNA sequence similarities. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, pp 249–258

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73:331–371

Kurtzman CP, Robnett CJ (2003) Phylogenetic relationships among yeasts of the '*Saccharomyces* complex' determined from multigene sequence analyses. FEMS Yeast Res 3:417–432

Kurtzman CP, Robnett CJ (2007) Multigene phylogenetic analysis of the *Trichomonascus, Wickerhamiella* and *Zygoascus* yeast clades, and the proposal of *Sugiyamaella* gen.nov. and 14 new species combinations. FEMS Yeast Res 7:141–151

Kurtzman CP, Robnett CJ (2010) Systematics of methanol assimilating yeasts and neighboring taxa from multigene sequence analysis and the proposal of *Peterozyma* gen. nov., a new member of the *Saccharomycetales*. FEMS Yeast Res 10:353–361

Kurtzman CP, Robnett CJ (2012) *Saitoella coloradoensis* sp. nov., a new species of the Ascomycota, subphylum Taphrinomycotina. Antonie Van Leeuwenhoek 101:795–802

Kurtzman CP, Robnett CJ (2013) Relationships among genera of the Saccharomycotina (Ascomycota) from multigene phylogenetic analysis of type species. FEMS Yeast Res 13(1):23–33

Kurtzman CP, Sugiyama J (2001) Ascomycetous yeasts and yeastlike taxa. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol VII (Systematics and evolution), Part A. Springer, Berlin, pp 179–200

Kurtzman CP, Suzuki M (2010) Phylogenetic analysis of ascomycete yeasts that form coenzyme Q-9 and the proposal of the new genera *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces* and *Scheffersomyces*. Mycoscience 51:2–14

Kurtzman CP, Robnett CJ, Ward JN, Brayton C, Gorelick P, Walsh TJ (2005) Multigenic phylogenetic

analysis of pathogenic *Candida* species in the *Kazachstania (Arxiozyma) telluris* complex and description of their ascosporic states as *Kazachstania bovina* sp. nov., *K. heterogenica* sp. nov., *K. pintolopesii* sp. nov. and *K. slooffiae* sp.nov. J Clin Microbiol 43:101–111

Kurtzman CP, Albertyn J, Basehoar-Powers E (2007) Multigene phylogenetic analysis of the Lipomycetaceae and proposed transfer of *Zygozyma* species to *Lipomyces* and *Babjevia anomala* to *Dipodascopsis*. FEMS Yeast Res 7:1027–1034

Kurtzman CP, Robnett CJ, Basehoar-Powers E (2008) Relationships among species of *Pichia*, *Issatchenkia* and *Williopsis* determined from multigene phylogenetic analysis and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. FEMS Yeast Res 8:939–954

Kurtzman CP, Price NP, Ray KJ, Kuo TM (2010) Production of sophorolipids biosurfactants by multiple species of the *Starmerella* (*Candida*) *bombicola* yeast clade. FEMS Microbiol Lett 311:140–146

Kurtzman CP, Fell JW, Boekhout T, Robert V (2011) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 87–110

Lachance M-A, Daniel H-M, Meyer W, Prasad GS, Gautam SP, Boundy-Mills K (2003) The D1/D2 domain of the large-subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. FEMS Yeast Res 4:253–258

Landvik S (1996) *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rRNA sequences. Mycol Res 100:199–202

Landvik S, Eriksson OE, Gargas A, Gustafsson P (1993) Relationships of the genus *Neolecta* (Neolectales ordo nov., Ascomycotina) inferred from 18S rDNA sequences. Syst Ascomyc 11:107–118

Langeron M, Guerra P (1940) Valeur et nature des variations et dissociations de colonies chez les champignons levuriformes. Ann Parasitol Hum Comp 17:447–469

Legras J-L, Medinoglu D, Cornuet J-M, Karst F (2007) Bread beer and wine: *Saccharomyces* diversity reflects human history. Mol Ecol 16:2091–2102

Liu Y, Leigh JW, Brinkmann H, Cushion MT (2009) Phylogenomic analyses support the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. Mol Biol Evol 26:27–34

Martin EM (1940) The morphology and cytology of *Taphrina deformans*. Am J Bot 27:743–751

Martini A, Phaff HJ (1973) The optical determination of DNA–DNA homologies in yeasts. Ann Microbiol 23:59–68

McClary DO, Nulty WL, Miller GR (1959) Effect of potassium versus sodium in the sporulation of *Saccharomyces*. J Bacteriol 78:362–368

McGovern PE, Zhang J, Tang J, Zhang Z, Hall GR, Moreau RA, Nuñez A, Dutyrm ED, Richards MP, Wang C-S, Cheng G, Zhao Z, Wang C (2004) Fermented beverages of pre-proto-historic China. Proc Natl Acad Sci U S A 101:17593–17598

Mikata K, Banno I (1989) Preservation of yeast cultures by L-drying: viability after 5 years of storage at 5 °C. Inst Ferment Osaka Res Commun 14:80–103

Mix AJ (1949) A monograph of the genus *Taphrina*. Univ Kansas Sci Bull 23:1–167

Moore RT (1987) Micromorphology of yeasts and yeast-like fungi and its taxonomic implications. In: de Hoog GS, Smith MT, Weijman ACM (eds) The expanding realm of yeast-like fungi. Elsevier, Amsterdam, pp 203–226

Moore RT (1990) The genus *Lalaria* gen. nov.: Taphrinales anamorphosum. Mycotaxon 38:315–330

Mortimer R, Polsinelli M (1999) On the origins of wine yeast. Res Microbiol 150:199–204

Mulvany JG (1969) Membrane filter techniques in microbiology. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 1. Academic, New York, pp 205–253

Naehring J, Kiefer S, Wolf K (1995) Nucleotide sequence of the *Schizosaccharomyces japonicus* var. *versatilis* ribosomal RNA gene cluster and its phylogenetic implications. Curr Genet 28:353–359

Nakase T, Komagata K (1968) Taxonomic significance of base composition of yeast DNA. J Gen Appl Microbiol 14:345–357

Nguyen NH, Suh SO, Marshall CJ, Blackwell M (2006) Morphological and ecological similarities: wood-boring beetles associated with novel xylose-fermenting yeasts, *Spathaspora passalidarum* gen. sp. nov. and *Candida jeffriesii* sp. nov. Mycol Res 110:1232–1241

Nishida H, Sugiyama J (1993) Phylogenetic relationships among *Taphrina*, *Saitoella*, and other higher fungi. Mol Biol Evol 10:431–436

Nishida H, Sugiyama J (1994a) Phylogeny and molecular evolution among higher fungi. Nippon Nogeikagaku Kaishi 68:54–57 (in Japanese)

Nishida H, Sugiyama J (1994b) Archiascomycetes: detection of a major new lineage within the Ascomycota. Mycoscience 35:361–366

Nishida H, Sugiyama J (1995) A common group I intron between a plant parasitic fungus and its host. Mol Biol Evol 12:883–886

Nishida H, Blanz PA, Sugiyama J (1993) The higher fungus *Protomyces inouyei* has two group I introns in the 18S rRNA gene. J Mol Evol 37:25–28

Nishida H, Tajiri Y, Sugiyama J (1998) Multiple origins of fungal group I introns located in the same position of nuclear SSU rRNA gene. J Mol Evol 46:442–448

Nishida H, Ogura A, Yokota A, Yamaguchi I, Sugiyama J (2000) Group I intron located in PR protein homologue gene in *Youngia japonica*. Biosci Biotechnol Biochem 64:606–609

Norvell LL (2011) Melbourne approves a new Code. Mycotaxon 116:481–490

Passoth V, Fredlund E, Druvefors UA, Schnurer J (2006) Biotechnology, physiology and genetics of the yeast *Pichia anomala*. FEMS Yeast Res 6:3–13
Peterson SW, Kurtzman CP (1991) Ribosomal RNA sequence divergence among sibling species of yeasts. Syst Appl Microbiol 14:124–129
Phaff HJ, Miller MW, Shifrine M (1956) The taxonomy of yeasts isolated from *Drosophila* in the Yosemite region of California. Antonie Van Leeuwenhoek 22:145–161
Phaff HJ, Starmer WT, Tredick-Kline J (1987) *Pichia kluyveri* sensu lato—a proposal for two new varieties and a new anamorph. In: de Hoog GS, Smith MT, Weijman ACM (eds) The expanding realm of yeast-like fungi. Elsevier, Amsterdam, pp 403–414
Pitt JI, Miller MW (1968) Sporulation in *Candida pulcherrima*, *Candida reukaufii* and *Chlamydozyma* species: their relationships with *Metschnikowia*. Mycologia 60:663–685
Porter TM, Schadt CW, Rizvi L, Martin AP, Schmidt SK, Scott-Denton L, Vilgalys R, Moncalvo JM (2008) Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. Mol Phylogenet Evol 46:635–644
Price CW, Fuson GB, Phaff HJ (1978) Genome comparison in yeast systematics: delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces* and *Pichia*. Microbiol Rev 42:161–193
Prillinger H, Dörfler C, Laaser G, Eckerlein B, Lehle L (1990) Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze: Hefe-Typen der Basidiomyceten. Teil I: Schizosaccharomycetales, *Protomyces*-Typ. Z Mykol 56:219–250
Redhead SA (1977) The genus *Neolecta* (Neolectaceae fam. nov., Lecanolales, Ascomycetes) in Canada. Can J Bot 55:301–306
Redhead SA, Malloch D (1977) The Endomycetaceae: new concepts, new taxa. Can J Bot 55:1701–1711
Rosling A, Cox F, Cruz-Martinez K, Ihrmark K, Grelet GA, Lindahl BD, Menkis A, James TY (2011) Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. Science 333:876–879
Rubio-Texeira M (2006) Endless versatility in the biotechnological applications of *Kluyveromyces* LAC genes. Biotechnol Adv 24:210–223
Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359–1361
Scheda R (1966) Merkmalsveränderungen bei Hefen der Gattung *Saccharomyces*. Monatsschr Brau 19:256–258
Schoch CL, Sung G-H, López-Giráldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Yahr R, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh S-O, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lücking R, Büdel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston P, Stenroos S, Zuccaro A, Dyer P, Crittenden P, Cole MS, Hansen K, Trappe JM, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239
Sietsma JH, Wessels JGH (1990) Occurrence of glucosaminoglycan in the wall of *Schizosaccharomyces pombe*. J Gen Microbiol 136:2261–2265
Sjamsuridzal W, Tajiri Y, Nishida H, Thuan T, Kawasaki H, Hirata A, Yokota A, Sujiyama J (1997) Evolutionary relationships of members of the genera *Taphrina*, *Protomyces*, *Schizosaccharomyces*, and related taxa within the Archiascomycetes: integrated analysis of genotypic and phenotypic characters. Mycoscience 38:267–280
Slininger PJ, Bolen PL, Kurtzman CP (1987) *Pachysolen tannophilus* properties and process considerations for ethanol production from D-xylose. Enzyme Microb Technol 9:5–15
Staib F, Seibold M, Antweiler E, Frölich B (1989) Staib agar supplemented with a triple antibiotic combination for the detection of *Cryptococcus neoformans* in clinical specimens. Mycoses 32:448–452
Stalpers JA (1987) Pleomorphy in holobasidiomycetes. In: Sugiyama J (ed) Pleomorphic fungi: the diversity and its taxonomic implications. Kodansha, Tokyo, pp 201–220
Starmer WT, Ganter PF, Aberdeen V (1992) Geographic distribution and genetics of killer phenotypes for the yeast *Pichia kluyveri* across the United States. Appl Environ Microbiol 58:990–997
Streiblová E (1971) Cell division in yeasts. In: Pérez-Miravette A, Peláez D (eds) Recent advances in microbiology. Proc. 10th int congress on microbiology, Mexico City, Mexico, pp 131–140
Sugiyama J (1998) Relatedness, phylogeny, and evolution of the fungi. Mycoscience 39:487–511
Sugiyama J, Hamamoto M (2011) *Saitoella* S. Goto, Sugiyama, Hamamoto & Komagata (1987). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 1313–1315
Sugiyama J, Nishida H (1995) The higher fungi: their evolutionary relationships and implications for fungal systematics. In: Arai R, Kato M, Doi Y (eds) Biodiversity and evolution. National Science Museum Foundation, Tokyo, pp 177–195
Sugiyama J, Fukagawa M, Chiu S-W, Komagata K (1985) Cellular carbohydrate composition, DNA base composition, ubiquinone systems and diazo-

nium blue B color test in the genera *Rhodosporidium*, *Leucosporidium*, *Rhodotorula* and related basidiomycetous yeasts. J Gen Appl Microbiol 31:519–550

Sugiyama J, Hosaka K, Suh SO (2006) Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. Mycologia 98:996–1005

Sugiyama J, Nishida H, Suh S-O (1993) The paradigm of fungal diagnoses and descriptions in the era of molecular systematics: *Saitoella complicata* as an example. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 261–269

Sullivan DJ, Moran GP, Coleman DC (2005) *Candida dubliniensis*: ten years on. FEMS Microbiol Lett 253:9–17

Syrop M, Beckett S (1976) Leaf curl disease of almond caused by *Taphrina deformans*. III. Ultrastructural cytology of the pathogen. Can J Bot 54:293–305

Takano I, Oshima Y (1970) Mutational nature of an allele-specific conversion of the mating type by the homothallic gene *HOα* in *Saccharomyces*. Genetics 65:421–427

Taylor JW, Swann EC, Berbee ML (1994) Molecular evolution of ascomycete fungi: phylogeny and conflict. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, pp 201–212

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32

Tomaszewski EK, Logan KS, Snowden KF, Kurtzman CP, Phalen DN (2003) Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. Int J Syst Evol Microbiol 53:1201–1205

Tripathi AK, Chauhan RKS, Bartaria AM, Chauhan S (2003) Quantitative and qualitative loss in coriander due to *Protomyces macrosporus*. Indian Phytopathol 56:451–452

Tubaki K (1957) Biological and cultural studies of three species of *Protomyces*. Mycologia 49:44–54

Tubaki K (1978) *Taphrina wiesneri* (Rathay) Mix. In: Udagawa S, Tubaki K, Horie Y, Miura K, Minoura K, Yamazaki M, Yokoyama T, Watanabe S (eds) Kinruizukan ('Compendium of Fungi'), part 1. Kodansha, Tokyo, pp 329–330 (in Japanese)

Vandenkoornhuyse P, Baldauf SL, Leyval C, Straczek J, Young JPW (2002) Extensive fungal diversity in plant roots. Science 295:2051

van der Walt JP (1970) Criteria and methods used in classification. In: Lodder J (ed) The yeasts, a taxonomic study, 2nd edn. North-Holland, Amsterdam, pp 34–113

van der Walt JP, Scott DB (1971) *Saccharomycopsis synnaedendra*, a new yeast from South African insect sources. Mycopathol Mycol Appl 44:101–106

van der Walt JP, van Kerken AE (1961) The wine yeasts of the Cape. Part V. Studies on the occurrence of Brettanomyces intermedius and Brettanomyces schanderlii. Antonie Van Leeuwenhoek 27:81–90

van Dijken JP, Harder W (1974) Optimal conditions for the enrichment and isolation of methanol-assimilating yeasts. J Gen Microbiol 84:409–411

Vaughan-Martini A, Kurtzman CP (1985) Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces* sensu stricto. Int J Syst Bacteriol 35:508–511

Vaughan-Martini A, Martini A (2011) *Schizosaccharomyces* Lindner (1893). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 779–784

Veenhuis M, van Dijken JP, Harder W (1983) The significance of peroxisomes in the metabolism of one-carbon compounds in yeasts. Adv Microb Physiol 24:1–82

Vega FE, Blackwell M (2005) Insect–fungal associations: ecology and evolution. Oxford University Press, Oxford

Verstrepen KJ, Chambers PJ, Pretorius IS (2006) The development of superior yeast strains for the food and beverage industries: challenges, opportunities, and potential benefits. In: Querol A, Fleet GH (eds) Yeasts in foods and beverages. Springer, Berlin, pp 399–444

von Arx JA, van der Walt JP (1987) Ophiostomatales and endomycetales. In: de Hoog GS, Smith MT, Weijman ACM (eds) The expanding realm of yeast-like fungi. Elsevier, Amsterdam, pp 167–176

von Arx JA, Weijman ACM (1979) Conidiation and carbohydrate composition in some *Candida* and *Torulopsis* species. Antonie Van Leeuwenhoek 45:547–555

Walker WF (1985) 5S ribosomal RNA sequences from ascomycetes and evolutionary implications. Syst Appl Microbiol 6:48–53

Watanabe J, Hori H, Tanabe K, Nakamura Y (1989) Phylogenetic association of *Pneumocystis carinii* with the "Rhizopoda/Myxomycota/Zygomycota group" indicated by comparison of 5S ribosomal RNA sequences. Mol Biochem Parasitol 32:163–168

Wells JM (1977) Sour rot of peaches caused by *Monilia implicata* and *Geotrichum candidum*. Phytopathology 67:404–408

Wickerham LJ (1951) Taxonomy of yeasts. Tech Bull 1029, U.S. Department of Agriculture, Washington, DC

Wickerham LJ (1958) Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science 128:1504–1505

Wickerham LJ (1969) New homothallic taxa of *Hansenula*. Mycopathol Mycol Appl 37:15–32

Wickerham LJ, Burton KA (1954) A clarification of the relationships of *Candida guilliermondii* to other yeasts by a study of their mating types. J Bacteriol 68:594–597

Wickerham LJ, Flickinger MH, Johnston RM (1946) The production of riboflavin by *Ashbya gossypii*. Arch Biochem 9:95–98

Yamada Y, Banno I (1987) *Hasegawaea* gen. nov., an ascosporogenous yeast genus for the organism whose ascospores have smooth surfaces without papillae and which are characterised by the absence of coenzyme Q and by the presence of linoleic acid in cellular fatty acid composition. J Gen Appl Microbiol 33:295–298

Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 77–100

Yoshida Y (1989) Ultrastructural studies of *Pneumocystis carinii*. J Protozool 36:53–60

2 Pezizomycotina: Pezizomycetes, Orbiliomycetes

Donald H. Pfister[1]

CONTENTS

I. **Introduction** ... 35
II. **Orbiliomycetes: An Overview** ... 37
III. **Occurrence and Distribution** ... 37
A. Species Trapping Nematodes and Other Invertebrates ... 38
B. Saprobic Species ... 38
IV. **Morphological Features** ... 38
A. Ascomata ... 38
B. Asci ... 39
C. Ascospores ... 39
D. Paraphyses ... 39
E. Septal Structures ... 40
F. Nuclear Division ... 40
G. Anamorphic States ... 40
V. **Reproduction** ... 41
VI. **History of Classification and Current Hypotheses** ... 41
VII. **Growth in Culture** ... 41
VIII. **Pezizomycetes: An Overview** ... 41
IX. **Occurrence and Distribution** ... 41
A. Parasitic Species ... 42
B. Mycorrhizal Species ... 42
C. Saprobic Species ... 42
X. **Morphological Features** ... 42
A. Ascomata ... 42
B. Asci ... 43
C. Ascospores ... 43
D. Paraphyses ... 43
E. Septal Structures ... 44
F. Anamorphic States ... 44
XI. **Reproduction** ... 44
XII. **History of Classification and Current Hypotheses** ... 44
A. Families of the Pezizomycetes ... 46
1. Ascobolaceae ... 46
2. Ascodesmidiaceae ... 46
3. Caloscyphaceae ... 46
4. Chorioactidaceae ... 46
5. Discinaceae ... 47
6. Glaziellaceae ... 47
7. Helvellaceae ... 47
8. Karstenellaceae ... 47
9. Morchellaceae ... 47
10. Pezizaceae ... 48
11. Pyronemataceae ... 48
12. Rhizinaceae ... 49
13. Sarcoscyphaceae ... 49
14. Sarcosomataceae ... 49
15. Tuberaceae ... 49
XIII. **Growth in Culture** ... 50
XIV. **Conclusion** ... 50
References ... 50

I. Introduction

Members of two classes, Orbiliomycetes and Pezizomycetes, of Pezizomycotina are consistently shown in molecular phylogenetic studies to diverge at the base of the Pezizomycotina phylogeny (Gernandt et al. 2001; Hansen and Pfister 2006; Spatafora et al. 2006). Kumar et al. (2012) give ultrastructural data on the septal structure, ascus wall construction, and nuclear division that suggest the Orbiliomycetes represent the earliest diverging lineage of the Pezizomycotina. The relationship of these two classes and the family diversity within the Pezizomycetes are shown in Fig. 2.1. These two groups show very few shared characteristics other than the formation, for the most part, of well-developed apothecial ascomata in which asci are generally arranged in a hymenium with paraphyses. Orbiliomycetes was recognized as a class only recently (Baral 2003). There is a single order, Orbiliales, and a single family, Orbiliaceae, with perhaps 300 species currently arranged in three teleomorphic

[1]Farlow Herbarium and Library of Cryptogamic Botany, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, 02138, USA; e-mail: dpfister@oeb.harvard.edu

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

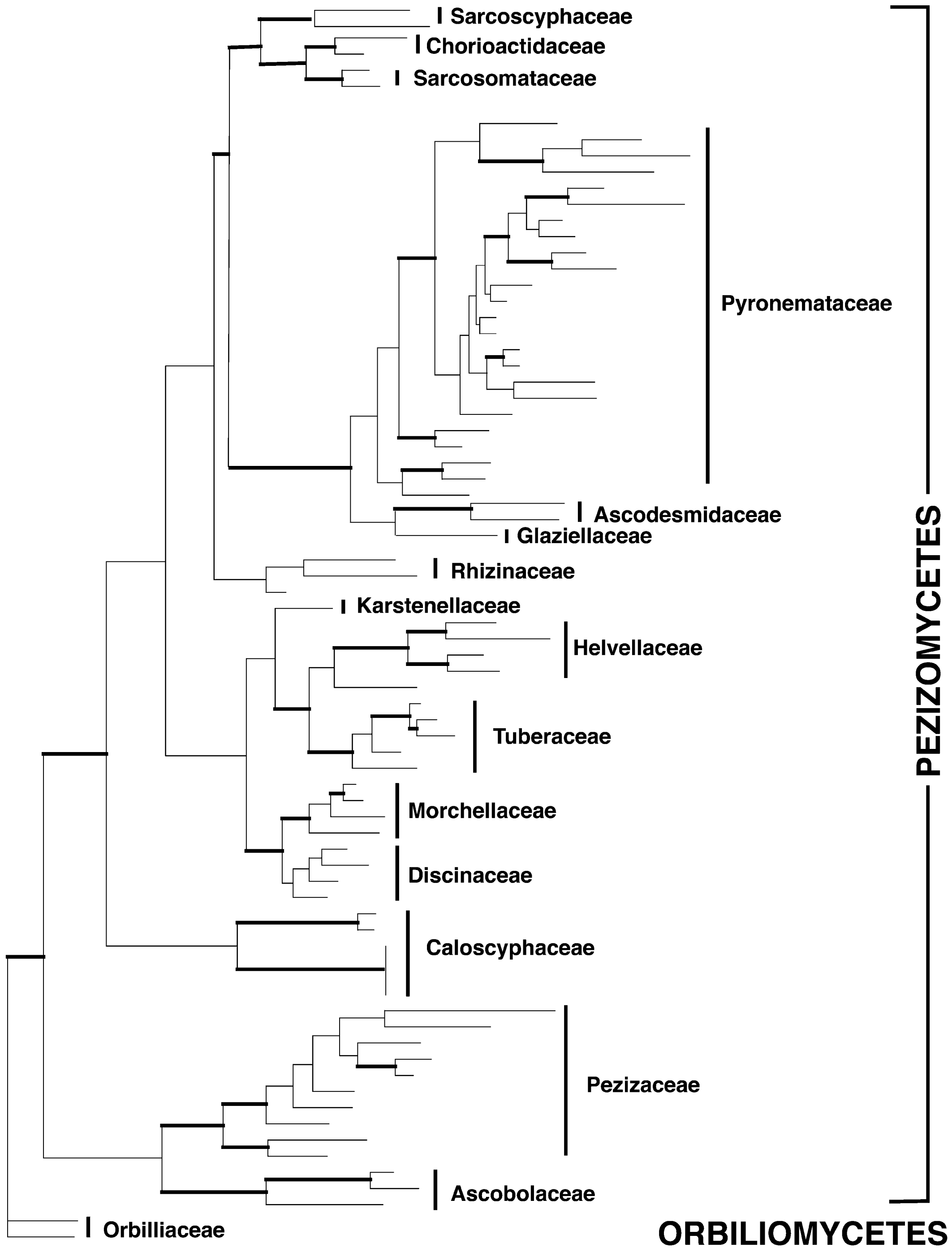


Fig. 2.1 A diagrammatic representation of the relationships of the Pezizomycetes and Orbiliomycetes, including the families of the Pezizomycetes, based on Hansen et al. (2013) and Pfister et al. (2013). Branches that received maximum parsimony, posterior probability, and maximum-likelihood support ≥75 %, 95 %, and 75 %, respectively, are in *bold*

genera. An early diverging position of the class has been confirmed by morphological studies and molecular phylogenetic analyses (Kumar et al. 2012; Spatafora 2006; Wang et al. 2006). Previously, the Orbiliaceae was placed among the Helotiales as a family based on Nannfelt's (1932) classic work on the nonlichenized inoperculate discomycetes. The Pezizomycetes have long been recognized to include a single order, the Pezizales, and include 16 families and approximately 1,200 species. The group, in whole or in part, has been the subject of recent surveys (Hansen and Pfister 2006; Harrington et al. 1999; Perry et al. 2007; Pfister et al. 2008).

In regard to morphological characteristics and ecology, the two classes also are distinct. The Orbiliomycetes are saprobic and in some cases supplement their nutrition through the capture and consumption of small invertebrates. Their spores are small, nonseptate (save for a single example), uninucleate, and contain a membrane-bound structure, the spore body (Baral 1994; Benny et al. 1978; Kumar et al. 2012). Spores are discharged actively. Asci are small and generally lack a well-developed apical apparatus. Anamorphic states are frequently formed, and their number has increased steadily in the literature in recent years. Species are found on dung, on wet wood on the forest floor, in water-soaked areas where they are somewhat ephemeral, or on dead wood hanging in trees where, because of their drought resistance, they are persistent (Baral 2011). No species are plant parasitic.

The Pezizomycetes are saprobic, plant parasitic, or associated with plants as ectotrophic mycorrhizae (Hansen and Pfister 2006; Tedersoo et al. 2006; Wei et al. 2010). The ascomata are always to some degree ephemeral. Spores are large, generally more than 10 μm and may reach a length of 60 μm, and single-celled. Spores generally are actively discharged through a well-developed opening created by the rupture of the ascus wall forming an operculum that is either terminal or eccentric in position. The trufflelike habit has developed independently in several lineages across the class (Læssøe and Hansen 2007). These hypogeous taxa generally do not have active spore release.

In this chapter, the two classes are treated independently in separate sections.

II. Orbiliomycetes: An Overview

The family Orbiliaceae was proposed by Nannfeldt (1932) and was restricted to those fungi with small so-called inoperculate asci, minute ascospores, and a waxy texture. The asci and paraphyses are often agglutinated. Nannfeldt illustrated several species showing details of the thin-walled globose to angular cells of the excipulum. A monograph on the genus *Orbilia* by Svrcek (1954) included 16 species that were distinguished based on ascospore size and shape, the color of the ascomata, and the form of the paraphyses. The groundbreaking work of Hans-Otto Baral and his collaborator, Guy Marson, has led to a refinement of the family and recognition of many more species. Kirk et al. (2008) proposed 288 species, but the extent of the diversity to be described is in flux. A monograph has been in preparation by Baral for many years. Modern methods employ morphological and histochemical characters previously not used and incorporate ecological characters as well. Of particular note is the reliance on fresh, living material (Baral 1992). There has been a burst of activity focusing on the description of teleomorphic taxa and associated anamorphs (e.g., Kohlmeyer et al. 1998; Liu et al. 2005a, b, 2006; Mo et al. 2005; Pfister 1994; Pfister and Liftik 1995; Qiao et al. 2011; Qin et al. 2010; Su et al. 2011; Tanabe et al. 1999; Webster et al. 1998; Wu et al. 2007; Yang and Liu 2005; Yu et al. 2007a, b, c, 2011; Zhang et al. 2007), and in this work there has been an intense interest in the diversity and functional morphology of the anamorphic states. This is particularly the case regarding those that capture nematodes, i.e., species referred to *Arthrobotrys* and allied form genera.

III. Occurrence and Distribution

Members of the Orbiliomycetes are found around the world in boreal to tropical habitats. Furthermore, several species complexes, such

as *O. luteorubella* and *O. xanthostigma*, that represent morphologically very similar collections seem to be found broadly distributed around the world and across latitudes. There are two major ecological groups—those that occur in moist habitats such as on dung, old polypores, and moist wood on the forest floor or near streams and species that are drought tolerant. These drought-tolerant species are found on dead twigs and small woody branches in the canopy of living trees. Often species of both ecological types are found in association with unicellular green algae, but there is no evidence that the fungi are lichenized.

A. Species Trapping Nematodes and Other Invertebrates

Since the classic work of Drechsler (1937a) on the nematode-trapping hyphomycetes, these fungi have attracted many researchers to this fascinating system. The work of Barron (1977, 1981) further set the stage for ecological and descriptive work on these fungi. Pfister (1994, 1997) and Pfister and Liftik (1995) documented the life history connection between these nematode-trapping hyphomycetes and teleomorphic members of the Orbiliomycetes. The several anamorphic form genera involved are discussed in what follows.

Trapping of nematodes and other invertebrates is accomplished by the formation of constricting rings, hyphal networks, or sticky knobs. These trapping structures are often, but not exclusively, initiated in the presence of prey. When nematodes become ensnarled in the trap, the fungus then grows and penetrates the body of its prey and proliferates within it. Conidia are formed in the presence or absence of nematodes and are generally dispersed across the medium, that is, they are not concentrated around trapped nematodes (Barron 1977). Earlier classification schemes for these fungi relied primarily on conidial morphology, particularly spore shape and septation. Phylogenetic studies have indicated that the form of the trapping devices gives important information for classification (Hagedorn and Scholler 1999; Rubner 1996; Scholler et al. 1999). In addition to trapping nematodes, some hyphomycetes in the orbiliaceous lineage trap other small invertebrates such as collembolans and rhizopods (e.g., Barron 1981; Drechsler 1936, 1937b). Even though these fungi may occur on wood or cellulose-rich substrates, there is evidence that few of the nematode-trapping fungi are able to break down cellulose (Park et al. 2002).

B. Saprobic Species

Until the work of Baral (2011) and Marson, most studies of diversity within the Orbiliomycetes were focused on those species on moist substrates. The discovery that these fungi are highly diverse in the dry habitats of hanging branches in living trees has greatly expanded the number of species that have been described through intense collecting in such habitats. Although these species are treated here as saprobic, there is little clear evidence of their mode of nutrition. Drechsler (1938) discussed at length hyphomycetes that are parasitic on oospores of Oomycota; these also seem now to be clearly referable to the Orbiliaceae. These fungi were "considered from a morphological viewpoint… [to] fit acceptably in the predacious series" (Drechsler 1938). Whether they fall in the main clade of nematode trapping Orbiliaceae or outside it remains to be studied, but that a variety of life styles and biotic modes are found within the family is unquestionable. It is important to note as well that no plant parasitic species have been discovered so far in this lineage.

IV. Morphological Features

A. Ascomata

Apothecial ascomata range in size from 0.5 to 5 mm and are saucer-shaped to pulvinate. Macroscopically they have been described as appearing waxy at sight or to touch. This is a reference at least in part to their shiny, translucent appearance. The sterile tissues of the ascomata are composed of an outer layer, or

ectal excipulum, of globose or angular cells that in some species give rise to superficial hairs. These cells may contain one or more cytoplasmic bodies that may take on several different shapes but disappear when material is mounted in KOH. Cells in the outer excipulum may be encrusted with granules or produce glassy projections or the cells may be embedded in an amorphous matrix. The inner layers of the ascomata are of interwoven hyphae or compressed angular cells (Nannfeldt 1932).

B. Asci

The asci of members of this class are small cylindrical to broad cylindrical with or without croziers; in some cases they have elongate, forked bases. Asci contain eight to many ascospores per ascus. Asci are inamyloid. The apex ranges from hemispherical conical to truncate. Walls are thin, composed of two layers in transmission electron microscopy (Benny et al. 1978; Kumar et al. 2012). The apex is generally little developed. Benny et al. (1978) reported no pores; Kumar et al. (2012) found an electron-light zone at the apex. In some taxa, an opening develops on the shoulder of the truncate asci (Zhang et al. 2007), and this was confirmed by Kumar et al. (2012). In some species, the apical walls may be thickened to form a cap (Kohlmeyer et al. 1998), as in *Orbilia* subgenus *Hemiorbilia*, in which the apex of the ascus is thickened (Baral 2011). In all cases, asci appear to dehisce by irregular tearing. Kumar et al. (2012) found similarities between the asci of the *Orbilia* species they studied and members of the Taphrinomycotina. Asci and paraphyses may be embedded in a gelatinous matrix that causes them to stick together and not fan out when crushed for microscopic observation. This is particularly the case in members of the genus *Hyalorbilia*.

C. Ascospores

The initial striking feature of the ascospores is their small size. They range in size from 3 to 8 μm. Observation of them is difficult in some situations and requires oil immersion and enhanced magnification. Spores are globose, ellipsoidal, ovoid, reniform, acicular, or filiform and mostly smooth, although spore wall ornamentations are known, for example, in *O. xanthostigma*. Ascospores of the Orbiliomycetes contain an inclusion, the spore body. This membrane-bound body was suggested to derive from the plasma membrane by Baral (2003), but Kumar et al. (2012) and Benny et al. (1978) showed that the spore body is derived from mitochondria. The spore body is attached to the cell membrane and is surrounded by a rough endoplasmic reticulum (Benny et al. 1978). The contents are electron dense (Benny et al. 1978). Under a light microscope it is seen in unstained living spores as a refractive body. Its appearance can be enhanced by application of aqueous brilliant cresyl blue or other vital stains. It disappears in dead spores and can be destroyed by application of KOH. The spore body is a synapomorphy for the class and can take on many different forms, from globose to tear-shaped to elongate. Its shape and orientation is consistent within the ascospores of a particular species. The shape and position of the spore body is considered a critical taxonomic character. Generally spores have a single spore body, but an example of a spore with two spore bodies is known (Wu et al. 2007). As mentioned previously, its contents are electron dense; it does not appear to be composed of lipids but may be proteinaceous (Kumar et al. 2012). No function has been attributed to the spore body, but Benny et al. (1978) offered three possible functions: for flotation and balance in a liquid environment before and during germination, storage of waste products, and storage of materials for use during germination. None of these functions has been documented, and ultimately the function of the spore body may have to do with special metabolic activities within the spore.

D. Paraphyses

Paraphyses are sparingly septate, broad, sometimes with a prominent capitate terminal cell. They may be encrusted or contain refractive materials. In some species, the paraphyses and

asci are closely adherent and are difficult to separate.

E. Septal Structures

Ascus, ascogenous, and nonascogenous hyphae have simple septa, with septal pores plugged by unelaborated electron-dense, nonmembranous occlusions. Globose Woronin bodies were located on both sides of the septum (Kumar et al. 2012).

F. Nuclear Division

Division is characterized by the retention of an intact nuclear envelope and a two-layered disk-shaped spindle pole body (Kumar et al. 2012).

G. Anamorphic States

Many members of the Orbiliomycetes have been shown to produce mitospores in culture; they are hyphomycetous or synnematous. All conidia are formed by holoblastic wall extension. A variety of named form genera have been applied to these conidial states. Anamorphs can provide some useful information on the biology and ecology of these fungi. Those that have been demonstrated to trap nematodes and other invertebrates fall into the following genera: *Arthrobotrys* (Li et al. 2005; Mo et al. 2005; Pfister 1994, 1997; Pfister and Liftik 1995; Qiao et al. 2011), *Drechslerella* (Li et al. 2005), *Dwayaangam* (Barron 1991), *Gamsylella*, known only in its anamorphic state (Scholler et al. 1999), *Lecophagus* (Tanabe et al. 1999), and *Monacrosporium* (Li et al. 2005; Pfister 1997). There remains debate about the application of some of these names. For general overviews the interested reader may consult Hagedorn and Scholler (1999), Liou and Tzean (1997), and Scholler et al. (1999). Through a multigene analysis Yang et al. (2007) postulated that two lineages evolved trapping mechanisms, with one lineage giving rise to constricting rings and the other adhesive traps, but, importantly, these fungi form a single clade. Yang et al. (2007) proposed a scheme for the evolution of trapping structures.

Other form genera are those that produce staurosporous or helicosporous conidia or are associated with aquatic habitats. These include *Angulospora* (Pfister 1997; Webster 1992; Webster and Descals 1979), *Dicranidion* (reports summarized by Pfister 1997), *Dwayaangam* (Kohlmeyer et al. 1998), *Helicoon* (Pfister 1997), *Trinacrium* (Matsushima 1995), and *Tridentaria*. Others are found on a variety of plant materials in terrestrial habitats, and these include *Brachyphoris* (Chen et al. 2007a, c), *Dactylellina* (Li et al. 2005; Qin et al. 2010), *Dactylella* (Chen et al. 2007a, b, c; Pfister 1997; Qin et al. 2010), and *Pseudotripoconidium* (Yu et al. 2011).

Staurosporous conidia and those reported from other aquatic anamorphs are detected in stem flow, through-fall, and treeholes (Gönczöl and Révay 2003, 2004, 2006; Révay and Gönczöl 2011). Among these are conidia referred to form genera of anamorphs associated with species of Orbiliomycetes. These include species of *Angulospora*, *Dactylaria*, *Dicranidion*, *Dwayaangam*, *Trinacrium*, and *Tridentaria*. Of these Gönczöl and Révay (2004) found that *Trinacrium* in particular was prevalent and that *Dwayaangam* and *Trinacrium* species were particularly diverse and geographically widespread (Gönczöl and Révay 2006). Such observations suggest that canopies are important habitats for these fungi. We expect that these are likely *Orbilia* anamorphs that are associated with the drought-tolerant, canopy-inhabiting Orbiliomycetes, which are fungi on dead branches in many woody plants. Gönczöl and Révay (2006) say, regarding their findings of aquatic hyphomycetes in these terrestrial habitats, that "this reinforces our belief that an ecological group of hyphomycetes distinct from those in lotic habitats exists and functions in canopies." By extension, we might assume that branches and twigs inhabited by Orbiliomycetes are a major part of this canopy ecosystem.

V. Reproduction

Although many of the Orbiliomycetes have been grown in culture, few reports have been published on apothecial production in the lab. Spontaneous production of ascomata has been noted (Drechsler 1937a; Rubner 1994). Zachariah (1983) was able to induce ascomatal primorida. Breeding systems have not been studied in part because of the unpredictability of producing ascomata in culture.

VI. History of Classification and Current Hypotheses

The family Orbiliaceae was created in 1932 in Nannfeldt's (1932) classic treatment of the inoperculate discomycetes. Recognizing the special features of this group, he removed the genera *Orbilia* and *Hyalina* from the core group of inoperculate discomycetes. Primary treatments of the family were those by Svrcek (1954) and Spooner (1987). Nannfeldt's circumscription of the family was used with few changes until the 1990s. The placement of the family outside the Leotiomycetes lineage initially began to be questioned by Pfister (1997), and subsequent work has confirmed the placement as an early diverging lineage within the Pezizomycotina (Gernandt et al. 2001; Spatafora et al. 2006). The position of the Orbiliomycetes as an early diverging lineage in the filamentous Ascomycota along with the Pezizomycetes was one of the major surprises in the era of molecular phylogenetic studies.

Within the Orbiliomycetes there is a single order, and only three teleomorphic genera are recognized. Other genera may well be proposed as more of these fungi come to be known through wider collection of them and collections come under deeper scrutiny. The described genera are *Orbilia*, the largest and most diverse genus with two subgenera and nine sections in Baral's treatment (2011), *Hyalorbilia* with six to eight species (Baral and Marson 2000), and *Pseudorbilia*, with a single species (Zhang et al. 2007). These genera are distinguished based on characteristics of the ascus, the ectal excipulum, spore bodies, the presence or absence of gelatinous material in the hymenium, and pigmentation [see Zhang et al. (2007) for a summary of the genera].

VII. Growth in Culture

One of the most remarkable features of the Orbiliomycetes is the ease with which most of the species can be cultivated on standard media. Spores deposited on the surface of agar media germinate readily, with the exception of the species of *Hyalorbilia* for which there are only a few reports of successful cultivation. Spores germinate quickly, and growth is often luxuriant on substrates containing glucose. Sporulation is common under room conditions.

VIII. Pezizomycetes: An Overview

The Pezizomycetes comprise a single order, Pezizales, with 16 families currently recognized. The full diversity of the order has not yet been completely recognized in the classification, and no doubt other lineages will of necessity be named. Ascomata are both epigeous and hypogeous. The epigeous types are apothecial, cleistothecial, or highly reduced, being composed of only a few asci in clusters on vegetative hyphae with little or no excipular tissue. In epigeous lineages, spores are generally forcibly discharged with the asci rupturing by the formation of an operculum. Hypogeous members occur in most families containing mycorrhizal members. To date, more than 15 independent origins of trufflelike members are known within a majority of the families (Læssøe and Hansen 2007).

IX. Occurrence and Distribution

Pezizomycetes can be found around the world, but representatives are unevenly distributed. Members of certain families, such as the Pezizaceae, Morchellaceae, Helvellaceae,

Rhizinaceae, and many of the Pyronemataceae, show a particularly high diversity in temperature regions. Others are more abundant in tropical areas, as exemplified by the Sarcosomataceae and Sarcoscyphaceae. Recent studies have made clear that, although collecting in this group is robust in many areas of the Northern Hemisphere, the Southern Hemisphere is poorly documented. Nutritionally, Pezizomycetes are saprobic or mutualistic (Tedersoo et al. 2006) or are parasitic on bryophytes and vascular plants (Hansen and Pfister 2006). Many of the species collected on soil occur in disturbed areas or in soil that has a high pH and low content of organic matter. Petersen (1985) provides a review of the edaphic factors involved in growth and reproduction.

A. Parasitic Species

The plant parasitic species are scattered in various lineages. *Rhizina undulata* is a serious root parasite of conifers (Ginns 1968). *Pithya* species may cause dieback on conifers. *Caloscypha fulgens* is implicated as a seed pathogen of conifers (Paden et al. 1978; Salt 1974). *Urnula craterium* has been implicated as the causal agent of strumella canker in oak (Davidson 1950). *Phymatothrichopsis ominivora* is a root pathogen of cotton and other dicotyledonous plants and is known only in its anamorphic state (Uppalapati et al. 2010). A clade in the Pyronemataceae, including *Octospora* and *Lamprospora*, is parasitic on bryophytes (Hansen and Pfister 2006). Apothecia occur on the thalli or surrounding soil if they are rhizoidal parasites (Döbbler 1979).

B. Mycorrhizal Species

With the advent of molecular approaches to studying mycorrhizal root tips a number of examples of mycorrhizal Pezizomycetes have been documented, and it is now known that mycorrhizal lineages are found throughout the class (Tedersoo et al. 2006). Morphologically, the mycorrhizae have a mantle that is often thin, have a well-developed Hartig net, and generally do not form rhizomorphs (Tedersoo et al. 2006; Wei et al. 2010). Mycorrhizal species seem to predominate in areas that experience xeric conditions (Smith et al. 2006). Mycorrhizal lineages are often also those that contain truffle or trufflelike taxa. There are no trufflelike species in the families Sarcoscyphaceae and Sarcosomataceae, and likewise there are no known mycorrhizal species.

C. Saprobic Species

Pezizomycetes occur on organic material of various types—decaying wood, dung, leaf litter, and twigs. The diversity of Pezizales on dung is well documented, with many studies and keys available (Bell 1983; Doveri 2004; Richardson and Watling 1997). Some clades are nearly exclusively found on dung, most notably species of Ascobolaceae (Brummelen 1967). Dung-inhabiting species are found in several clades of the Pyronemataceae and Pezizaceae (Hansen and Pfister 2006; Hansen et al. 2001; Perry et al. 2007). In most cases, little is known of the biology of saprobic species. Sarcoscyphaceae, Sarcosomataceae, and Chorioactidaceae are found exclusively on wood, leaves, and plant debris.

X. Morphological Features

A. Ascomata

Apothecia are the basic type of ascomata. These range in size from several millimeters to several centimeters and vary from sessile cups of small to large size to stalked cups to the stipitate piliate structures found in the Helvellaceae, Discinaceae, and Morchellaceae. Some highly reduced members are little more than small fascicules of asci such as in *Ascodesmis*. Further reductions include highly reduced forms found in Eleutherascus and Monascella (Guarro and Arx 1986; Stchigel et al. 2001). Here the asci are formed in clusters on unspecialized hyphae. Ascomata are often highly pigmented, particularly the hymenium. Carotenoid pigments have been characterized (Arpin 1969).

Variations are found in typical apothecial ascomata. On the one hand, there are highly reduced types that are merely asci scattered on hyphae as mentioned previously, but small cleistothecial ascomata have also been found in a few cases (Hansen et al. 2005b). In the case of *Orbicula parietina*, active discharge is lost and spores are presented in a powdery mass. *Heydenia* species form stalked cleistothecial fruit bodies closely related to *O. parietina* (Lechtmann and Clémençon 2011). The ascomata of *Heydenia*, which was recently placed in the Pyronemataceae (Lechtmann and Clémençon 2011), deserve special comment. *H. alpina* is a small fungus that occurs on plant debris and mosses. It is stipitate with a dark stipe that at the top expands to hold a pale mass of hyaline spores and radiating hyphae. No asci are present, but molecular evidence confirms that this species belongs in the Pyronemataceae as a close neighbor of *Orbicula*, another fungus that produces dry powdery masses of spores at maturity. The two genera share a cleistothecial fruit body and asci that seem to disintegrate early in the development of the ascomata but diverge on important morphological features (Lechtmann and Clémençon 2011). These genera, often placed outside of the Pezizales, have been demonstrated through molecular systematic studies to be members of the order.

Truffleoid fruit bodies are found in several lineages. Following the terminology of Weber et al. (1997), these are stereothecia, exothecia, or ptycothecia, depending on the arrangement of the asci and the presence or absence of well-developed hymenial layers. Truffle-type ascomata are derived from apothecial forms. Læssøe and Hansen (2007) summarize much of the literature on these taxa.

Some highly reduced forms are found among bryophyte parasites. The fruit bodies are partly closed and have only a few asci. They give the appearance of perithecia.

B. Asci

Brummelen (1978, 1994) summarized the structural characters of the ascus in the Pezizomycetes. The apical apparatus, when present, is in the form of an operculum, and its position, the reaction of the wall layers in various staining agents in light microscopy, and the ultrastructure of the ascus wall are all important characters. Amyloid asci are found in Pezizaceae and Ascobolaceae (Hansen et al. 2001), but this character has been lost several times, most notably in the hypogeous members of the Pezizaceae. Amyloid asci are not found outside these families.

The layering of the wall as seen in transmission electron microscopy helps delimit groups, particularly the taxa with thick lateral walls in the Sarcoscyphaceae and Sarcosomataceae. The asci of these fungi were termed suboperculate by Le Gal (1946a, b, 1953). Subsequently, Ecblad (1968) and Samuelson (1975) discounted the term.

Not all members of the class have opercula. Hypogeous (Læssøe and Hansen 2007) and some cleistothecial taxa (Hansen et al. 2005a, b) have lost the operculum and spores remain either within asci until eaten and the fruit body is broken down, or the asci disintegrate and a powdery mass is formed.

C. Ascospores

No member of the Pezizomycetes has septate spores at maturity. Septa may form in germinating spores, but only when a germ tube has already been established. The spores may take on a variety of shapes from globose to naviculate, with smooth or ornamented surfaces. Generally, the ornamentations are derived from secondary wall material, although a few examples are known of spore ornamentation without a contribution of secondary walls. The spore walls are multilayered and may be thin or thick (Merkus 1976). The number of nuclei per spore varies from one, two, or four to many, and such variation has been shown to have some taxonomic value (Berthet 1964; Korf 1972, 1973).

D. Paraphyses

The pigmentation of the hymenium is attributed to the pigments found in the paraphyses.

Yellow, red, and orange carotenoid pigments are distinctive features of some of these fungi. These pigments are dissolved in oil droplets in the paraphyses. A variety of carotenoid pigments are known to occur in these fungi, as summarized by Arpin (1969). Paraphyses are generally septate and may be either uninucleate or multinucleate, this latter character being used in the classification (Berthet 1964; Pfister et al. 2008). In some cases, paraphyses are interspersed with hyaline or darkly pigmented, often thick-walled elements or setae. Examples of such setae are found in members of the Rhizinaceae, Sarcosomataceae, Chorioactidaceae, and Sarcoscyphaceae.

E. Septal Structures

Ultrastructural characters of septal pore plugs and Woronin bodies correlate to some degree with families and lineages in the order. Kimbrough (1994) summarizes much of the information. Septal pores in vegetative hyphae generally have lamellae embedded in a matrix. These septa generally have associated Woronin bodies. The Woronin bodies take on several forms—globular, hexagonal, or cylindrical, sometimes considerably elongate. The form of the Woronin bodies is an important taxonomic and phylogenetic character. The septa at the base of the asci also provide important characters. These septa are occluded by dome-shaped, pyramidal, or dumbbell-shaped structures. These plugging structures show electron-dense bands, lamellae, and raylike extensions.

F. Anamorphic States

Anamorphic states have been reported across many families. Conidia are blastic in development and are generally hyphomycetous. Table 2.1 provides a summary of the known anamorphs. In many of these anamorphs, the germination of conidia has not been observed, suggesting that they may rather act as spermatia. The investigations by Healy et al. (2013) identify anamorphic spore mats in several clades in the Pezizaceae and Tuberaceae. Attempts to germinate mitospores from these mats have failed, and the function of these spores remains unknown. Several anamorphic states have been discovered through molecular phylogenetic studies to belong within the Pezizomycetes, but they have no known teleomorphs. Most notable among these is *Phymatotrichopsis omnivora*, the cotton root pathogen. This falls within the Rhizinaceae based on molecular studies, but despite intensive study of this important pathogen, no evidence of a teleomorph has been discovered. Another example is *Cephaliophora*, a dung-inhabiting fungus and one that has been implicated in causing keratitis (Hoog 2000).

XI. Reproduction

Extensive studies have been carried out on several members of the class. *Ascobolus* was early incorporated as a genetic tool. *Pyronema* has also been extensively studied. Early cytological studies on members of the Pyronemataceae were used to explore mitotic events in asci.

XII. History of Classification and Current Hypotheses

The current classification has its origins in the work of Boudier (1885, 1907), who was the first to use the presence of an operculum to define this group. Hypogeous, truffloid members previously treated in the order Tuberales have now been placed in the Pezizales based on morphology, cytology (Trappe 1975), and molecular sequence analyses (Læssøe and Hansen 2007). Wide-ranging phylogenetic work within the order led to the recognition of three primary lineages (Landvik et al. 1997). Subsequent work has expanded this framework and has suggested groupings of families that in part reflect those previously based on morphological, cytological, and pigment data and are noted in earlier work (Korf 1972, 1973; Rifai 1968). The most recent comprehensive overview of the class is that of Hansen and Pfister (2006). Currently, 15 families are recognized (Fig. 2.1),

Table 2.1 Summary of known anamorphs of Pezizomycetes

Family	Anamorphic form genus	Teleomorphic genus	References
Ascobolaceae	*Rhizostilbella*	*Ascobolus*	Seifert (1985)
	Papulospora	*Ascobolus*	Brummelen (1967)
	An oidial state	*Ascobolus*	Brummelen (1967)
Caloscyphaceae	*Geniculodendron*	*Caloscypha*	Paden et al. (1978)
Chorioactidaceae	*Kumanasamuha geaster*	*Chorioactis geaster*	Nagao et al. (2009)
	Verticicladium	*Desmazierella acicola*	Paden (1972)
Morchellaceae	*Costantinella*	*Morchella*	Paden (1972)
Pezizaceae	*Glischroderma*	Pezizaceae	Norman and Egger (1999), Hansen et al. (2001), Healy et al. (2013)
	Chromelosporium	*Peziza*, *Plicaria*, *Muciturbo*	Hennebert (1973), Paden (1972), Warcup and Talbot (1989), Healy et al. (2013)
	Oedocephalum, including highly reduced forms	*Peziza*, *Pachyella*, *Iodophanus*, *Cleistoiodophanus*	Hennebert (1973), Hennebert and Bellemère (1979), Paden (1972), Bezerra and Kimbrough (1976)
Pyronemataceae	*Actinosporella megalspora*	*Miladina lechithina*	Descals et al. (1998)
	Alciphila vulgaria	*Byssonectria*	Harmaja (2002a, b)
	Ascorhizoctonia	*Tricharina*	Yang and Korf (1985), Barrera and Romero (2001)
	Cephaliophora	None known	Tanabe et al. (1999)
	Complexipes	*Wilcoxina*	Yang and Korf (1985)
	Dichobotrys	*Trichophaea*	Hennebert (1973)
	Micronematobotrys	None known	Sun and Guo (2010)
	"*Nodulosporium*"-like	*Geopyxis majalis*	Paden (1972)
Rhizinaceae	*Phymatotrichopsis*	None known	Uppalapati et al. (2010)
Sarcosomataceae	*Conoplea*	*Urnula craterium*, *Plectania*, *Sarcosoma latahensis*	Hughes (1958), Paden (1972)
Sarcoscyphaceae	*Molliardiomyces*	*Phillipsia*, *Sarcoscypha*, *Nanoscypha*	Paden (1984), Pfister (1973b)
Tuberaceae	Unnamed sympodulosporous type	*Tuber*	Urban et al. (2004), Healy et al. (2013)

though the relationship among those families in many instances is not well resolved. Although this group is reasonably well known, future sampling will certainly change our understanding of the relationships and of these lineages. A particular focus is on the large and heterogeneous family Pyronemataceae, in which several lineages are recognizable based on both sequence analyses and expanded knowledge of nutritional modes, morphology, and anamorphic states.

General overviews of the families can be found in Cannon and Kirk (2007). The following augmented descriptions include available structural and cytological information that has been used in refining the classification.

A. Families of the Pezizomycetes

1. Ascobolaceae

Only epigeous members are known, and these occur primarily on dung, but a few species are found on plant material; *Ascobolus carbonarius* is found on burned material (Brummelen 1967). All species are saprobic and ascomata have multiple cell layers. Developmental patterns have been characterized by Brummelen (1967). Cleistothecial developmental types are found. Asci blue diffusely along the walls in iodine solutions. Ascospores are uninucleate (Berthet 1964); in *Ascobolus* and *Saccobolus* the spore walls are ornamented with purple brown pigments that become fissured at maturity (Brummelen 1967). The placement here of *Thecotheus* species, with their hyaline ascospores, has been suggested on morphological grounds and has been confirmed in molecular phylogentic studies (Landvik et al. 1997). Septa associated with the ascogenous system are dome-shaped (Kimbrough 1994; Kimbrough and Curry 1985). Three genera are generally recognized.

2. Ascodesmidiaceae

Ascomata are much reduced in most genera; for example, in Eleutherascus species the asci are scattered on hyphae. In *Ascodesmis*, several asci are formed on a much-reduced excipulum (Brummelen 1981). These species are saprobic on dung and often are isolated from soil. Asci are inamyloid and are saccate or pyriform. Ascospores are brownish and have reticulate spore ornamentations. Septa in ascogenous cells and the ascus bases are dome-shaped with arrays of radiating tubular elements and a striate structure associated with the septal pore rim in vegetative cells (Brummelen 1989; Kimbrough 1994). Four genera are placed in the family (Hansen et al. 2005b). A recent study by Hansen et al. (2013) places this family within the larger and highly diverse sister group to the Pyronemataceae *sensu stricto*.

3. Caloscyphaceae

The brightly colored cupulate ascomata stain blue green when bruised. No hypogeous members are known. *Caloscypha fulgens* is a parasite of conifer seeds and seedlings. The ascomata have a well-developed inner layer of interwoven hyphae and an outer layer of globose or angular cells. Ascospores are globose, and their walls are smooth (Harmaja 2002a). The genus *Caloscypha* was previously placed in the Pyronemataceae, in part because of its carotenoid pigments and because of the globose spores (Arpin 1969; Korf 1972, 1973). For details on septal construction see Kimbrough and Curry (1986b). Recently a second genus was added to the family, *Kallistoskypha*, with a single species, *K. incarnata*, which is associated with *Eucalyptus* found around the Mediterranean region (Pfister et al. 2013).

4. Chorioactidaceae

The ascomata are cupulate or deep urnulate and dark on the outer surface; the hymenium is beige, brownish, or orange. No hypogeous members are known in this family, and all the species are considered to be saprobic. The ascomata have a well-developed inner layer and an outer layer of globose or angular cells that give rise to thick, brown hairs encrusted with granular material. The asci are inamyloid and thick-walled with a terminal operculum. Ascospore

ornamention is in the form of warts or ribs. Four genera have been included (Pfister et al. 2008).

5. Discinaceae

The ascomata of this family are discoid or gyromitroid. Both epigeous and hypogeous members are known. Many species in the family are mycorrhizal (Tedersoo et al. 2006, 2010). The ascomata are characteristically constructed with an inner layer of interwoven hyphae and an outer layer of elongate cells oriented perpendicularly to the outer surface. Asci are inamyloid, and ascospores are tetranucleate (Berthet 1964), smooth, or elaborately ornamented with warts, often with apiculae. Woronin bodies are elongate and often surround the septal pore. The septa at the base of asci are accompanied by an electron-opaque, hemispherical structure that becomes cone- to dumbbell-shaped with V-shaped structures; an electron-translucent torus separates the pore plug from the septal pore border (Kimbrough 1991, 1994). The septal ultrastructure is similar to that of the Helvellaceae. Generally, five or six genera are recognized.

6. Glaziellaceae

The ascomata in this family are large and hollow with a basal opening. The unisporic, clavate to globose asci are embedded in the rindlike wall. Ascus walls break down, but the spores remain embedded in this peridiumlike wall. The single species, *Glaziella aurantiaca*, is presumed to be mycorrhizal. See Gibson et al. (1986) for ultrastructural details that initially supported the placement of *Glaziella* in the Ascomycota.

7. Helvellaceae

Ascomata are sessile cupulate or stipitate cupulate, saddle-shaped, or columnar (Abbott and Currah 1997; Dissing 1966). Both epigeous and hypogeous species are found in the family (Hansen and Pfister 2006; Kimbrough et al. 1996). Many or most are mycorrhizal (Tedersoo et al. 2006). The ascomata are characteristically constructed with an inner layer of interwoven hyphae and an outer layer of elongate cells oriented perpendicularly to the outer surface. Asci are inamyloid. Ascospores are tetranucleate (Berthet 1964), smooth, or ornamented with irregular warts, and Woronin bodies are elongate, hexagonal, or globose (Kimbrough 1994). Septa at the base of the asci are electron-opaque. There are hemispherical structures that become cone- to dumbbell-shaped with V-shaped striations; in vegetative cells, an electron-translucent torus separates the pore plug from the septal pore border. Vegetative cells possess globular or slightly angled Woronin bodies (Kimbrough 1994; Kimbrough and Gibson 1989; Kimbrough et al. 1996).

8. Karstenellaceae

In this family, the ascomata are little more than a very thin, resupinate crust situated on leaf litter and wood surfaces. Asci are scattered on this mycelial mat. Nothing is known of its mode of nutrition. Asci are inamyloid and operculate. Ascospores are binucleate or multiguttulate (Hansen et al. 2008; Harmaja 1969). No ultrastructural details are known for the vegetative septal construction or for the septa of the asci. Molecular data place it in an unresolved position near Helvellaceae and Tuberaceae. There is a single genus with a single species recognized, *Karstenella vernalis*.

9. Morchellaceae

The morels are characterized by stipitate apothecia, with an interrupted hymenial layer giving a honeycomb appearance, or by cupulate forms such as *Disciotus*. Hypogeous members include *Leucoangium* (Li 1997) and *Kalapuya* (Trappe et al. 2010). Species are saprobic and have been implicated in mycorrhizal relationships (Buscot 1994; Dahlstrom et al. 2000). Asci are inamyloid. Ascospores are multinucleate (Berthet 1964) and lack both prominent wall ornamentation and internal oil droplets. Traditional classifications of the species accept six to eight species, but phylogenetic studies indicate

the presence of many species (Du et al. 2012; Kuo et al. 2012; Taskin et al. 2010) that seem restricted to specific continents (O'Donnell et al. 2011). Some of these species are morphologically distinct; others are cryptic (O'Donnell et al. 1997, 2011). The septal construction is similar to that found in the Helvellaceae. At the base of the asci, dome-shaped structures with V-shaped striations are found at the septal pore. Lamellate structures are found within septal pores of most vegetative cells. Elongate Woronin bodies have been found along with hexagonal ones (Kimbrough 1994). Five or six genera are recognized.

10. Pezizaceae

Both epigeous and hypogeous taxa are found; there are multiple origins of the hypogeous taxa within several clades (Hansen et al. 2001, 2002, 2005a; Healy et al. 2009). Saprobic members include those on dung, wood, and other plant debris. Mycorrhizal taxa are found in several lineages. Some taxa are found exclusively on burned areas. The ascomatal structure varies, but multiple layers composed of large, thin-walled globose cells are often present. Asci generally become blue in iodine solutions. This bluing can take several forms. The reaction may be diffuse along the entire wall or concentrated in the upper portion of the ascus but most intensely at the tip, or in the upper portion with a more intense ring surrounding the opercular region (Hansen et al. 2001). The bluing reaction has been lost in several taxa, including some that are hypogeous. Ascospores are uninucleate (Berthet 1964), with or without secondary wall ornamentations (Hansen et al. 2001, 2002). Oil droplets may or may not be present in the ascospores. Septa in vegetative cells are lamellate with large globose Woronin bodies. At the ascus bases, septa are occluded by convex or biconvex bands that become covered with electron-opaque amorphous material or by an additional secondary wall (Curry and Kimbrough 1983; Kimbrough 1994; Kimbrough et al. 1991). Approximately 30 genera are currently recognized in the family, but it is well established that the genus *Peziza* is nonmonophyletic and will need to be broken down into several additional genera (Hansen et al. 2001, 2005a, b).

11. Pyronemataceae

Pyronemataceae is the largest of the families of the class and the most diverse ecologically and morphologically. As recognized here, the family includes Aleuriaceae, Humariaceae, Otideaceae, and Geneaceae. These fungi are saprobic, mycorrhizal vascular plants or parasitic on bryophytes. Epigeous members are cupulate, often possessing hairs on the outer surface. Hypogeous members are scattered in the family. The ascomata show considerable variation in construction and development. Asci are inamyloid, and ascospores vary in shape and ornamentation from smooth to variously ornamented (Perry et al. 2007). Several different septal types are present; at least four types of septa plugging have been associated with asci (Kimbrough 1994). These are the aleurioid type, which have a granular, opaque matrix that borders both the ascal and ascogenous hyphal sides of pores; later this becomes fan-shaped with a lamellate electron-translucent torus adjacent to the pore rim. The otideoid type, is characterized by double translucent bands in a granular, opaque pore matrix. Later the pore plug is differentiated into two zones, an inner dense zone and an outer less opaque zone. The pulvinuloid type, is similar to the Helvellaceae at maturity with V-shaped striations, but these are fewer and less prominent than in Helvellaceae. The scutellinioid type has a large hemispherical septal pore plug that becomes zonate; the inner zone adjacent to the pore lumen is electron-opaque, and an outer, thicker zone is composed of less dense material. In general, in this family, vegetative cells have a lamellate structure in the septal pores. Woronin bodies are small and largely globular, but often hexagonal.

The pyronema type has asci and ascogenous hyphae that are similar to those of the Ascobolaceae but with a different electron density core and small, radiating tubular bands similar to those of *Ascodesmis* (Kimbrough 1994; Kimbrough and Curry 1986a, b).

The family includes approximately 80 genera. It is perhaps highly heterogeneous and one that will see future division and refinement. The family was recently studied by Hansen et al. (2013), and this work provides the most accurate overview of the family.

12. Rhizinaceae

The ascomata in members of this family are flat, recurved, or pulvinate. *Rhizina undulata* is a pathogen of conifer roots. Also included here is *Psilopezia*, whose species are found on water-soaked wood and which is presumed to be a saprobe (Pfister 1973a). The construction of the ascomata follows a pattern of interwoven hyphae forming the medulla, with more densely interwoven hyphae contributing to the cortical layer. These fungi are characterized by indeterminate marginal growth. The asci are inamyloid, and ascospores are smooth or ornamented with warts and apiculae. The spores are presumed to be tetranucleate. Studies are needed on the septal structure. The family encompasses the two genera mentioned earlier as well as *Phymatotrichopsis omninvora*, which is known only as an anamorph.

13. Sarcoscyphaceae

Ascomata are generally cupulate, bright colored, stipitate, or sessile. No hypogeous members are known; all are considered saprobic. The ascomata have a well-developed inner layer and an outer layer of globose or angular cells or of tightly interwoven hyphoid cells oriented parallel to the outer surface. Cells on the outer surface sometimes give rise to single or fasciculate hairs. Ascospores are smooth or ornamented with ridges and ribs. In vegetative cells, lamellate structures are poorly differentiated or missing. Septa in vegetative cells are imperforate, plugged by an electron-opaque, fan-shaped matrix surrounded by a number of globose electron-dense Woronin bodies. The Woronin bodies are globose or occasionally hexagonal and electron-opaque. At maturity obscure V-shaped striations may be found (Benny and Samuelson 1980; Kimbrough 1994; Li and Kimbrough 1995b). Twelve genera are commonly recognized within this family. Romero et al. (2012) review the classification within the family.

14. Sarcosomataceae

In this family, ascomata are cupulate or urnulate, tough and leathery of moderate to large size, and black or dark brown on the outside, often with a paler hymenium. No hypogeous members are known. Most species are presumed to be saprobic on plant debris, but in the case of *Urnula*, the species is said to be parasitic. The ascomata have a well-developed inner layer, with gelatinous material, and an outer layer of globose or angular cells that give rise to dark hyphoid hairs. Asci are inamyloid and thick-walled with a terminal operculum. The ultrastructure of the asci and ascospores have been studied by Bellemère et al. (1990). The ascospores are smooth or with wrinkled or folded walls or with low warts. In addition, they are multinucleate. Septal structures are composed of an electron-dense matrix and globose Woronin bodies that are often accompanied by short or long, cylindrical, hexagonal Woronin bodies. Septa at the ascus bases are composed of a dumbbell-shaped matrix with V-shaped bands. The septal structure was studied by Li and Kimbrough (1995b). Five or six genera are commonly accepted.

15. Tuberaceae

Until recently, all members of this family were considered to be hypogeous. The recent placement of *Nothojafnea thaxteri* among these fungi is notable as the first epigeous member of the family (Bonito et al. 2013). The truffles of commerce (*Tuber melanosporum* and *T. magnatum*) belong here. Ascomata are highly convoluted and folded, often with chambers or pockets of asci. The large, primarily Northern Hemisphere genus *Tuber* has been investigated by Bonito et al. (2010). All members are mycorrhizal on a range of plant families (Tedersoo et al. 2006). The asci are inamyloid, globose, or pyriform with one to

eight spores per ascus. The ascospores, in addition to being often multinucleate, are often elaborately ornamented with reticula, ridges, and warts. Septal structure was studied by Li and Kimbrough (1995b), who determined that there was some heterogeneity among those species examined. In that study, the researchers found long, cylindrical Woronin bodies in some of the species, similar to those of the Morchellaceae, Discinaceae, and Helvellaceae, and rectangular ones in another, similar to those of the Pyronemataceae. Septa associated with ascogenous hyphae and ascus bases also differed among the sampled taxa. O'Donnell et al. (1997) included a series of genera in addition to *Tuber*. Seven genera are commonly recognized. Bonito et al. (2013) estimate a late Jurassic origin of the genus *Tuber* and discuss the Southern Hemisphere diversity.

XIII. Growth in Culture

The ascospores of saprobic species germinate, and often an anamorphic state is produced. Fungi in mycorrhizal lineages do not easily germinate, and few examples are known of their anamorphic states. In some cases, field collections of anamorphs have proven to be pezizalean, and there are many examples of environmental samples linked with Pezizomycetes (Healy et al. 2013).

XIV. Conclusion

The Orbiliomycetes and Pezizomycetes are early divergent lineages within the filamentous Ascomycota. The two classes are remarkably different in their ecology and in the structure of their asci. So far as is known, there are no mycorrhizal taxa in the Orbiliomycetes, whereas the mycorrhizal life style is found in most families of the Pezizomycetes. Although anamorphs are known in the Pezizomycetes, their diversity is low; in the Orbiliomycetes, on the other hand, there is a wide range of conidial morphologies. Within the Pezizomycetes there are no known examples of predatory behavior, whereas some members of the Orbiliomycetes trap and consume invertebrates. Recent studies have placed many hypogeous Pezizomycetes within families. These studies highlight the evolutionary patterns within the class in which suites of changes, including loss of active spore discharge mechanisms and extended ascomata developmental times, take place in distantly related clades. Most hypogeous Pezizomycetes are found within clades with other mycorrhizal members. The diversity in both Orbiliomycetes and the Pezizomycetes has proven to be greater than previously anticipated, and much researches remains to be done regarding the biogeography and evolutionary histories of the group.

References

Abbott SP, Currah RS (1997) The Helvellaceae: systematic revision and occurrence in northern and northwestern North America. Mycotaxon 63:1–125

Arpin N (1969) Les caroténoïdes des Discomycètes: essai chimiotaxinomique. Bull Mens Soc Linn Lyon 38(suppl):1–169

Baral H-O (1992) Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. Mycotaxon 44:333–390

Baral H-O (1994) Comments on "Outline of the ascomycetes—1993". Syst Ascomycetum 13:113–128

Baral H-O (2003) Notes on ascomycetes systematics. no. 3591. Myconet 9:96

Baral H-O (2011) Ecology of xerotolerant fungi, with special emphasis on Orbiliomycetes. http://www.gbif-mycology.de/HostedSites/Baral/ecology.htm

Baral H-O, Marson G (2000) Monographic revision of *Gelatinopsis* and *Calloriopsis* (Calloriopsideae, Leotiales). Mycologia 92:23–46

Barrera VA, Romero AI (2001) *Tricharina striispora* from Argentina and the finding of its anamorph, *Ascorhizoctonia*. Mycotaxon 77:31–37

Barron GL (1977) The nematode destroying fungi. Canadian Biological Publications Ltd, Lancaster, PA

Barron GL (1981) Predators and parasites of microscopic animals. In: Cole GT, Kendrick B (eds) Biology of conidial fungi, vol 2. Academic, New York, pp 167–199

Barron GL (1991) A new species of *Dwayaangam* parasitic on eggs of rotifers and nematodes. Can J Bot 69:1402–1406

Bell AE (1983) Dung fungi: an illustrated guide to coprophilous fungi in New Zealand. Victoria University Press, Wellington

Bellemère A, Malherbe MC, Chacun H, Meléndez-Howell LM (1990) L'Etude ultrastructurale des asques et des ascospores de *l'Urnula helvelloides* Donadini, Berthet et Astier et les conscepts d'asque subopercułé et de Sarcosomataceae. Cryptog Mycol 11:203–238

Benny GL, Samuelson DA (1980) Ultrastructure of septa in the vegetative hyphae of *Chaetomidium arxii* and *Phillipsia domingensis* (Ascomycotina). Mycologia 72:836–840

Benny GL, Samuelson DA, Kimbrough JW (1978) Ultrastructural studies on *Orbilia luteorubella* (Discomycetes). Can J Bot 56:2006–2012

Berthet P (1964) Essai Biotaxonomique sur les Discomycètes. Joanny Lorge, Lyon

Bezerra JL, Kimbrough JW (1976) Structure and development of *Cleistoiodonphanus conglutinatus* gen. & sp. n. (Ascobolaceae). Am J Bot 63:838–844

Bonito G, Trappe JM, Rawlinson P, Vilgalys R (2010) Improved resolution of major clades within *Tuber* and taxonomy of species within the *Tuber gibbosum* complex. Mycologia 102:1042–1057

Bonito G, Smith ME, Nowak M, Healy RA, Guevara G, Czares E, Kinoshita A, Nouhra ER, Dominguez LS, Tedersoo L, Murat C, Wang Y, Arroyo Moren B, Pfister DH, Nara K, Zambonelli A, Trappe JM, Vilgalys R (2013) Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified Southern Hemisphere sister lineage. PLoS One 8:1–15

Boudier JLÉ (1885) Nouvelle classification naturelle des Discomycètes charnus. Bull Soc Mycol Fr 1:97–120

Boudier JLÉ (1907) Histoire et classification des discomycètes d'Europe. Klincksieck, Paris

Buscot F (1994) Ectomycorrhizal types and endobacteria associated with ectomycorrhizas of *Morchella elata* (Fr.)Boudier with *Picea abies* (L.)Karst. Mycorrhiza 4:223–232

Cannon PF, Kirk PM (2007) Fungal families of the world. CAB International, Wallingford, pp i–xiii, 1–456

Chen J, Xu L-L, Liu B, Liu X-Z (2007a) Taxonomy of *Dactylella* complex and *Vermispora*. I. Generic concepts based on morphology and ITS sequences data. Fungal Divers 26:73–83

Chen J, Xu L-L, Liu B, Liu X-Z (2007b) Taxonomy of *Dactylella* complex and *Vermispora*. II. The genus *Dactylella*. Fungal Divers 26:85–126

Chen J, Xu L-L, Liu B, Liu X-Z (2007c) Taxonomy of *Dactylella* complex and Vermispora. III. A new genus *Brachyphoris* and revision of *Vermispora*. Fungal Divers 26:127–142

Curry KJ, Kimbrough JW (1983) Septal structures in apothecial tissues of the Pezizaceae (Pezizales, ascomycetes). Mycologia 75:781–794

Dahlstrom JL, Smith JE, Weber NS (2000) Mycorrhiza-like interaction by *Morchella* with species of the Pinaceae in pure culture synthesis. Mycorrhiza 9:279–285

Davidson RW (1950) *Urnula craterium* is possibly the perfect state of *Strumella coryneoidea*. Mycologia 42:735–742

de Hoog GS (2000) Atlas of clinical fungi, 2nd edn. Centraalbureau voor Schimmelcultures, Utrecht

Descals E, Marvanov L, Webster J (1998) New taxa and combinations of aquatic hyphomycetes. Can J Bot 76:1647–1659

Dissing H (1966) The genus *Helvella* in Europe with special emphasis on the species found in Norden. Dansk Bot Ark 25:1–172

Döbbler P (1979) Untersuchangen on mooseparasitishen Pezizales aus der Verwandtschaft von *Octosopora*. Nova Hedwigia 31:817–864

Doveri F (2004) Fungi fimicoli italici: a guide to the recognition of basidiomycetes and ascomycetes living on faecal material. Associazione micologica Bresadola, Trento

Drechsler C (1936) A *Fusarium*-like species of *Dactylella* capturing and consuming testaceous Rhizopods. J Wash Acad Sci 26:397–404

Drechsler C (1937a) Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552

Drechsler C (1937b) A species of *Tridentaria* preying on *Difflugia constricta*. J Wash Acad Sci 27:391–398

Drechsler C (1938) Two hyphomycetes parasitic on oospores of root-rotting Oomycetes. Phytopathology 28:81–103

Du X-H, Zhao Q, O'Donnell KO, Rooney AP, Yang ZL (2012) Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. Fungal Genet Biol 49:455–469

Ecblad F-E (1968) The genera of operculate discomycetes: a re-evaluation of their taxonomy, phylogeny and nomenclature. Nytt Mag Bot 15:1–191

Gernandt DS, Platt JL, Stone JK, Spatafora JW, Holst-Jensen A, Hanlin RC, Kohn LM (2001) Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. Mycologia 93:915–933

Gibson JL, Kimbrough JW, Benny GL (1986) Ultrastructural observations on Endogonaceae (Zygomycetes). II. Glaziellales ord. nov. and Glaziellaceae fam. nov.: new taxa based upon light and electron microscopic observations of *Glaziella aurantiaca*. Mycologia 78:941–954

Ginns JH (1968) *Rhizina undulata* pathogenic on Douglas-fir seedlings in Western North America. Plant Dis Rep 52:579–589

Gönczöl J, Révay Á (2003) Treehole fungal communities: aquatic, aero-aquatic and dematiaceous hyphomycetes. Fungal Divers 12:19–34

Gönczöl J, Révay Á (2004) Fungal spores in rainwater: stemflow, throughfall and gutter conidial assemblages. Fungal Divers 16:67–86

Gönczöl J, Révay Á (2006) Species diversity of rainborne hyphomycete conidia from living trees. Fungal Divers 22:37–54

Guarro J, von Arx JA (1986) Monascella, a new genus of Ascomycota. Mycologia 78:869–871
Hagedorn G, Scholler M (1999) A reevaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data. Sydowia 51:27–39
Hansen K, Pfister DH (2006) Systematics of the Pezizomycetes—the operculate discomycetes. Mycologia 98:1029–1040
Hansen K, Læssoe T, Pfister DH (2001) Phylogenetics of Pezizaceae, with an emphasis on *Peziza*. Mycologia 93:958–990
Hansen K, Læssoe T, Pfister DH (2002) Phylogenetic diversity in the core group of *Peziza* inferred from ITS sequences and morphology. Mycol Res 106:879–902
Hansen K, Lobuglio KF, Pfister DH (2005a) Evolutionary relationships of the cup-fungus *Peziza* and Pezizaceae inferred from multiple nuclear genes: RPB2, beta-tubulin, and LSU rDNA. Mol Phylogenet Evol 36:1–23
Hansen K, Perry BA, Pfister DH (2005b) Phylogenetic origins of two cleistothecial fungi, *Orbicula parietina* and *Lasiobolidium orbiculoides*, within the operculate discomycetes. Mycologia 97:1023–1033
Hansen K, Weber NS, Landvik S (2008) Phylogenetic relationships and distribution of *Karstenella* (Pezizomycetes). Karstenia 48:13–19
Hansen K, Perry BA, Dranginis AW, Pfister DH (2013) A phylogeny of the highly diverse cup-fungus family Pyronemataceae (Pezizomycetes, Ascomycota) clarifies relationships and evolution of selected life history traits. Mol Phylogenet Evol 67:311–336
Harmaja H (1969) *Karstenella vernalis* Harmaja, a new genus and species of discomycetes from Finland. Karstenia 9:20–22
Harmaja H (2002a) Caloscyphaceae, a new family of the Pezizales. Karstenia 42:27–28
Harmaja H (2002b) *Alciphila vulgaria*. Karsentia 42:35
Harrington FA, Pfister DH, Potter D, Donoghue MJ (1999) Phylogenetic studies within the Pezizales. I. 18S rRNA sequence data and classification. Mycologia 91:41–50
Healy RA, Bonito G, Trappe JM (2009) *Calongea*, a new genus of truffles in the Pezizaceae. An Jard Bot Madr 33:25–32
Healy RA, Smith ME, Bonito GM, Pfister DH, Ge Z-W, Guevara GG, Williams G, Stafford K, Kumar L, Lee T, Hobart C, Trappe J, Vilgalys R, McLaughlin DJ (2013) High diversity and widespread occurrence of mitotic spore mats in ectomycorrhizal Pezizales. Mol Ecol 22:1717–1732
Hennebert GL (1973) *Botrytis* and *Botrytis*-like genera. Persoonia 7:183–204
Hennebert GL, Bellemère A (1979) Les formes conidiennes ds discomycètes. Rev Mycol 43:259–315
Hughes SJ (1958) Revisiones Hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can J Bot 36:727–836
Kimbrough JW (1991) Ultrastructural observations on Helvellaceae (Pezizales, ascomycetes). V. Septal structures in *Gyromitra*. Mycol Res 95:421–426
Kimbrough JW (1994) Septal ultrastructure and ascomycete systematics. In: Hawskworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York
Kimbrough JW, Curry KJ (1985) Septal ultrastructure in the Ascobolaceae (Pezizales, ascomycetes). Mycologia 77:219–229
Kimbrough JW, Curry KJ (1986a) Septal structures in apothecial tissues of the tribe Aleurieae in the Pyronmataceae (Pezizales, ascomycetes). Mycologia 78:407–417
Kimbrough JW, Curry KJ (1986b) Septal structures in apothecial tissues of the tribes Scutellineae and Sowerbyelleae (Pyronemataceae, Pezizales). Mycologia 78:734–743
Kimbrough JW, Gibson JL (1989) Ultrastructural observations on Helvellaceae (Pezizales: ascomycetes). III. Septal structures in Helvella. Mycologia 81:914–920
Kimbrough JW, Wu CG, Gibson JL (1991) Ultrastructural evidence for a linkage of the truffle genus *Hydnobolites* to the Pezizaceae (Pezizales). Bot Gaz 152:408–420
Kimbrough JW, Li L-T, Wu C-G (1996) Ultrastructural evidence for the placement of the truffle *Barssia* in the Helvellaceae (Pezizales). Mycologia 88:38–46
Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Ainsworth & Bisby's dictionary of the fungi, 10th edn. CABI, Europe
Kohlmeyer J, Baral H-O, Volkmann-Kohlmeyer B (1998) Fungi on *Juncus roemerianus*. 10. A new *Orbilia* with ingoldian anamorph. Mycologia 90:303–309
Korf RP (1972) Synoptic key to the genera of the Pezizales. Mycologia 64:937–994
Korf RP (1973) Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The fungi: an advanced treatise IV. Academic, New York, pp 249–319
Kumar TKA, Healy R, Satafora JW, Blackwell M, McLaughlin DJ (2012) *Orbilia* ultrastructure, character evolution and phylogeny of Pezizomycotina. Mycologia 104:462–476
Kuo M, Dewsburry DR, O'Donnell K, Carter MC, Rehner SA, Moore JD, Moncalvo J-M, Canfield SA, Stephenson SL, Methven AS, Volk TJ (2012) Taxonomic revision of true morels (Morchella) in Canada and the United States. Mycologia 104:1159–1177
Læssøe T, Hansen K (2007) Truffle trouble: what happened to the Tuberales. Mycol Res 111:1075–1099
Landvik S, Egger KN, Schumacher T (1997) Towards a subordinal classification of the Pezizales (Ascomycota): phylogenetic analysis of SSU rDNA sequences. Nord J Bot 17:403–418

Le Gal M (1946a) Mode de déhehiscence des asques chez les Cookeina et les Leotia, et ses conséquences du point de vue phylogénétique. Compt Rend Hebd Séances Acad Sci 222:755–757

Le Gal M (1946b) Les Discomycètes suboperculés. Bull Soc Mycol Fr 62:218–240

Le Gal M (1953) Les discomycètes de Madagascar. Prod Fl Mycol Madagascar 4:1–465

Lechtmann A, Clémençon H (2011) The taxonomic position of the genus *Heydnia* (Pyronemataceae, Pezizales) based on molecular and morphological data. Mycol Prog. doi:10.1007/s11557-011-0779-5

Li LT (1997) Ultrastructural studies of Leucangium carthusianum (hypogeous Pezizales). Int J Plant Sci 158:189–197

Li LT, Kimbrough JW (1995a) Septal structures in the Sarcoscyphaceae and Sarcosomataceae (Pezizales). Int J Plant Sci 156:841–848

Li LT, Kimbrough JW (1995b) Septal ultrastructure in three species of *Tuber* (Hypogeous Pezizales). Int J Plant Sci 156:849–856

Li Y, Hyde KD, Jeewon R, Cai L, Vijaykrishna D, Zhang KQ (2005) Phylogenetics and evolution of nematode-trapping fungi (Orbiliales) estimated from nuclear and protein coding genes. Mycologia 97:1034–1046

Liou GY, Tzean SS (1997) Phylogeny of the genus *Arthrobotrys* and allied nematode-trapping fungi based on rDNA sequences. Mycologia 89:876–884

Liu B, Liu XZ, Zhang WY (2005a) A new species of *Hyalorbilia* and its anamorph from China. Nova Hedwigia 81:145–155

Liu B, Liu XZ, Zhang WY (2005b) *Orbilia querci* sp. nov. and its knob-forming nematophagous anamorph. FEMS Microbiol Lett 245:99–105

Liu B, Liu XZ, Zhang WY, Baral H-O (2006) Orbiliaceous fungi from Tibet, China. Fungal Divers 22:107–120

Matsushima T (1995) Matsushima mycological memoirs, vol 8. Matsushima Fungus collection, Kobe

Merkus E (1976) Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)—IV. Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae. General discussion. Persoonia 9:1–38

Mo MH, Huang XW, Zhou W, Huang Y, Hao YE, Zhang KQ (2005) *Arthrobotrys yunnanensis* sp. nov., the fourth anamorph of *Orbilia auricolor*. Fungal Divers 18:107–115

Nagao H, Kurogi S, Kiyota E, Sasatomi K (2009) *Kumanasamuha geaster* sp. nov., an anamorph of *Chorioactis geaster* from Japan. Mycologia 101:871–877

Nannfeldt JA (1932) Studien über die Morphologie und Systematic der Nicht-Lichenisierten Inoperculaten Discomyceten. Societät der Wissenschaften, Uppsala, Königl

Norman JE, Egger KN (1999) Molecular phylogenetic analysis of *Peziza* and related genera. Mycologia 91:820–829

O'Donnell K, Cigelnik E, Weber NS, Trappe JM (1997) Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89:48–65

O'Donnell K, Rooney AP, Mills GL, Kuo M, Weber NS, Rehner SA (2011) Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early Cretaceous origin and high continental endemism and provincialism in the Holarctic. Fungal Genet Biol 48:252–265

Paden JW (1972) Imperfect states and the taxonomy of the Pezizales. Persoonia 6:405–414

Paden JW (1984) A new genus of Hyphomycetes with teleomorphs in the Sarcoscyphaceae (Pezizales, Sarcoscyphineae). Can J Bot 62:211–218

Paden JW, Sutherland JR, Woods TAD (1978) *Caloscypha fulgens* (Ascomycetidae, Pezizales): the perfect state of the conifer seed pathogen *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes). Can J Bot 56:2375–2379

Park J-O, Gams W, Scholler M, Ghisalberti EL, Sivasithamparam K (2002) Orbiliaceous nematode-trapping fungi and related species in Western Australia and their biological activities. Aust Mycol 21:45–52

Perry BA, Hansen K, Pfister DH (2007) A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol Res 111:549–571

Petersen PM (1985) The ecology of Danish soil inhabiting Pezizales with emphasis on edaphic conditions. Opera Bot 77:1–38

Pfister DH (1973a) The psilopezioid fungi. III. The genus *Psilopezia* (Pezizales). Am J Bot 60:355–365

Pfister DH (1973b) Notes on Caribbean Discomycetes. III. Ascospore germination and growth in culture of *Nanoscypha tetraspora* (Pezizales, Saarcoscyphineae). Mycologia 65:952–956

Pfister DH (1994) *Orbilia fimicola*, a nematophagoous discomycete and its arthrobotrys anamorph. Mycologia 86:451–453

Pfister DH (1997) Castor, Pollux and life histories of fungi. Mycologia 89:1–23

Pfister DH, Liftik ME (1995) Two *Arthrobotrys* anamorphs from *Orbilia auricolor*. Mycologia 87:684–688

Pfister DH, Slater C, Hansen K (2008) Chorioactidaceae: a new family in the Pezizales (Ascomycota) with four genera. Mycol Res 112:513–527

Pfister DH, Agnello C, Lantieri A, LoBuglio KF (2013) The Caloscyphaceae (Pezizomycetes, Ascomycota), with a new genus. Mycol Prog. doi:10.1007/s11557-012-0874-2

Qiao M, Zhang Y, Li S-F, Baral H-O, Weber E, Su H-y, Xu J-P, Zhang K-Q, Yu Z-F (2011) *Orblia blumenaviensis* and its *Arthrobotrys* anamorph. Mycol Prog. doi:10.1007/s11557-011-07443

Qin L, Qiao M, Yang Y, Yang G-Z, Lu K-P, Zhang K-Q, Xu J-P, Yu Z-F (2010) *Dactylella pseudobrevistipitata*, a new species from China. Ann Microbiol 61:591–595

Révay Á, Gönczöl J (2011) Canopy fungi ("terrestrial aquatic hyphomycetes") from twigs of living evergreen and deciduous trees in Hungary. Nova Hedwigia 92:303–316

Richardson MJ, Watling R (1997) Keys to fungi on dung. British Mycological Society, Stourbridge, West Midlands

Rifai MA (1968) The Australasian Pezizales in the herbarium of the Royal Botanic Gardens Kew. Verh Kon Ned Akad Wetensch, Afd Natuurk Tweede Sect 57:1–295

Romero AI, Robledo G, LoBuglio KF, Pfister DH (2012) *Rickiella edulis* and its phylogenetic relationships within Sarcoscyphaceae. Kurtziana 37:79–89

Rubner A (1994) Predacious fungi from Ecuador. Mycotaxon 51:143–151

Rubner A (1996) Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. Stud Mycol 39:1–134

Salt GA (1974) Etiology and morphology of *Geniculodendron pyriforme* gen. et sp. nov., a pathogen of conifer seeds. Trans Br Mycol Soc 63:339–351

Samuelson DA (1975) The apical apparatus of the suboperculate ascus. Can J Bot 53:2660–2679

Scholler M, Hagedorn G, Rubner A (1999) A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51:89–113

Seifert K (1985) A monograph of *Stilbella* and some allied Hyphomycetes. Stud Mycol 27:1–235

Smith ME, Trappe JM, Rizzo DM (2006) *Genea*, *Genabea* and *Gilkeya* gen. nov: ascomata and ectomycorrhiza formation in *Quercus* woodland. Mycologia 98:699–716

Spatafora JW, Sung G-H, Johnson D, Hesse C, O'Rourke B, Serdani R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser D, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch C (2006) A five-gene phylogeny of Pezizomycotina. Mycologia 98:1018–1028

Spooner BM (1987) Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. Bibl Mycol 116:1–711

Stchigel AM, Cano J, MacCormack W, Guarro J (2001) *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. Mycol Res 105:377–382

Su HY, Zhang Y, Baral H-O, Yang XY, Mo MH, Cao YH, Chen MH, Yu ZF (2011) Four new species of Orbiliaceae from Yunnan, China. Mycol Prog 10:373–381

Sun X, Guo L-D (2010) *Micronematobotrys*, a new genus and its phylogenetic placement based on rDNA sequence analyses. Mycol Prog 9:567–574

Svrcek M (1954) Revise Velenovské drusni Rodu Orbilia (Discomycetes). Revisio critica J Velenovskyi specierum generis Orbilia. Sborník Nrodního Musea v Praze B 10:3–23

Tanabe Y, Nagahamat SM, Sugiyama J (1999) Phylogenetic relationship of *Cephaliophora* to nematophagous hyphomycetes including taxonomic and nomenclatural emendation of the genus *Lecophagus*. Mycologia 91:830–835

Taskin H, Büyükalaca S, Dogan HH, Rehner SA, O'Donnell K (2010) A multigene molecular phylogenetic assessment of true morels (*Morchella*) in Turkey. Fungal Genet Biol 47:672–682

Tedersoo L, Hansen K, Perry BA, Kjøller R (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytol 170:581–596

Tedersoo L, May TM, Smith ME (2010) Ectomycorrhizal life style in fungi: global distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Trappe JM (1975) The orders, families, and genera of hypogeous Ascomycotina (truffles and their relatives). Mycotaxon 9:297–340

Trappe MJ, Trappe JM, Bonito GM (2010) *Kalapuya brunnea* gen. & sp. nov. and its relationship to other sequestrate genera in Morchellaceae. Mycologia 102:1058–1065

Uppalapati SR, Young CA, Marek SM, Mysore KS (2010) Phymatotrichum (cotton) root rot caused by *Phymatotrichopsis omnivora*. Mol Plant Pathol 11:325–334

Urban A, Neuner-Plattner I, Krisai-Greilhuber I, Haselwandter K (2004) Molecular studies on terricolous microfungi reveal novel anamorphs of two *Tuber* species. Mycol Res 108:749–758

van Brummelen J (1967) A world-monograph of the genus *Ascobolus* and *Saccobolus* (Ascomycetes, Pezizales). Persoonia Suppl 1:1–260

van Brummelen J (1978) The operculate ascus and allied forms. Persoonia 10:113–128

van Brummelen J (1981) The genus *Ascodesmis* (Pezizales, Ascomycetes). Persoonia 11:333–358

van Brummelen J (1989) Ultrastructure of the ascus and the ascospore wall in Eleutherascus and *Ascodesmis* (Ascomycotina). Persoonia 14:1–17

van Brummelen J (1994) Septal ultrastructure and ascomycete systematics. In: Hawksworth D (ed) Ascomycete systematics. Problems and perspectives in the nineties, vol 269, NATO ASI series. Plenum, New York, pp 127–141

Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, Hibbett DS (2006) Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): a nuclear rDNA phylogeny. Mol Phylogenet Evol 41:295–312

Warcup JH, Talbot PHB (1989) *Muciturbo*: a new genus of hypogeous ectormycorrhizal Ascomycetes. Mycol Res 92:95–100

Weber NS, Trappe JM, Denison WC (1997) Studies on Western American Pezizales. Collecting and describing ascomata-macroscopic features. Mycotaxon 61:153–176

Webster J (1992) Anamorph-teleomorph relationships. In: Barlocher F (ed) The ecology of aquatic hyphomycetes. Springer, Berlin, pp 99–117

Webster J, Descals E (1979) The teleomorphs of water-borne hyphomycetes from fresh water. In: Kendrick B (ed) The whole-fungus: the sexual-asexual synthesis. National Museum of Natural Sciences, National Museums of Canada and the Kananaskis Foundation, Ottawa, pp 419–451

Webster J, Henrici A, Spooner B (1998) *Orbilia fimicoloides* sp. nov., the teleomorph of *Dactylella* cf. *oxyspora*. Mycol Res 102:99–102

Wei J, Persoh D, Agerer R (2010) Four ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*): morpho-anatomical and molecular-phylogenetic analyses. Mycol Prog 9:267–280

Wu M-L, Su Y-C, Baral H-O, Liang S-H (2007) Two new species of *Hyalorbilia* from Taiwan. Fungal Divers 25:233–244

Yang CS, Korf RP (1985) A monograph of the genus *Tricharina* and of a new, segregate genus, *Wilcoxina* (Pezizales). Mycotaxon 24:467–531

Yang Y, Liu XZ (2005) *Dactylella coccinella* sp. nov., an anamorphic species. Mycotaxon 91:127–132

Yang Y, Yang E, An Z, Liu X (2007) Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. Proc Natl Acad Sci U S A 104:8379–8384

Yu ZF, Qiao M, Zhang Y, Baral HO, Zhang KQ (2007a) *Orbilia vermiformis* sp. nov. and its anamorph. Mycotaxon 99:271–278

Yu ZF, Zhang Y, Qiao M, Baral HO, Weber E, Zhang KQ (2007b) *Drechslerella brochopaga*, the anamorph of *Orbilia (Hyalinia) orientalis*. Mycotaxon 96:163–168

Yu ZF, Zhang Y, Qiao M, Zhang KQ (2007c) *Orbilia dorsalia* sp.nov., the teleomorph of *Dactylella dorsalia* sp. nov. Cryptog Mycol 28:55–63

Yu ZF, Qiao M, Zhang Y, Qin L, Zhang K-Q (2011) *Pseudotripoconidium*, a new anamorph genus connected to *Orbilia*. Mycologia 103:164–173

Zachariah K (1983) Ascocarp induction in a natural auxotroph of a predatory fungus. Can J Bot 61:3262–3266

Zhang Y, Yu Z-F, Baral H-O, Qiao M, Zhang K-Q (2007) *Pseudorbilia* gen. nov. (Orbiliaceae) from Yunnan, China. Fungal Divers 26:305–312

3 Pezizomycotina: Sordariomycetes and Leotiomycetes

NING ZHANG[1], ZHENG WANG[2]

CONTENTS

I. **Introduction** ... 57
II. **Sordariomycetes** ... 57
A. Ecology ... 59
B. Morphology ... 60
1. Ascomata ... 60
2. Centrum Development ... 60
3. Asci ... 61
4. Ascospores ... 61
5. Anamorphs ... 61
6. Ultrastructure ... 61
C. Molecular Phylogeny ... 61
D. Classification ... 62
1. Hypocreomycetidae ... 62
2. Sordariomycetidae ... 65
3. Xylariomycetidae ... 67
4. Orders Incertae Sedis ... 68
III. **Leotiomycetes** ... 69
A. Classification History ... 69
1. History of Leotiomycetes ... 69
2. Current Classification of Leotiomycetes ... 70
B. Molecular Phylogeny Update ... 72
1. Higher-Level Relationships of Leotiomycetes ... 72
2. Phylogeny Within Leotiomycetes ... 73
C. Characters ... 75
1. Apothecium (Ascoma) Morphology, Anatomy, and Ultrastructure ... 75
2. Ecology and Biogeography ... 76
IV. **Geoglossomycetes** ... 77
V. **Laboulbeniomycetes** ... 78
A. Laboulbeniales ... 78
B. Pyxidiophorales ... 78
VI. **Problems and Perspectives** ... 78
A. Genome Project ... 78
B. Environmental Study ... 79
VII. **Culture and Maintenance** ... 80
VIII. **Conclusion** ... 80
References ... 81

I. Introduction

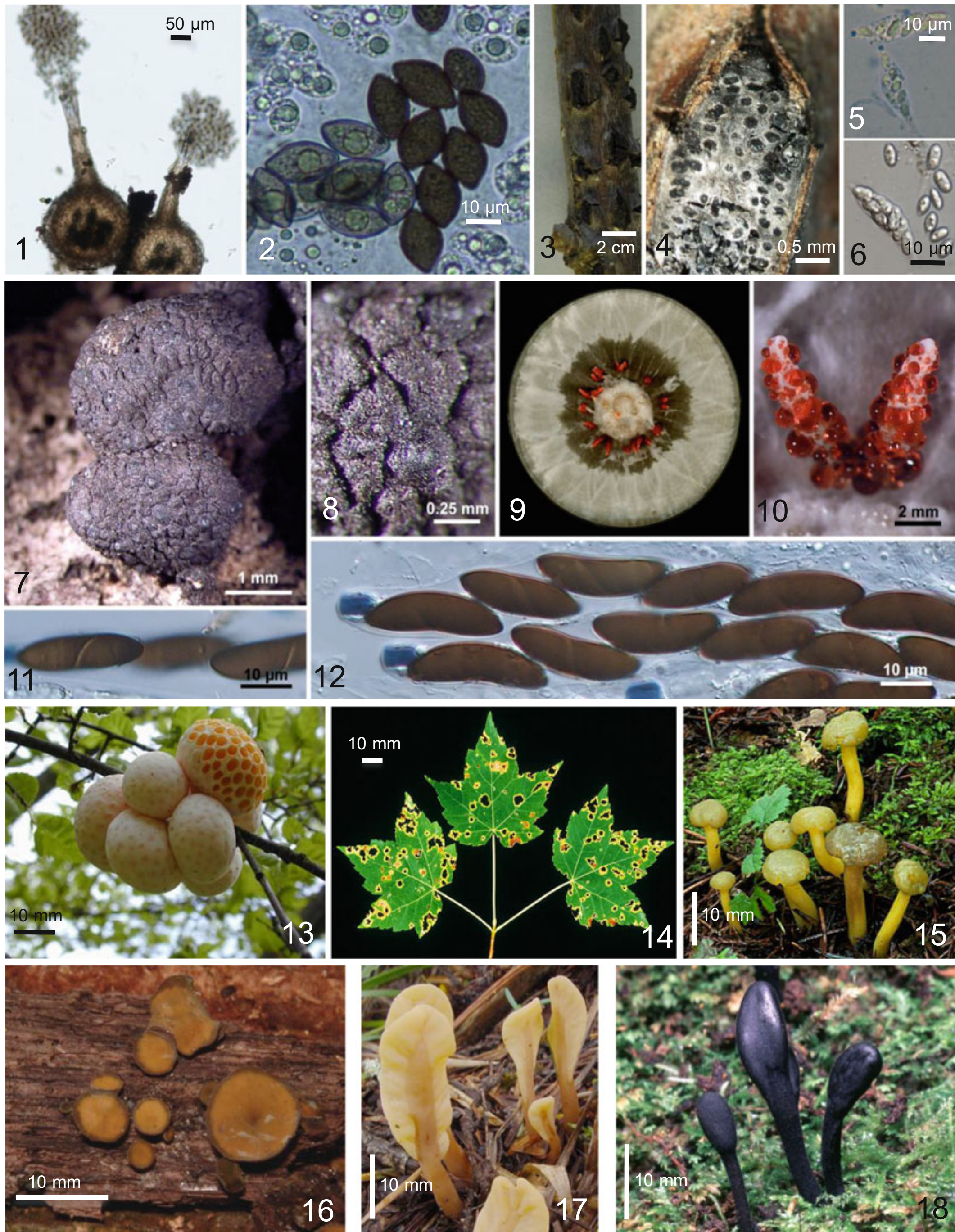
A close evolutionary relationship between the Sordariomycetes and Leotiomycetes was initially supported through phylogenetic analyses of DNA sequence data and subcellular characters. Using various data sets and tree reconstruction methods, several recent studies corroborated that the two classes are sister clades in Ascomycota (Celio et al. 2006; Fitzpatrick et al. 2006; Lumbsch et al. 2005; Robbertse et al. 2006; Schoch et al. 2009a; Spatafora et al. 2006; Wang et al. 2009; Zhang et al. 2006). A rankless taxon, "**Sordariomyceta,**" was proposed by Schoch et al. (2009a) for the clade that includes the classes **Sordariomycetes, Laboulbeniomycetes,** and **Leotiomycetes**. Although Sordariomycetes and Laboulbeniomycetes are characterized by the production of **perithecial ascoma** (Figs. 3.1–3.13) and Leotiomycetes by **apothecial ascoma** (Figs. 3.14–3.18), and members in these classes have diverse ecology and nutritional modes, they likely evolved from a common ancestor that was terrestrial, saprotrophic, and nonlichenized. A putative **synapomorphy** of "Sordariomyceta" is their **inoperculate, unitunicate, and poricidal asci.**

II. Sordariomycetes

Based on the 10th edition of the *Dictionary of the Fungi* (Kirk et al. 2008), Sordariomycetes

[1]Department of Plant Biology & Pathology, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901, USA; e-mail: zhang@aesop.rutgers.edu
[2]Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT 06520, USA; e-mail: wang.zheng@yale.edu

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015



Figs. 3.1–3.18 Morphology of species in Sordariomycetes (1–13) and Leotiomycetes (14–18). **Fig. 3.1** Perithecia of *Melanospora* sp. **Fig. 3.2** Ascospores of *Melanspora* sp. **Fig. 3.3** Stromata of *Anisogramma anomala* (Peck) E. Müll. on hazelnut stem. Courtesy of Guohong Cai. **Fig. 3.4** *Anisogramma anomala* perithecia in a stroma. Courtesy of Guohong Cai. **Figs. 3.5** and **3.6** Asci and ascospores of *Anisogramma anomala*. **Figs. 3.7–3.12** *Xylaria globosa* (Spreng. ex Fr.) Mont. Courtesy of Yu-Ming Ju. **Fig. 3.7** Stromata. **Fig. 3.8** Reticulately cracked stromatal surface showing ostioles. **Fig. 3.9** Colony on 9-cm petri dish containing Difco oatmeal agar. **Fig. 3.10** Stromata produced on OA with red exudates. **Fig. 3.11** Ascospores showing an

represents a large clade in Ascomycota, with 1,119 genera and over 10,000 known species. It contains most nonlichenized ascomycetes with perithecial (flask-shaped) or, less frequently, cleistothecial (nonostiolate) ascomata and inoperculate unitunicate or prototunicate asci (Alexopoulos et al. 1996).

The term "Pyrenomycetes" was formerly used to unite fungi with perithecial ascomata and unitunicate asci (Luttrell 1951), and its use was discontinued based on the placement of perithecial species outside of the clade and the inclusion of species with prototunicate asci (e.g., *Corollospora* and *Ophiostoma*) in order to avoid confusion (Eriksson and Winka 1997).

Sordariomycetes share some features with Dothideomycetes and Leotiomycetes but are unique in having a true perithecium and inoperculate, unitunicate asci. In the 10 years since the first edition of the Mycota VII, 115 articles with the keywords "Sordariomycet* OR pyrenomycet*" and "phylogen*" have been published, when a search in the Web of Science was conducted. A number of new taxa were discovered from nature and placed in the Sordariomycetes, from both terrestrial and aquatic habitats (Campbell et al. 2009; Raja and Shearer 2008; Samuels et al. 2009).

Since the early 1990s, molecular sequence data have been used to test fungal systematics and evolutionary hypotheses. Until recently, most molecular phylogenetic studies had comprised single-gene phylogenies, usually of rRNA genes. In general, **nuclear small subunit (SSU) rRNA genes were used to infer relationships at family and higher taxonomic levels, large subunit (LSU) rRNA genes at the genus and family levels, and the internal transcribed spacer (ITS) region at species or infrageneric levels.** In the past decade, through initiatives such as **Assembling the Fungal Tree of Life (AFTOL)**, **Deep Hypha**, and **Myconet**, mycologists and bioinformatists have pursued collaborative efforts to generate **a relatively stable fungal systematic framework using multilocus phylogenies and other traits**, such as subcellular and other morphological characters.

In the current classification sensu Lumbsch and Huhndorf (2010) (outline of Ascomycota, initiated by Eriksson and Winka in 1997, now hosted at the Field Museum: http://fieldmuseum.org/explore/myconet), Sordariomycetes comprises 18 orders with core taxa in 3 subclasses, i.e., Hypocreomycetidae, Sordariomycetidae, and Xylariomycetidae. The subclasses were first proposed by Eriksson and Winka (1997) based on morphological characters and SSU phylogenies. This classification is further supported by a number of multilocus phylogenies, such as the four-gene phylogeny by Zhang et al. (2006).

Classes and orders in Sordariomycetes have been classified based on ascoma shape and developmental types. However, convergent evolution often results in similar ascoma morphology and even ontogenesis, as shown in recent reviews by Liu and Hall (2004) and Lumbsch and Huhndorf (2007a).

A. Ecology

Members of Sordariomycetes are ubiquitous and cosmopolitan. They function in virtually all ecosystems as saprotrophs involved in decomposition and nutrient cycling, as endophytes and pathogens of plants, arthropods, and mammals, and even as mycoparasites attacking other fungi. Most plant pathogens in Sordariomycetes are distributed in Diaporthales, Glomerellales, Hypocreales, Microascales, Ophiostomatales, Phyllachorales, and Xylariales. These include the best-known **plant pathogens**, for example, *Cryphonectria parasitica* (the causal agent of **chestnut blight**), *Magnaporthe oryzae* (the cause of **rice blast**), *Ophiostoma ulmi* and *O. novo-ulmi* (the **Dutch elm disease** causal agents), *Fusarium* species, and *Rosellinia* species (Alexopoulos et al. 1996; Samuels and Blackwell 2001).

Figs. 3.1–3.18 (Continued) oblique germ slit. **Fig. 3.12** Asci with an apical apparatus staining blue in an iodine solution and ascospores. **Fig. 3.13** *Cyttaria espinosae* Lloyd. (Courtesy of G. Palfner) **Fig. 3.14** *Rhytisma americanum* Hudler & Banik. (Courtesy of G. W. Hudler) **Fig. 3.15** *Leotia lubrica* Fr. (Courtesy of M. Wood) **Fig. 3.16** *Chlorencoelia versiformis* (Pers.) J. R. Dixon. (Courtesy of E. Bosman) **Fig. 3.17** *Spathularia flavida* Pers. **Fig. 3.18** *Trichoglossum hirsutum* (Fr.) Boudier

In addition to pathogens of plants, Sordariomycetes includes **endophytes** that live inside apparently healthy plants (Alexopoulos et al. 1996; Carroll 1988). Numerous endophytic fungal survey studies have demonstrated that Sordariomycetes is one of the most frequently isolated fungal classes in land plant foliage (Arnold and Lutzoni 2007; Carroll 1988). The best-studied endophytes belong to Hypocreales (e.g., *Balansia* and *Epichloë*), Xylariales (e.g., *Nemania* and *Xylaria*), and Glomerellales (e.g., *Colletotrichum*). Their infected host plants often benefit from increased drought resistance, reduced feeding by insects, and limited pathogen infections (Alexopoulos et al. 1996).

Some members of Hypocreales, Ophiostomatales, and Microascales are associated with **opportunistic infections of humans and other animals** (e.g., *Sporothrix schenkii, Fusarium solani* species complex, and *Trichoderma* spp.) (Gugnani et al. 1976; O'Donnell et al. 2010; Summerbell 2003). As symbionts of arthropods, Sordariomycetes comprise a diverse assemblage of species that range from antagonistic to mutualistic (Samuels and Blackwell 2001).

Members of Microascales and Ophiostomatales (e.g., *Ceratocystis*, *Ophiostoma*, and *Ambrosiella*) are associated with bark beetles in fungal spore dispersal. Species of Hypocreales (e.g., *Cordyceps* and *Torrubiella*) directly parasitize a broad range of arthropods. The Hypocreales is also known to be rich in **mycoparasites.** Most species of *Hypomyces* are parasitic on fleshy fungal fruit bodies such as mushrooms and large apothecia (Rogerson and Samuels 1989). Some species of *Trichoderma*, used alone or combined with other **biocontrol** agents, have been applied in agriculture for plant disease management as an alternative to pesticides (Harman et al. 2004).

Saprobic Sordariomycetes function in the decomposition and nutrient cycling of plant litter, including wood, herbaceous stems, and dung. Important taxa include *Neurospora crassa*, the model organism widely used in molecular and genetic studies (Alexopoulos et al. 1996), and *Chaetomium*, an important cellulolytic organism responsible for the destruction of paper and fabrics. Sordariomycetes also contains species known as producers of some of the most important fungal **secondary metabolites**. These include the trichothecene **mycotoxins** produced by many members of Nectriaceae and the *Stachybotrys* clade and the ergot and other alkaloids produced by *Claviceps* and *Epichloë*. Marine fungi are also a good source of novel bioactive compounds (Bugni and Ireland 2004).

B. Morphology

1. Ascomata

The majority of Sordariomycetes produce perithecial ascomata (Fig. 3.1). **The shape, size, pigmentation, texture, and position of ascomata are examined in traditional taxonomy.** For example, the position of ascomata in relation to substrates was used in the family delimitation of Diaporthales by Barr (1978), but this classification was not supported by phylogenetic analyses using molecular characters (Castlebury et al. 2002; Zhang and Blackwell 2001). A phylogenetic study by Miller and Huhndorf (2005) suggested that ascomal wall morphology is a better character than ascospore morphology in defining genera in Sordariales. Leal et al. (2010) assessed fungal wall heteromannans as a phylogenetically informative character in ascomycetes, including Sordariomycetes.

2. Centrum Development

Nannfeldt (1932) and Luttrell (1951) first applied **ontogenetic characters** in filamentous ascomycete classification. Although ontogenetic characters do not always correspond well with molecular phylogenies, they are still informative characters in ordinal level classification within Sordariomycetes (Hibbett et al. 2007; Samuels and Blackwell 2001). For a detailed description and illustration of perithecium ontogeny and centrum types in Sordariomycetes, see Samuels and Blackwell (2001). For a review and recent progress on ontogenic studies of Sordariomycetes, see Lord and Read (2011). In Samuels and Blackwell (2001), nine **centrum types** were outlined and illustrated: ***Melanospora* type, *Nectria* type, *Ophiostoma* type, *Xylaria* type, *Epichloë* type, *Sordaria* type, *Eutypa* type, *Meliola* type, and *Phaeotrichum* type.**

3. Asci

Asci usually arise from ascogenous hyphae through the mediation of a **crozier**, a structure that functions to ensure that only two compatible nuclei are sequestered in a developing ascus. The arrangement of asci in Sordariomycetes is basal or peripheral in a hymenium (Fig. 3.1). Asci of Sordariomycetes either are capable of forceful ejection and wind dissemination of ascospores or lack any apical discharge mechanism. A sphincterlike ring at the ascal apex is likely to effect **forceful ejection** (Read and Beckett 1996; Samuels and Blackwell 2001). The presence or absence of an **apical ring** (amyloid or chitinoid) is an important feature in the classification of Sordariomycetes (Figs. 3.5 and 3.12). Typically, the asci of Sordariomycetes are **octosporous** (Figs. 3.2, 3.5, and 3.6).

4. Ascospores

Traditionally, mycologists have put considerable weight on ascospore morphology (e.g., **ascospore shape, size, pigmentation, wall ornamentation**) in delimiting ascomycete genera. Sordariomycetes is no exception; however, many molecular phylogenetic studies suggest that these generic deliminations do not always accurately reflect evolutionary history.

For instance, in Melanosporales, genera delimited based on ascospore shape and ornamentation are not monophyletic, likely owing to convergent evolution (Zhang and Blackwell 2002). In Sordariales, ascomal wall morphology was found to be a better character than ascospore morphology in defining genera (Miller and Huhndorf 2005). In Hypocreales, asexual states seem to be more informative in defining monophyletic taxa because the sexual stages tend to be more conserved (Chaverri et al. 2003).

5. Anamorphs

Sordariomycetes is an anamorph-rich class, with significant diversity represented by **hyphomycete** and **coelomycete** species. Many species of Hypocreales, Ophiostomatales, and Chaetosphaeriales have two or more distinguishable anamorphs (synanamorphs). Hyphomycetes occur throughout the class, but coelomycete anamorphs also occur, most notably in the Glomerellaceae and Diaporthales. In common with teleomorph characters, many characters used to delimit anamorph genera (e.g., variations in conidiomata, pigmentation, conidiophore branching, and conidial septation) are homoplastic in Sordariomycetes. Despite this, recognizable patterns of anamorph morphological characters often allow recognition of phylogenetic groups (Seifert and Gams 2001).

6. Ultrastructure

Subcellular or ultrastructural and biochemical characters also play a major role in understanding fungal evolution, but reports on these characters are scattered in the literature. As part of the AFTOL project, a searchable structural and biochemical database for selected fungal taxa is now available at http://aftol.umn.edu. This database includes descriptions and illustrations of characters such as **nuclear division, septum/pore cap, sterol data, hyphal tip organization, and meisporangium.**

According to Celio et al. (2006), **Sordariomycetes was strongly supported by the ascogenous hypha/ascus pore occlusions in immature and mature hyphae.** Unique characters of the Sordariomycetes include the following: (1) for immature ascogenous hypha/ascus: endoplasmic reticulum associated with toroid occlusion (donutlike with central pore), torus with radiating tubular cisternae; (2) for mature ascoenous hypha/ascus: subspherical pore cap membrane, simple membrane enclosing cytoplasm. Leotiomycetes and Sordariomycetes appear to have the same spindle pole body form and nuclear envelope organization during nuclear division, i.e., unlayered disc with intact nuclear envelope and internal microtubule organizing center.

C. Molecular Phylogeny

Recent comprehensive taxonomic and phylogenetic studies of families and higher taxa of Sordariomycetes were published by Samuels and Blackwell (2001), Eriksson (2006), and Zhang et al. (2006); they were based on both morphological characters and molecular sequence data and reviewed in Hibbett et al.

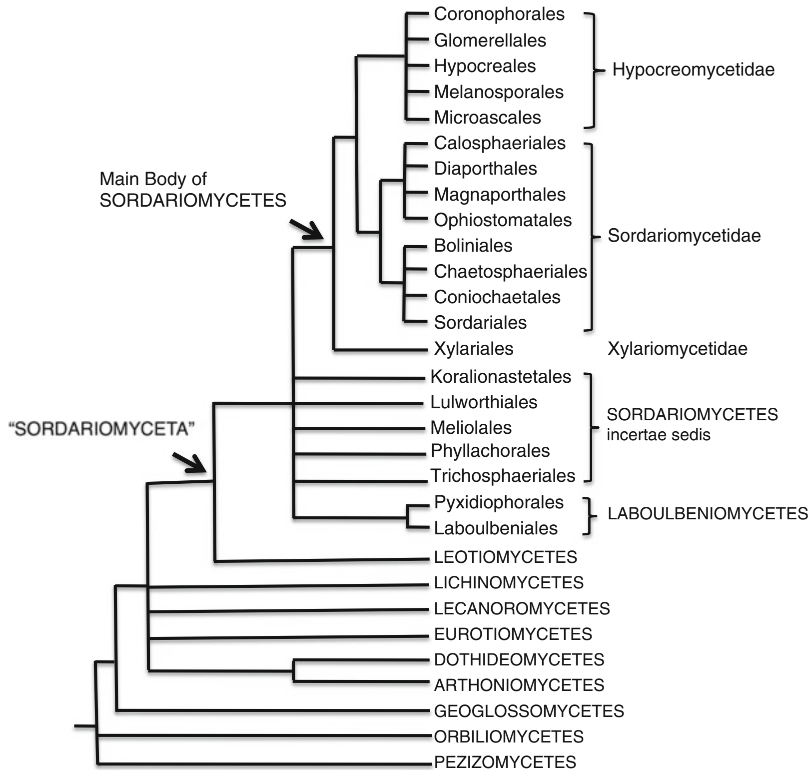


Fig. 3.19 Phylogeny and classification of Sordariomycetes. Branch lengths are not proportional to genetic distances

(2007) and Lumbsch and Huhndorf (2010). We provide here a compiled classification to reflect the well-supported phylogenetic relationship of Sordariomycetes and allied taxa (Fig. 3.19).

There are three major clades in Sordariomycetes, corresponding to the three subclasses of Eriksson (2005) (Fig. 20). **Xylariomycetidae** (one order) is placed as the earliest diverging group of Sordariomycetes, followed by the monophyletic clade comprising **Hypocreomycetidae** (five orders) and **Sordariomycetidae** (eight orders), which are supported by several multilocus phylogenetic studies (Schoch et al. 2009a; Tang et al. 2007; Zhang et al. 2006). These three well-delimited subclasses constitute the main body of Sordariomycetes. The position of five sordariomycetous orders, including Lulworthiales, Koralionastetales, Meliolales, Phyllachorales, and Trichosphaeriales, is uncertain. Erysiphales and Coryneliales sensu Barr (1990) are excluded from the Sordariomycetes (Samuels and Blackwell 2001). Erysiphales is now placed in Leotiomycetes, which was supported by rRNA gene sequences (Wang et al. 2006b), and Coryneliales is confirmed as a member of Eurotiomycetes (Geiser et al. 2006; Schoch et al. 2009a). The extralimital pyrenomycete Laboulbeniales sensu Samuels and Blackwell (2001) is now recognized as a separate class close to Sordariomycetes (Lumbsch and Huhndorf 2010; Schoch et al. 2009a; Weir and Blackwell 2001).

D. Classification

1. Hypocreomycetidae

Hypocreomycetidae is recognized as a monophyletic subclass based on recent molecular phylogenetic studies (Lumbsch and Huhndorf 2010; Réblov et al. 2011; Schoch et al. 2009a; Tang et al. 2007; Zhang et al. 2006). It includes five orders: **Coronophorales, Glomerellales,**

Hypocreales, Melanosporales, and Microascales (Fig. 3.19). Members of Hypocreomycetidae typically possess **light-colored perithecia,** but with many exceptions. Asci are amyloid or chitinoid or lack apical rings, and true paraphyses are lacking in most members. *Melanospora* and allied species were excluded from Hypocreales and recognized as a distinct order by Zhang and Blackwell (Hibbett et al. 2007). Réblov et al. (2011) validated and delimited the order Glomerellales based on morphology and a three-gene phylogeny.

a) Coronophorales

Coronophorales is composed of primarily wood-inhabiting taxa and defined by species with erumpent to superficial ascomata that often collapse upon drying and that sometimes possess an extensive hyphal subiculum or basal stroma. Many taxa contain **Munk pores** (small pores each of which is surrounded by a ring or thickening) in their ascomatal cell walls and a **Quellkörper** (a gelatinous mass of cells in the apical region of an ascoma, believed to function in rupturing the ascoma) in their centrum (Huhndorf et al. 2004a). While Munk pores are found in a few taxa outside the order, the Quellkörper is a character unique to Coronophorales. Filiform paraphyses are absent in the group, and in most species the asci are thin-walled, clavate, and stipitate and lack an apical ring. Ascospores usually are hyaline, small, and allantoid. Anamorphs are hyphomycetous when known.

b) Glomerellales

Glomerellales is a monotypic order characterized by dark perithecia, well-developed periphysate ostioles, abundant thin-walled paraphyses, ascal apex thickened without visible discharge mechanism or with a distinct apical annulus, and hyaline or pigmented ascospores. Known anamorphs include *Colletotrichum*, *Monilochaetes*, *Cylindrotrichum*, and *Sporoschismopsis*. *Glomerella* had been placed in Phyllachorales, but some of its features are clearly distinct from those of other Phyllachorales, such as its lack of stromatic tissue and its exclusively *Colletotrichum* anamorphs. Molecular phylogenetic analyses confirmed these distinctions, and Wanderlei-Silva et al. (2003) recognized *Glomerella* as a family in Hypocreomycetidae. Further investigation is required to test whether *Verticillium dahliae*, a sister taxon to *G. cingulata* according to Zhang et al. (2006), is actually a member of Glomerellales.

The family Glomerellaceae was invalidly published by Locquin (1984) and was validated by Seifert and Gams (Zhang et al. 2006). Réblov et al. (2011) validated and delimited order Glomerellales.

c) Hypocreales

Hypocreales is a monophyletic order that includes virulent plant and insect pathogens as well as useful mycoparasitic, endophytic, and saprobic species (Rossman et al. 1999). This order is defined by species with soft-textured, generally light- to bright-colored perithecial fruiting bodies, and unitunicate asci that develop within apical (and centripetal in some species) paraphyses that often dissolve at maturity. The colorless ascospores vary from nonseptate, globose to one- to multiseptate, ellipsoidal to filiform. **Hypocreales includes over 200 genera in seven families: Bionectriaceae, Clavicipitaceae, Cordycipitaceae, Hypocreaceae, Nectriaceae, Niessliaceae, and Ophiocordycipitaceae** [see details in Kirk et al. (2008), Lumbsch and Huhndorf (2010), and Rossman et al. (1999)].

Bionectriaceae and Nectriaceae consist primarily of species previously placed in the very large and artificial genus *Nectria*, which is characterized by its nonstromatic perithecia. The genus *Nectria* (anamorph: *Tubercularia*) is now restricted to a related group of 23 species. **Nectriaceae** (e.g., *Haematonectria*, *Nectria*, and *Pseudonectria*) is defined by the orange to red, KOH+ perithecia. Bionectriaceae (e.g., *Bionectria*, *Hydropisphaera*, and *Roumegueriella*) consists primarily of pallid, *Nectria*-like species such as *Bionectria* (anamorph: *Clonostachys*), some of which are used to control greenhouse pathogens. **Hypocreaceae** includes *Hypocrea* (anamorph: *Trichoderma*), increasingly useful in the **biological control** of plant pathogens (Samuels 2006), and *Hypomyces* with diverse anamorphic states. **Clavicipitaceae** produces a

wide range of secondary metabolites and plant-associated (e.g., *Claviceps*, *Epichloe*) and insect-associated genera including *Metacordyceps* and *Hypocrella*. Cordycipitaceae and **Ophiocordycipitaceae** comprise the majority of arthropod pathogenic species of Hypocreales (e.g., *Cordyceps* and *Ophiocordyceps*) as well as several lineages of mycoparasites (e.g., *Elaphocordyceps* of Ophiocordycipitaceae). The anamorphs of Hypocreales are generally phialidic, producing powdery to slimy conidia; chlamydospore-like anamorphs are also produced by many species. The anamorphs include the well-known phialidic genera *Gliocladium*, *Trichoderma* (Hypocreaceae), *Clonostachys*, *Stilbella* (Bionectriaceae), *Cylindrocarpon*, *Cylindrocladium*, *Fusarium*, *Tubercularia* (Nectriaceae) (Rossman et al. 1999) and *Beauveria*, *Hirsutella*, *Metarhizium*, and *Tolypocladium* (**Cordycipitaceae**, **Clavicipitaceae**, **Ophiocordycipitaceae**) (Hodge 2003; Sung et al. 2007). The "**toxic mold**" fungus, *Stachybotrys chartarum*, represents part of a lineage separate from any of the defined families within Hypocreales (Castlebury et al. 2004).

d) Melanosporales

The typical characteristics of Melanosporales include **translucent** perithecial or cleistothecial ascomata, pseudoparenchymatous centra, **absence of paraphyses** in development, clavate evanescent asci, and **dark-colored ascospores**. Previous phylogenetic studies based on rDNA sequences suggested that *Melanospora* was within or near Hypocreales (Spatafora and Blackwell 1994; Zhang and Blackwell 2002). When analyzed with more taxa of the Sordariomycetes, *Melanospora* formed a distinct clade outside Hypocreales (Castlebury et al. 2004). A four-gene phylogeny (Zhang et al. 2006) supported the exclusion of *Melanospora* from the Hypocreales. Furthermore, a close relationship between Coronophorales and Melanosporales was recognized. Similar morphological and ecological features of the two orders include a pseudoparenchymatous ascomal wall, clavate, deliquescent asci, lack of paraphyses (with a few exceptions in Coronophorales), and often a **mycoparasitic** habit.

e) Microascales

Microscales is an order of primarily saprobic fungi in soil, rotting vegetation, dung, and marine environments. A few species of this order cause plant diseases such as *Ceratocystis fimbriata*, transmitted by beetles to living trees and causing cacao wilt and many other economically important diseases. Other members, such as species of *Pseudallescheria*, cause incurable diseases in humans. The order is characterized by nonstromatic black **perithecial ascomata with very long necks** or, rarely, with cleistothecial ascomata that lack paraphyses, and by globose and evanescent asci, developing singly or in chains. The nonseptate, colorless ascospores often bear ornamenting ridges or wings. The anamorphs of **Microascaceae** produce percurrently proliferating conidiogenous cells (annellides) and sometimes chlamydosporelike or aleurioconidial synanamorphs that are mostly classified in the hyphomycete genera *Scopulariopsis*, *Graphium*, and *Scedosporium* (Abbott et al. 1998; Okada et al. 1998).

Halosphaeriaceae, a marine clade within Microascales, is characterized by usually submerged perithecial ascomata; an interascal tissue is absent, but the **centrum is filled by a thin-walled pseudoparenchyma** that dissolves or breaks up to form filamentous catenophyses. A small number of species in this family are found in freshwater, but the majority consists of marine species. The ascus wall in most species deliquesces, releasing the ascospores, which are forced into the neck of the ascocarp by the production of additional asci and ascospores. Several anamorphs have been attributed to Halosphaeriaceae, including chlamydosporelike anamorphs and several genera of so-called Ingoldian hyphomycetes. The hyphomycetous anamorph *Varicosporina ramulosa* produces conidia and sclerocarps, sclerotium-like fruiting bodies that lack sexual capacity. It was postulated that *V. ramulosa* was an ascomycete that had lost its ability to reproduce sexually (Kohlmeyer and Charles 1981). Sclerocarps are morphologically similar to ascomata of *Corollospora* species and well adapted to **extreme conditions of sandy bea-**

ches, where they may be exposed to long periods of drying and extreme high or low temperatures.

2. Sordariomycetidae

The members of Sordariomycetidae possess light- to dark-colored perithecia. Asci are non-amyloid, amyloid, or lack apical rings, and true paraphyses are present in some members. The subclass consists of eight orders divided into two clades. **Calosphaeriales, Diaporthales, Magnaporthales, and Ophiostomatales belong to one clade, and Boliniales, Chaetosphaeriales, Coniochaetales, and Sordariales belong to the other clade.** Representative taxa of the Annulatascaceae and Papulosaceae were found to be close to the Ophiostomatales, according to recent multilocus phylogenetic analyses (Schoch et al. 2009a; Zhang et al. 2006).

a) Calosphaeriales
Species in Calosphaeriales are saprobes associated with woody plants. They produce perithecial ascomata, which are often gregarious, with separate or convergent periphysate ostioles. The peridium is two-layered, externally dark, and internally pallid. Asci are formed in fascicles or spicate clusters, usually with a refractive nonamyloid apical ring. Paraphyses are lacking or few when present. Ascospores are light colored, allantoid or ellipsoid, and biseriate or crowded in the ascus. Anamorphs are hyphomycetous where known. The six-gene Ascomycota phylogeny by Schoch et al. (2009a) grouped Calosphaeriales with Diaporthales, Magnaporthales, and Ophiostomatales, which is followed here.

b) Diaporthales
Diaporthales is a strongly supported monophyletic order and includes **primarily plant-associated** fungi, such as the agent of **chestnut blight** (*Cryphonectria parasitica*) and **dogwood anthracnose** (*Discula destructiva*). The order is characterized by black perithecial fruiting bodies that may or may not be aggregated and immersed in a stroma, evanescent interthecial elements, and asci that float free at maturity and often have conspicuous refractive apical rings. The ascospores vary from colorless, allantoid (*Valsa*), one-septate, ellipsoidal (*Diaporthe*), or elongated (*Cryptosporella*) to large, multiseptate, and black (*Melanconis*). Commonly encountered anamorphs are coelomycetes, such as *Cytospora* (*Valsa*), *Phomopsis* (*Diaporthe*), and *Melanconium* (*Melanconis*), producing pycnidia with phialidic conidiogenous cells, often on the same stroma as the sexual state.

c) Magnaporthales
Magnaporthales is characterized by nonstromatic black perithecia, usually with long hairy necks, tapering paraphyses, persistent asci, and elongate fusiform or filiform ascospores. The anamorphs are hyphomycetous and variable but can be categorized as two types: *Pyricularia*-like or *Phialophora*-like. This order contains devastating fungal cereal and grass pathogens, such as *Magnaporthe oryzae* (**rice blast fungus**, formerly known as *M. grisea*), *M. poae* (summer patch pathogen of turf grasses), and *Gaeumannomyces graminis* (**take-all fungus** of various cereals and grasses). Historically, these species were placed in various orders in Ascomycota because of a lack of convincing morphological and developmental characters, such as Diaporthales (Krause and Webster 1972), Phyllachorales (Barr 1977), and Xylariales (Barr 1977). A new single-family order, Magnaporthales, was recently established for these fungi (Thongkantha et al. 2009). Molecular phylogenetic studies indicated that this group of fungi belongs to the subclass Sordariomycetidae (Huhndorf et al. 2008; Thongkantha et al. 2009; Zhang and Blackwell 2001; Zhang et al. 2006). A recent study by Zhang et al. (2011) indicated that **anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among taxa in Magnaporthales.**

d) Ophiostomatales
Ophiostomatales includes fungi associated with wood and bark such as the agents of **Dutch elm disease** (*Ophiostoma ulmi* and *O. novo-ulmi*) and blue stain of hardwood and softwood timber (*O. piliferum*). Most species of the order, however, are saprobic and usually are asso-

ciated with beetle dispersers, while related anamorphs, i.e., *Sporothrix schenckii*, are reported to cause diseases in humans (Summerbell 2003). The order is characterized by solitary, black perithecia, and most species have long necks from which sticky ascospores ooze and are transported by insects to new substrates. The asci are globose and dissolve early in development, while the ascospores often have ornamenting sheaths, ridges, or wings. Many of the hyphomycete genera once associated with Ophiostomatales have been simplified by the recognition that the apparent variety in conidium ontogeny represents a minor variation of a common pattern. In genera such as *Leptographium* and *Pesotum*, there is percurrent proliferation of the conidiogenous cells and delayed secession of conidia, giving a false impression of sympodial proliferation. In addition, some species have exclusively sympodially proliferating, denticulate conidiogenous cells that are classified in *Sporothrix* (Wingfield et al. 1993). *Ophiostoma* was once considered synonymous with *Ceratocystis* in Microascales, and their long-necked, fleshy perithecia with sticky ascospores associated with insects in wood represent a morphological homoplasy (Cain and Weresub 1957; Samuels and Müller 1978; Spatafora and Blackwell 1994).

e) Boliniales
Members of Boliniales occur primarily on wood. It is the only order in the Sordariomycetidae that contains taxa with large, stromatic, carbonaceous ascomata. Other taxa in the group may possess soft-textured stromata that may be brightly colored. Perithecia may be polystichous or monostichous, wherein they often will be distinctively vertically elongate. Members of this order possess small asci and brown, ellipsoidal, frequently flattened ascospores that often have polar germ pores. No anamorphs are known in this order. Boliniales in the four-gene phylogeny is represented by four species of *Camarops* encompassing disparate morphologies and is more strongly allied with *Lasiosphaeriella nitida* and *Linocarpon appendiculatum* (taxa that currently are placed in Sordariomycetidae inc. sed.).

f) Chaetosphaeriales
Chaetosphaeriales includes saprobic, often wood-inhabiting fungi. Small, dark perithecial ascomata that are often setose are found in a number of genera in the group. Ascomata are typically associated with dematiaceous, hyphomycetous anamorphs. Members of the Chaetosphaeriales have filiform paraphyses and cylindrical asci, usually with a pronounced apical refractive ring. Ascospores are often hyaline and can range from ellipsoidal, nonseptate to elongate, almost filiform, and septate in species of *Chaetosphaeria*, with fusiform, one- to three-septate ascospores being typical of most members of the order (Fernndez and Huhndorf 2005).

g) Coniochaetales
Members of Coniochaetales typically possess small, setose perithecial ascomata and occur on wood, dung, or soil. The asci are more or less clavate without an apical ring. Ascospores are brown, ellipsoidal, fusoid, or discoid, and most possess a germ slit. The relatively simple ascomatal morphology can cause members of the group to be confused with taxa in Chaetosphaeriales or Sordariales. The known anamorphs of this order are classified as species of *Lecythophora*. These are phialidic hyphomycetes with the phialide often reduced to a collarette lateral on a hyphal cell (Gams 2000).

h) Sordariales
Sordariales consists of mostly **wood- and dung-inhabiting species** with relatively large, erumpent, or superficial ascomata with large-celled, membraneous, or coriaceous ascomatal walls. Filiform papraphyses are present in some taxa, as are subapical globules within the asci. Ascospores in this group show variation on a distinct developmental theme and range from cylindrical, hyaline ascospores to ellipsoid, brown ascospores, often with appendages or sheaths. Many of the species in this order lack anamorphs, but some have lightly pigmented, phialidic, *Phialophora*-like anamorphs, or the ascospores germinate by directly producing phialides (Gams 2000; Réblov and Winka 2000). The most important discovery in these

four-gene analyses is that the internode representing Sordariales is strongly supported by both bootstrap and posterior probability, which was not shown in previous studies (Huhndorf et al. 2004b; Miller and Huhndorf 2005). The present taxon sampling encompasses some of the great morphological variation within the group, but the use of four genes does not distinguish any well-supported new lineages. In previous studies, well-supported clades correlated with distinct ascomal wall characters were uncovered [see details in Miller and Huhndorf (2005)]. Sordariaceae (e.g., *Neurospora* and *Sordaria*) remains a well-supported monophyletic group, and this is the only traditional family recognized in the order. While *Chaetomium* is monophyletic with strong support, the genera *Podospora* and *Cercophora* are polyphyletic.

3. Xylariomycetidae

This well-supported monophyletic subclass contains a single order, Xylariales, which is characterized by well-developed stromata, dark-colored perithecia, persistent asci often with amyloid apical rings, and true paraphyses (Figs. 3.7–3.12). Most species of Xylariales are **saprotrophs or plant parasites** in terrestrial habitats. While most marine species are classified in Halosphaeriaceae and Lulworthiales (Schoch et al. 2007; Spatafora et al. 1998), some species, for example, *Anthostomella torosa*, represent aquatic lineages in Xylariales (Kohlmeyer and Volkmann-Kohlmeyer 2002; Zhang et al. 2006). Xylariales is one of the largest orders in Sordariomycetes, which includes over 2,400 species in 209 genera and 9 families according to the *Dictionary of the Fungi* (Kirk et al. 2008). Some of its families are larger and more diverse than small orders in Sordariomycetes. Therefore, we will discuss each family so as not to oversimplify the picture.

Xylariaceae is a large assemblage and the type family of Xylariales. Perithecia are embedded in stromata, asci usually possess amyloid apical rings, and mature ascospores are usually unicellular with a prominent germ slit. A number of recent reports have dealt with phylogenetics within the family (e.g., Snchez-Ballesteros et al. 2000; Smith et al. 2003; Triebel et al. 2005; Hsieh et al. 2005, 2010; Pelaez et al. 2008; Ju et al. 2011). Anamorphs of Xylariaceae have sympodially proliferating conidiogenous cells with characteristic scars and holoblastic, single-celled conidia with detachment scars corresponding with those on the conidiogenous cells. Variation in conidiophore branching and the nature of the conidiogenous cell proliferation (ampulliform, irregularly swollen, geniculate) allows the distinction of anamorph genera, such as *Nodulisporium* and *Geniculosporium*, and several others, which often delimit monophyletic groups (Ju and Rogers 1996). **Amphisphaeriaceae** in the sense of Müller and von Arx (1973) included taxa that greatly resemble members of Xylariaceae, but with ascospores that (mostly) lack defined germination sites. Barr (1990) presented a much more restricted circumscription of the family, and Kang et al. (2002) published a molecular-based phylogeny, including the restricted group. The versicolored, multiappendaged conidia of the *Pestalotia* complex seem to be a synapomorphic character for these coelomycetous anamorphs of Amphisphaeriaceae (Kang et al. 2002).

Cainiaceae was established for a small assemblage of monocot inhabitants with two-celled ascospores ornamented with striations or multiple germination slits and asci with amyloid apical rings (Kang et al. 1999). The two-locus phylogeny of Lutzoni et al. (2004) places *Cainia* among Xylariales. **Clypeosphaeriaceae** sensu Barr (1990) includes genera with mostly iodine-negative ascal apices and germination sites, when present, that are pores. Important genera include *Clypeosphaeria* and *Endoxyla*. **Graphostromatace** contains *Graphostroma*, and although species have a stroma reminiscent of Diatrypaceae and ascospores somewhat suggestive of that family, its affinities are clearly with Xylariaceae. **Diatrypaceae** has long been recognized as a natural assemblage allied with Xylariaceae. A cardinal feature is the pale brown allantoid ascospore. The anamorphs of Diatrypaceae also have sympodially proliferating, as well as percurrently proliferating, conidiogenous cells (sometimes on the same conidiophore) but lack conspicuous scars, and

often they have tiny conidiogenous cells and conidia densely packed into rudimentary coelomycetous conidiomata, sometimes little more than cavities in stroma (Glawe and Rogers 1982). Although circumscription of the family is clear and widely accepted (Barr 1990), the delimitation of genera is, in part, artificial (Acero et al. 2004). **Hyponectriaceae** as circumscribed by Barr (1990) contains 13 genera. The type genus *Hyponectria* falls within Xylariales in the two-locus phylogeny of Lutzoni et al. (2004). Phylogenetic analyses based on a single locus, ITS, found Hyponectriaceae to be monophyletic (Triebel et al. 2005). **Myelostromataceae** is monotypic; it is generally assigned to Xylariales, but its true affinities remain uncertain. **Iodosphaeriaceae** is a recently established small family containing only two species (Hilber and Hilber 2002).

4. Orders Incertae Sedis

a) Koralionastelales

Based on rDNA sequences, Campbell et al. (2009) assigned two marine genera, *Koralionastes* and *Pontogeneia*, to a new order, Koralionastelales, a sister group to Lulworthiales. Species in Koralionastelales have distinct paraphyses and periphyses, while species of Lulworthiales lack hamathecia in mature ascomata. Furthermore, unlike most Lulworthiales, species in the Koralionastelales do not have apical ascospore structures, such as apical mucus-containing chambers or gelatinous sheaths. Koralionastelales differs from other Sordariomycetes by the characteristic formation of antheridia on germinating ascospores.

b) Lulworthiales

Lulworthiales was recently segregated from Halosphaeriaceae based on molecular data (Kohlmeyer et al. 2000). Lulworthiales is characterized by dark ostiolate ascomata, with or without a subiculum, and the absence of interascal tissue, although the centrum is initially filled with a thin-walled pseudoparenchyma. Asci are thin-walled and deliquesce early, and ascospores are usually filamentous, mostly with mucus-containing apical chambers or appendages. The members of Lulworthiales degrade wood and marsh plants in **marine and estuarine environments**. Recently, two species of an enigmatic genus, ***Spathulospora*****, obligate parasites on red algae**, were placed in this order based on nuclear ribosomal data (Inderbitzin et al. 2004). It was noted by Inderbitzin et al. (2004) that *Spathulospora* shares the apical mucus-filled chambers of the ascospores with several lulworthialean species. However, major features of *Spathulospora* species, absent in other members of Lulworthiales, include the presence of sterile ascomatal hairs, antheridia, and trichogynes. ***Haloguignardia*****, another genus with algae-inhabiting species** and ascospores characterized by mucus-filled apical chambers, also is nested within Lulworthiales (Campbell et al. 2005). The only reported anamorphs in this order are the hyphomycetes, *Zalerion maritimum* (teleomorph *Lulworthia uniseptata*) (Nakagiri 1984), with helically coiled conidia, and *Anguillospora marina* (teleomorph *Lindra obtusa*) (Nakagiri and Tubaki 1983), with filiform conidia. The placement of Lulworthiales in Sordariomycetes is not settled. According to the four-gene phylogeny of Zhang et al. (2006), this order is an early diverging clade of Sordariomycetes, suggesting a new subclass-level lineage. Other phylogenetic studies placed it either within or close to Hypocreomycetidae, but with no statistical support (Schoch et al. 2007, 2009a; Tang et al. 2007).

c) Meliolales

Species in Meliolales are **biotrophic** and have never been grown in pure culture. They are all **leaf parasites** in **tropical regions**. Perithecia are superficial on host leaves, nonstromatic, but associated with coarse hyphae that bear "**hyphopodia**" that themselves are modified conidiophores. Pigmentation is black. Asci form a basal hymenium and tend to be broadly clavate, and they lack an apical discharge mechanism. Ascospores are ellipsoidal to cylindrical, dark brown, and multiseptate. Conidiogenesis is enteroblastic. They have been suggested as being Dothideomycetes, but developmental and phylogenetic studies place the order among Sordariomycetes (Luttrell 1989; Vijaykrishna

et al. 2004). Hansford (1961, 1963) has monographed this group.

d) Phyllachorales
The ordinal limits of Phyllachorales are ambiguous because of a lack of reliable morphological characters that clearly delimit this group (Alexopoulos et al. 1996; Samuels and Blackwell 2001). Recent molecular data strongly support the segregation of the fleshy stroma *Glomerella* (anamorph *Colletotrichum*) from the nonfleshy black stroma *Phyllachora* (Réblov et al. 2011; Wanderlei-Silva et al. 2003). Glomerellales is now a member of Hypocreomycetidae, but the phylogenetic position of the remaining Phyllachorales senso lato still needs further investigation. Here we follow the Myconet (Lumbsch and Huhndorf 2010) and treat this order as Sordariomycetes incertae sedis.

e) Trichosphaeriales
Trichosphaeriales is a small single-family order whose members have carbonized, relatively thin perithecial walls and tend to be lignicolous, less frequently fungicolous. Perithecia are superficial, nonstromatic, often with hairs, or setose conidiophores arising from the surface of the peridium. Asci have a reflective, inamyloid apical ring. Ascospores are variable. Phylogenetic analysis of LSU sequences of *Cryptadelphia*, a constituent genus, suggested a close relationship with Sordariomycetidae (Réblov and Seifert 2004).

III. Leotiomycetes

Following Eriksson (2005), Leotiomycetes, including Geoglossaceae, was referred to as the "**inoperculate discomycetes,**" a group of **nonlichenized ascomycetes characterized by the production of open ascomata (apothecia) and asci opening with an apical perforation or pore (inoperculate) for releasing ascospores.** The **types of dehiscence** in asci have been one of the central issues in discomycetes classification, and sophisticated terms have been developed to describe and to classify structural characters revealed by light microscopy and transmission electron microscopy (e.g., Baral 1987; Bellemère 1994; Mengoni 1986; Minter and Cannon 1984; Verkley 1994). Although ultrastructural characters like the ascus dehiscence mechanism, along with other morphological characters, and ecological or biochemical features can be informative for classification at different levels for some ascomycetous groups, the difficulties in gathering and interpreting such data across the highly diverse Leotiomycetes cause these traditional methods to fail to keep up with molecular approaches in resolving the phylogeny of the class, especially at higher taxonomic ranks.

The Assembling the Fungal Tree of Life (AFTOL) project represents a peak of effort and achievement in studying molecular phylogeny in fungi (Blackwell et al. 2006; Hibbett et al. 2007), and the traditional classification of Leotiomycetes at high levels has experienced considerable challenges from hypotheses based on molecular systematics (Schoch et al. 2009a, b; Wang et al. 2006a, b). However, suggestions such as the inclusion of Erysiphales, *Myxotrichum*, and *Pseudogymnoascus* and the exclusion of Geoglossaceae were solely or mainly based on molecular data with little evidence from ecology and morphology, and this makes it very difficult to teach or to study Leotiomycetes in a taxonomic context (Weber 2009). The traditional classification of Leotiomycetes was reviewed by Pfister and Kimbrough (2001) within the morphological group Discomycetes for *The Mycota VII* (McLaughlin et al. 2001). Additional studies with a wide taxon sampling in Leotiomycetes at different levels have been published since then, and reconciliation is thus indispensable for Leotiomycetes and Geoglossomycetes between the traditional classification and recent developments in molecular phylogenies.

A. Classification History

1. History of Leotiomycetes

Leotiomycetes was based on Leotiales (Helotiales) for nonlichenized inoperculate discomycetes, and the inclusion of the other three orders, **Cyttariales, Erysiphales, and**

Rhytismatales, was primarily supported with SSU rDNA data (Eriksson and Winka 1997). However, some members of Helotiales were positioned paraphyletically among other ascomycetes and within Leotiomycetes in published phylogenies, and Leotiomycetes was not accepted by some classifications (e.g., Pfister and Kimbrough 2001). As discussed subsequently, the monophyly of Leotiomycetes in its current concept has not yet been verified with a proper taxon sampling and a robust phylogeny, and the use of Leotiomycetes should be considered more as a practicable solution for managing the extreme diversity within these fungi than a solid rank for a fully matured classification. The contents of the order Helotiales and Leotiomycetes have changed dramatically during the history of major classifications in Ascomycetes, and a summary of these changes is updated from Spooner (1987) in Table 3.1.

2. Current Classification of Leotiomycetes

Because of the important and diverse ecological roles of these fungi, morphological classification of Leotiomycetes has been extensively studied, and their classification has experienced many changes over a century, especially recently, as molecular characters have become available. Many of these revisions have been controversial. The classification of Leotiomycetes published recently by Lumbsch and Huhndorf (2007b) on Myconet and by Kirk et al. (2008) in the *Dictionary of the Fungi* are widely accessible and the most inclusive ones beyond the family level. Kirk et al. (2008) accepted five orders, **Cyttariales, Erysiphales, Helotiales, Leotiales, and Rhytismatales**, and included Thelebolales with uncertainty, and these orders, except Leotiales, were recognized in Lumbsch and Huhndorf (2007b) for Leotiomycetes as well. Accommodating the SSU rRNA phylogeny in Hambleton and Sigler (2005), Eriksson (2006) treated Myxotrichaceae as family incertae sedis for the class, and this placement was followed in Lumbsch and Huhndorf (2007b) (Table 3.1).

Although the orders Cyttariales, Erysiphales, and Thelebolales are distinct in morphology and ecology, their interordinal relationships within Leotiomycetes have not yet been well established regarding the relationships among them and other members of the class (Schoch et al. 2009a; Wang et al. 2006a, b).

Cyttariales hosts a group of weak parasites in southern South America and southeastern Australasia that produce the largest fruiting bodies (apothecia) in Leotiomycetes on *Nothofagus* trees, and the biogeography of *Cyttaria* species has been of special interest to mycologists and evolutionary biologists since Charles Darwin.

Close relationships between Cyttariales and members of Helotiales were hypothesized based on morphological characters, and molecular phylogenies supported the placement of Cyttariales in Leotiomycetes [reviewed in Peterson and Pfister (2010) and Peterson et al. (2010)]. In contrast, the smallest but delicately structured fruiting bodies (cleistothecia) are found in Erysiphales (Takamasu 2004). Given the dramatic differences in morphology, connections between powdery mildew fungi and other Leotiomycetes are difficult to interpret directly at the morphological level, and the inclusion of Erysiphales in Leotiomycetes has been only inferred with molecular phylogeny (LoBuglio and Pfister 2010; Wang et al. 2006a, b). Although Erysiphales has a high diversity at the species level, monophyly of the order is strongly supported with morphology, ecology, and molecular phylogeny, and the single family Erysiphaceae consists of 16 genera and approximately 650 species (Takamasu et al. 2010). Similar to the case of Erysiphales, the placement of Thelebolales in Leotiomycetes is solely suggested with molecular characters from one or two *Thelebolus* species (Gernandt et al. 2001; Landvik et al. 1998; LoBuglio and Pfister 2010; Peterson and Pfister 2010). Thelebolaceae had been included in the Pezizales for a long time because the species produce small, globose to disk-shaped ascomata with sometimes polysporus asci, and the development of the ascomata varies from a cleistohymenial type to a eugymnohymenial type (de Hoog et al. 2005; Wang et al. 2006b). Some species of Thelebolales might be true members of Pezizales with respect to their ascus dehiscence mechanisms (Hansen and Pfister 2006).

Table 3.1 Family structure of Helotiales (left six columns) and the development of the Leotiomycetes (right four columns) in major classifications (updated from Spooner 1987)

Helotiales (Leotiales)						Leotiomycetes (Leotiomycetidae)			
Nannfeldt (1932)	Korf (1973)	Barr (1976)	Dennis (1978)	Hawksworth et al. (1983)	Eriksson (1983)	Eriksson (1999)	Kirk et al. (2001)	Eriksson (2006), Lumbsch and Huhndorf (2007)	Kirk et al. (2008)
Dermateaceae	+	+	+	+	+	+	+	+	+
Geoglossaceae	+	+	+	+	+	+	+	+	–
Hyaloscyphaceae	+	N/A	+	+	+	+	+	+	+
Orbiliaceae	+	+	+	+	+	–	–	–	–
Helotiaceae	Leotiaceae	N/A	Helotiaceae	Helotiaceae	Leotiaceae	+	+	+	+
Phacidiaceae	–	–	–	+	+	+	+	+	+
	Sclerotiniaceae	+	+	+	+	+	+	+	+
	Ascocorticiaceae	+	+	+	?	?	+	?	+
	Hemiphacidiaceae	+	–	+	+	+	+	+	+
		Mycocaliciaceae	Caliciaceae	–	–	–	–	–	–
				Gelatinodiscaceae	+	–	–	–	–
				Medeolariaceae	–	–	+ (Medeolariales)	–	–
				Baeomycetaceae	+	–	–	–	–
						Leotiaceae	+	+	+ (Leotiales)
						Loramycetaceae	+	+	+
						Rutstroemiaceae	+	+	+
						Vibrisseaceae	+	+	+
						Cyttariaceae (Cyttariales)	+ (Helotiales)	+	+
						Erysiphaceae (Erysiphales)	–	+	+
						Ascodichaenaceae (Rhytismatales)	+	+	+
						Cryptomycetaceae (Rhytismatales)	–	+	–
						Rhytismataceae (Rhytismatales)	+	+	+
							Bulgariaceae	+	+ (Leotiales)
							Cudoniaceae	+ (Rhytismatales)	+ (Rhytismatales)
								Thelebolaceae (Thelebolales)	?
								Myxotrichaceae?	–

The name Helotiales, instead of Leotiales suggested by Carpenter (1988), is used here following Korf (1973), but the content of the order is modified from Eriksson (2006) and Lumbsch and Huhndorf (2007b) with the exclusion of the family Geoglossaceae. The order Helotiales, one of the largest nonlichen-forming ascomycetous groups, is composed of fungi of diverse morphology and ecology, and helotialean fungi have been reported from a broad range of niches. To make the classification of this order more complicated, correlations between teleomophs and anamorphs in the families Dermateaceae, Hyaloscyphaceae, and Leotioaceae (as Helotiaceae) were regarded as heterogeneous (Huhtinen 1989; Sutton and Hennebert 1995), and the monophyletic status of Helotiales is also rejected based on ultrastructural study and molecular phylogeny (Pfister and Kimbrough 2001; Schoch et al. 2009a; Wang et al. 2006b). Twelve families, including **Ascocorticiaceae, Bulgariaceae, Dermateaceae, Helotiaceae, Hemiphacidiaceae, Hyaloscyphaceae, Leotiaceae, Loramycetaceae, Phacidiaceae, Rutstroemiaceae, Sclerotiniaceae, and Vibrisseaceae,** are accepted in Helotiales by Lumbsch and Huhndorf (2007b) and Kirk et al. (2008). Hosoya et al. (2010) accepted the Lachnaceae as well for lachnoid fungi based on an rDNA phylogeny. Except for monotypic or small families such as Ascocorticiaceae, Bulgariaceae, Leotiaceae, and Loramycetaceae, which reflect our lack of knowledge about relationships among these fungi and other helotialean species, monophyly of other families in Helotiales has been questioned with morphological and molecular characters from limited taxon samplings (Pfister and Kimbrough 2001; Schoch et al. 2009a), and retaining these ranks is primarily for practical purposes.

The order Rhytismatales hosted only leaf endophytic fungi and pathogens causing serious plant diseases, before the saprotrophic Cudoniaceae was added to Ascodichaenaceae, Cryptomycetaceae, and Rhytismataceae in this order (Pfister and Kimbrough 2001; Kirk et al. 2008; Wang et al. 2006a, b). Although challenged with ultrastructural observations, the traditional classification of Rhytismatales has been supported with the reduced but highly adaptive morphology and parasitic lifestyle (Hawksworth 1995; Johnston 1997; Livsey and Monter 1994; Minter and Cannon 1984). However, a recent study showed a parasitic or endophytic lifestyle playing critical roles via divergent, convergent, and parallel evolution in shaping the evolution of morphology in the Ascomycota (Schoch et al. 2009b; Wang et al. 2009). Molecular data of Ascodichaenaceae and Cryptomycetaceae were recently available for phylogenetic analysis, and significant changes in the classification of Rhytismatales are expected in the near future (Lantz et al. 2010; LoBuglio and Pfister 2010).

B. Molecular Phylogeny Update

1. Higher-Level Relationships of Leotiomycetes

The evolutionary history of Leotiomycetes was investigated haphazardly in the **AFTOL-1 project.** Only two out of six or seven orders were sampled using multilocus sequence data, resulting in an unresolved phylogeny with polytomies and long branches at diverse levels (James et al. 2006; Lutzoni et al. 2004; Schoch et al. 2009a; Wang et al. 2006a, b). Close relationships between members of Leotiomycetes and species of Sordariomycetes, rather than operculate discomycetes, are suggested by a study of the mechanisms of fertilization, ascomatal development, and ascal structures (Kimbrough 1981; Pfister and Kimbrough 2001; Verkley 1994, 1996). Recent molecular phylogenies have consistently recognized a sibling relationship between these two classes, but significant support for a monophyletic Leotiomycetes has only been achieved with multilocus sequence data from a very limited taxon sampling (e.g., James et al. 2006; LoBuglio and Pfister 2010; Lutzoni et al. 2004; Schoch et al. 2009a, b; Spatafora et al. 2006). Sibling relationships between Leotiomycetes and Sordariomycetes have not yet been established with an inclusive taxon sampling of Leotiomycetes that includes Cyttariales, Theleborales, and Erysiphales, as well as traditional inoperculate discomycetes (e.g., Gernandt et al. 2001; LoBuglio and Pfister 2010; Peterson and Pfister 2010; Saenz et al. 1994; Schoch et al. 2009a, b;

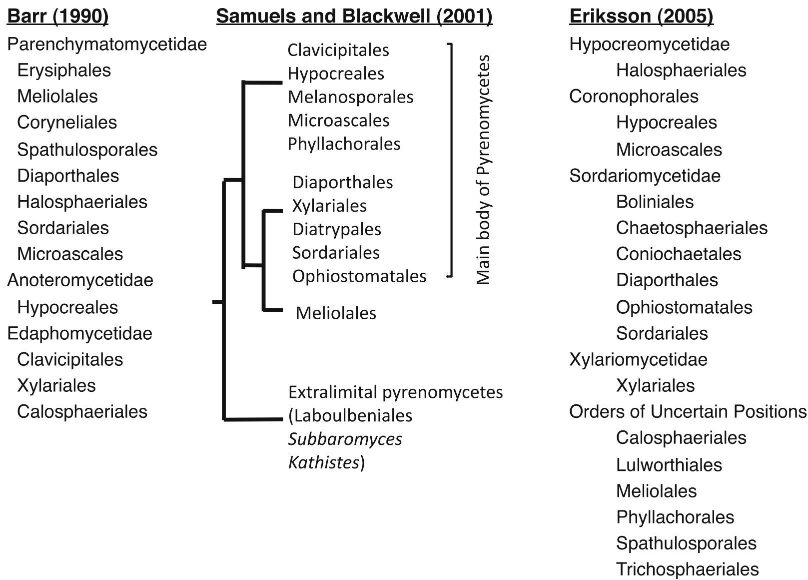


Fig. 3.20 Outline of previous classifications of Sordariomycetes

Spatafora et al. 2006; Wang et al. 2006a, b). To delimit Leotiomycetes and to resolve relationships among major clades of Leotiomycetes and Sordariomycetes, multilocus data for some lichenized lineages, for species of Cyttariales, Erysiphales, and Thelebolales, and for species of *Bulgaria*, *Chlorociboria*, and *Bisporella* in Helotiales are critical.

2. Phylogeny Within Leotiomycetes

In summary, phylogeny within Leotiomycetes is not well resolved with molecular data (Fig. 3.20), and polytomies and long branches encountered in Leotiomycetes phylogeny reflect the complicated natural history of these fungi. Three recent publications, by LoBuglio and Pfister (2010), Peterson and Pfister (2010), and Lantz et al. (2011), are among the most important developments toward resolving the phylogeny of Leotiomycetes.

Biogeographic isolation and disjunction may be one of the major reasons for the difficulties in resolving phylogenetic relationships within orders of Leotiomycetes. **Cyttariales**, a single-genus order with a dozen species, so far has been found only in the **Southern Hemisphere** (Peterson and Pfister 2010; Peterson et al. 2010). **Erysiphales** has two basal lineages restricted separately to **South American and eastern Asia** (Takamasu et al. 2005). Species of the genus *Thelebolus* (Thelebolales) have been frequently isolated from the Arctic and Antarctic, and the position of Thelebolales in Leotiomycetes is only weakly supported by rDNA data (de Hoog et al. 2005). Species of **Helotiales**, the largest order of nonlichenized ascomycetes and apparently not monophyletic, are reported from **all over the world**, but some major clades in this order may not historically reside in the Southern Hemisphere. Problems in resolving lower-level clades (family and genus) in Leotiomycetes may be caused by recent adaptation to novel ecological roles in new environments, with the result that almost all morpho families in Helotiales collapse in recently published gene trees. Evidence for this lack of resolution includes unresolved relationships among clades composed of taxa that are morphologically very different yet share similar ecological characters [e.g., the aquatic-saprobe clade in Wang et al. (2005)], and the evidence also includes lineages with very long branch lengths (e.g., worldwide distributed small or monotypic genera *Bisporella*, *Neobulgaria*, *Medeolaria*, *Chlorociboria*, or *Cordierites* (LoBuglio and Pfister 2010; Peterson and Pfister 2010; Wang et al. 2006a, b).

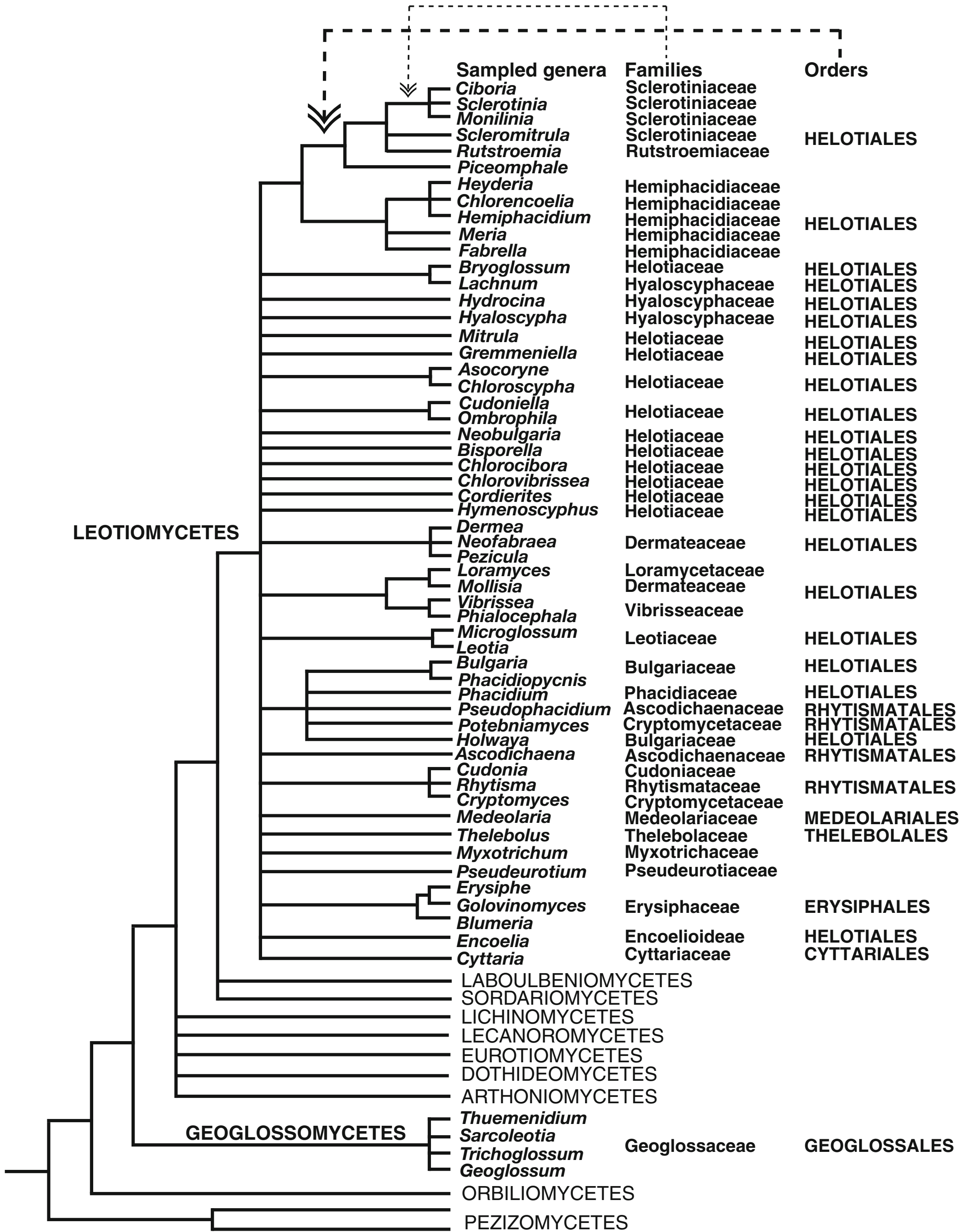


Fig. 3.21 Phylogeny of Leotiomycetes. Branch lengths are not proportional to genetic distances

Besides the order Medeolariales with only one species, the orders Cyttariales, Erysiphales, and Thelebolales have been supported as monophyletc with at least rRNA sequence data; however, **taxon sampling is not considered as sufficient for the species-rich**

Erysiphales and morphologically diverse Thelebolales (LoBuglio and Pfister 2010; Peterson and Pfister 2010; Schoch et al. 2009a). Although *Medeolaria farlowii* was reported almost a century ago, reliable rRNA sequence data were not available until LoBuglio and Pfister (2010), and its position in Leotiomycetes was confirmed. A close relationship between *M. farlowii* and the always problematic *Chlorociboria* was suggested without support in consensus trees in the study, and top hits resulting from a BLAST search with LSU-rRNA of *M. farlowii* are various leotiomycetous fungi but did not include any *Chlorociboria* species. Multilocus data, including the protein-coding gene EF1-alpha, were generated from the same lab for *Cyttaria* species (Peterson and Pfister 2010). In the study, the inclusion of Cyttariales in Leotiomycetes was further verified with multilocus data, and Encoelioideae of Helotiales, including species of *Ionomidotis*, *Encoelia*, and *Cordierites*, together with *Chlorociboria* species, were identified as sister groups to Cyttariales with moderate support (Peterson and Pfister 2010). The apothecia of Encoelioideae fungi excrete violet pigmentation in aqueous KOH solution, a so-called ionomidotic reaction, and Encoelioideae is likely polyphyletic based on morphology and molecular characters (Peterson and Pfister 2010; Zhuang 1988; Zhuang et al. 2000). It would also be desirable to have data of *M. farlowii* included in the *Cyttaria* studies.

Shortly after LoBuglio and Pfister (2010), who pointed out that there were no data from **Ascodichaenaceae** and **Cryptomycetaceae** for resolving the phylogeny of the order Rhytismatales, Lantz et al. (2011) reported a core clade of Rhytismatales using mitochondrial SSU rRNA and nuclear LSU rRNA data from representatives of all four families in the order. Not unanticipated, Rhytismatales and its families Ascodichaenaceae, **Rhytismataceae,** and probably Cryptomycetaceae did not form a monophyletic group, but data from a wider taxon sampling for genes that are more informative are required to further accept or reject these suggestions, which were based on a poorly resolved phylogeny.

Little progress has been made in molecular phylogeny for the polyphyletic and most problematic group in Leotiomycetes, the order Helotiales, and this is likely due to the difficulties in identifying and generating data from proper genetic markers for evolutionary events in this epoch, when there seems to be a quick radiation of helotialean diversity. Consistent with Wang et al. (2006a, b), Schoch et al. (2009a, b) proposed **eight lineages to be recognized in Helotiales** for future research; they are **(1) *Cyclaneusma* and *Naemacyclus* clade; (2) Leotiaceae (including *Leotia* and *Microglossum*); (3) Bulgariaceae (including *Bulgaria* and *Holwaya*); (4) Dermeataceae sensus stricto (including *Dermea*, *Neofabraea*, and *Pezicula*); (5) Sclerotineaceae, Rutstroemiaceae, and Hemiphacidiaceae; (6) gelatinous species such as *Ascocoryne* and *Chloroscypha*; (7) *Hymenoscyphus* and *Cudoniella* related species; and (8) aquatic *Vibrissea*, *Loramyces*, root endophytes *Phialocephala*, and *Mollisia*.** Among these lineages, the Sclerotineaceae, Rutstroemiaceae, and Hemiphacidiaceae clade should be justified as an order with morphology (including biochemistry), ecology, and molecular systematics.

C. Characters

1. Apothecium (Ascoma) Morphology, Anatomy, and Ultrastructure

Apothecial morphology has been well studied for major groups traditionally classified within Leotiomycetes, and a detailed review is provided in Pfister and Kimbrough (2001). Considerable differences in morphology among species in Leotiomycetes make it one of the most difficult groups for taxonomy; however, an ascoma morphology definition for the orders Cyttariales, Erysiphales, Medeolariales, and Thelebolales is well established. For most genera in Helotiales, macromorphological characters are reliable, but exceptions do exist, for example, in *Ascoryne* and *Vibrissea* (Bunyard et al. 2008; Wang et al. 2006b). **At the family level, current molecular phylogenies do not support traditional classification using ascoma morphology for the families Helotiaceae, Leotioaceae, Hyaloscyphaceae, and Dermateaceae in Helotiales and the families**

Ascodichaenaceae and Cryptomycetaceae in Rhytismatales. Anatomical characters, such as the structure of the excipulum, epithecium, gelatinous layer, paraphyses, and hymenium, are informative for classification at the genus or lower levels, as are histochemical characters of asci and ascospores. The ultrastructure of asci has been used in classifications in some groups in both Leotiomycetes and Geoglossomycetes (Pfister and Kimbrough 2001), but a lack of widespread use of these characters in many other groups makes it hard to evaluate the usefulness of these techniques, which usually require special equipment and training. Difficulties encountered in recognizing homologies between ultrastructural characters are further complicated by the complex terminology used by different researchers.

2. Ecology and Biogeography

More and more evidence, especially inferred from molecular data, suggest **ecology and biogeography have been critical for understanding the evolution of Leotiomycetes**. Within the incredible variety of ecology observed for Leotiomycetes, three groups that are highly adapted to unique niches require more attention, i.e., leaf epiphytic powdery mildews, leaf endophytes—plant pathogens, and aeroaquatic fungi—root endophytes.

The **powdery mildews** are a group of **obligate biotrophic** fungi attacking about 10,000 angiosperm species, and the host range is strictly confined to angiosperms for this fungal group that originated in the late Cretaceous (Amano 1986).

Molecular phylogenetic analyses suggested that Erysiphaceae is a monophyletic group (Wang et al. 2006b). The molecular phylogenies of powdery mildews also suggested that tree-parasitic species usually take basal positions and herb-parasitic species have derived positions, but multiple host shifts from trees to herbs may have then occurred during the Tertiary [a detailed review is provided in Takamasu et al. (2010)]. Because powdery mildews have been well documented as pathogens for various flowering plants for their identifications, hosts, and distribution, they are potentially good indicators for historical and ongoing events in global climate changes.

Species of Leotiomycetes also contribute a significant diversity to fungal **endophytes** of leaves (U'Ren et al. 2010; Wang et al. 2009), including conditional pathogens. **Leaf endophytes are concentrated in the families Rhytismataceae, Cryptomycetaceae, and Ascodichaenaceae in the Rhytismatales and Dermateaceae, Hemiphacidiaceae, Rutstroemiaceae, and Sclerotiniaceae in the Helotiales.** Apparently many leaf endophytes are adapted to the leaf environment by producing highly reduced, dark-colored, and often covered ascomata, and their close saprotrophic relatives, in contrast, produce large and bright-colored fruiting bodies (Lantz et al. 2011; Wang et al. 2009). Thus, molecular data are critical in classifying Leotiomycetes that produce a small and highly reduced reproductive structure, because morphological characters in these fungi are generally limited and not very informative. A highly reduced morphology and life history are also found in Leotiomycetes **associated with roots** of certain groups of plants, such as the *Phialocephala fortinii* s.l.—*Acephala applanata* species complex, also known as dark-septate root endophytes, which are found in conifer roots (Queloz et al. 2011). Although the mating-type locus has been partially characterized, sexual reproduction has not yet been observed for this species complex (Zaffarano et al. 2010).

Molecular phylogenies identified fungi in the mostly **aeroaquatic Loramyces-Vibrissea-Mollisia clade** as closely related to these root endophytes (Grünig et al. 2008; Upson et al. 2009; Wang et al. 2006a, b), and a shared evolutionary history between root endophytes and aquatic hyphomycetes has been hypothesized based on molecular and morphological data (Sati and Belwall 2005; Selosse et al. 2008). Independent origins of ectomycorrhizae are observed in the Helotiales (Tedersoo et al. 2009), and this includes species in *Hymenoscyphus* and related genera often isolated from the roots of Ericaceae (Hambleton and Sigler 2005). Some species in Leotiomycetes are found to be associated with mosses, with the highest diversity of bryosymbiotic fungi being in

Leotiomycetes among all the major ascomyceteous lineages, as revealed by a five-gene phylogeny (Stenroos et al. 2009). Some Leotiomycetes associated with **mosses** were found to be capable of colonizing **lichens**, and a high similarity of moss endophytes and endolichenic fungal communities was observed (U'Ren et al. 2010).

Leotiomycetes also show biogeographic isolation and disjunction within major clades. The order Cyttariales is found only in the Southern Hemisphere, and a recent study based on the cophylogeny and biogeography of these fungal parasites and its southern beech hosts accepted both a vicariance hypothesis and long-distance dispersal model for the distribution of *Cyttaria* species (Peterson et al. 2010**). The basal lineages of the Erysiphales are restricted to South America and eastern Asia** (Takamasu et al. 2005). The distribution of *Thelebolus* (Thelebolales) is largely in the Arctic and Antarctic (de Hoog et al. 2005). Species of Helotiales are reported from all over the world, but some major clades, such as lineages in Sclerotiniaceae, may not historically reside in some areas of the Southern Hemisphere. It is also worth mentioning that Southern Hemisphere species of *Chlorovibrissea* and *Vibrissea albofusca* evolved a morphology that is remarkably similar to that of the also aquatic *Vibrissea* species found in the Northern Hemisphere, although the two biogeographic groups are not closely related in Helotiales (Wang et al. 2006b).

Many helotialean fungi are now known to be associated with **ectomycorrhizas**, and such relationships probably evolved independently among biogeographic groups, as inferred from molecular phylogenies (Tedersoo et al. 2009). Interestingly, no biogeographical pattern has been identified in the root-associated *Phialocephala fortinii* s.l.—*Acephala applanata* species complex after a worldwide sampling, and long-distance distribution mechanism, e.g., by conidia, has been shown to be rare, if it even occurs, for this root endophyte complex (Queloz et al. 2011). Furthermore, Leotiomycetes closely related to these root endophytes include some aeroaquatic species in *Vibrissea* and *Loramyces* (Grünig et al. 2008, 2009), whose long-distance gene flow seems also to be restricted because of the geographic isolation of freshwater bodies. It is worthy of mention that an isolate related to *Ascocoryne*, Helotiales, was found to produce hydrocarbon derivatives of potential interest for the biofuel industry (Strobel et al. 2010).

IV. Geoglossomycetes

The class Geoglossomycetes with a single family, Geoglossaceae, was created for *Geoglossum*, *Trichoglossum*, and *Sarcoleotia* to accommodate recent molecular phylogenies and a new interpretation of previously observed developmental morphology (Schoch et al. 2009a, b; Schumacher and Sivertsen 1987; Spooner 1987; Wang et al. 2006a, b). Geoglossaceae was initially proposed to include **club-shaped, unitunicate, inoperculate discomycetes (earth tongues)**, but the content of Geoglossaceae has experienced significant changes, especially with the development of molecular systematics (Korf 1973; Spooner 1987; Wang et al. 2002, 2006a, b).

Species of *Geoglossum*, *Trichoglossum*, and *Sarcoleotia* possess a hymenium that freely develops toward the base of the stalk. Other earth tongues, such as species of *Leotia*, *Microglossum*, *Bryoglossum* of Helotiales, or *Cudonia* of Rhytismatales, show a distinct bond between the developing hymenium and infertile stalk, implying an ancestral state of covered (cleistohymenial) hymenium that has not been lost in *Cyttaria*, *Erysiphales*, *Rhytismatales*, and probably some species in Thelebolales in Leotiomycetes.

The relationships between Geoglossomycetes and other basal lineages and lichenized ascomycetes in the Pezizomycotina require further investigation (Lutzoni et al. 2004; Schoch et al. 2009a, b; Spatafora et al. 2006).

Conflicting placements of Geoglossomycetes within Pezizomycotina were inferred from different genes in different analyses, and the relationships among Geoglossomycetes, Orbiliomycetes, Pezizomycetes, and two lichen lineages, Lecanoromycetes and Lichinomycetes, are not resolved given the so far poorly supported backbone in the phylogeny of

Pezizomycotina (Schoch et al. 2009a, b). In addition to the genera *Geoglossum*, *Trichoglossum*, and *Sarcoleotia*, Ohenoja et al. (2010) suggested that one of the three species of *Thuemenidium*, *T. arenarium*, is also a species in this class, while the other *Thuemenidium* species are probably related to *Microglossum*.

V. Laboulbeniomycetes

This class contains over 2,000 species in two orders, most of which are **minute obligate ecoparasites of living beetles or other arthropods** (Tavares 1985; Weir and Hammond 1997). These fungi had been placed in pyrenomycetes or ascomycetes incertae sedis (Alexopoulos et al. 1996). Recent molecular studies of Laboulbeniomycetes supported a distinct but close relationship with the main body of Sordariomycetes (Schoch et al. 2007; Weir and Blackwell 2001).

A. Laboulbeniales

More than 2,000 species in 146 genera have been described in Laboulbeniales, which are cosmopolitan. These fungal species are often attached to specific exoskeleton areas of a single host insect. Typical characteristics include hyaline to darkly pigmented thalli, perithecial ascomata, often surrounded by complex appendages, an absence of hamathecial structures, evanescent asci maturing sequentially, and two-celled ascospores. The species are usually haustorial parasites of arthropods. Four families are recognized: Ceratomycetaceae, Euceratomycetaceae, Herpomycetaceae, and Laboulbeniaceae (Kirk et al. 2008; Lumbsch and Huhndorf 2010). Monographs of Laboulbeniomycetes were written by Thaxter (1896–1918) and Tavares (1985).

B. Pyxidiophorales

This single-family order contains 22 species in five genera. Molecular phylogenetic analyses supported their sister group relationship with Laboulbeniales (Blackwell 1994; Blackwell and Mallach 1989). Species in Pyxidiophorales occur in diverse habitats, such as dung, bark beetle galleries, and plant debris. Unusual features of *Pyxidiophora* include perithecia consisting of a single layer of wall cells, and three-ascospore asci. Ascospores of *Pyxidiophora* are carried by mites (Alexopoulos et al. 1996).

VI. Problems and Perspectives

A. Genome Project

Genomics has a significant impact on biology as a whole, and fungal genome projects provide new insights into reproduction, gene gain and loss, horizontal gene transfer, gene structure, and regulation for a better understanding of the evolution of fungal diversity. In systematic biology, phylogenetic analysis of DNA or protein sequence data currently is considered the most powerful tool for the elucidation of evolutionary relationships. However, the analysis of single or few loci often yields conflicting phylogenies. A robust systematic framework that stands the test of time should be based on genetic information at the genomic scale, which includes various independently evolving regions. **It was estimated that at least 20 unlinked genes or 8,000 randomly selected orthologous nucleotides are required to reach this goal** (Rokas et al. 2003). Advancements in **high-throughput genome sequencing** technologies have made large-scale phylogenomic studies possible.

Neurospora crassa (Sordariales, Sordariomycetes), the **red bread mold**, is a classic model organism in genetic studies. The genome of *N. crassa*, which is approximately 43 megabases long and includes approximately 10,000 genes, was reported as completely sequenced in 2003 (Galagan et al. 2003).

Soon thereafter, tremendous progress was made in the genomics of a number of other model organisms in Sordariomycetes and Leotiomycetes (Couch et al. 2005; Aguileta et al. 2009; Fillinger et al. 2007; Xu et al. 2007). According to the Genome Online Database (www.genomesonline.org), on May 25, 2012,

there were 105 Sordariomycetes and 15 Leotiomycetes complete and ongoing genome projects, which constitue 22 % of the 544 genome projects for all ascomyceteous fungi.

Fungal species being sequenced for genomes are mostly economically important, and many of them are pathogens. For instance, the genome project for *Magnaporthe oryzae* (former name: *M. grisea*), the **rice blast fungus**, was completed by the International Rice Blast Genome Consortium and the BROAD Institute (http://www.broadinstitute.org). The genomes of its closely related species, *M. poae* (summer patch pathogen of turfgrass) and *Gaeumannomyces graminis* var. *tritici* (take-all pathgen of cereals), have been sequenced and are currently in the process of gene annotation. The BROAD Institute and Department of Energy Joint Genome Institute have sequenced several plant pathogenic *Fusarium* genomes (teleomorphs: *Nectria* and *Gibberella*), which enables comparative genomic studies (Ma et al. 2010). In 2005, the international consortium ***Botrytis* & *Sclerotinia* genome project**, led by M.H. Lebrun (INRA-Bioger), was initiated to support the sequencing and genome annotation of these two very closely related necrotrophic and polyphageous fungi (Fillinger et al. 2007). These are the first two Leotiomycetes to be fully sequenced. *Botrytis cinerea* was sequenced at the French National Sequencing Center, and *Sclerotinia sclerotiorum* was sequenced at the BROAD Institute. Genome sequences for these two species were completed and released for public access, and publications of these two genomes will be available soon. In addition, the genomes of three powdery mildew fungi were sequenced; they have a genome size of more than 120 Mb—more than four times larger than the median of other ascomycetes—and experienced many gene losses during the evolution of obligate biotrophy (Spanu et al. 2010). Two other Leotiomycetes, *Leotia lubrica* (Leotioaceae, Helotiales) and species of *Rhytisma* (Rhytismataceae, Rhytismatales), have been nominated as candidates to be sequenced for genomes in the near future (http://www.broadinstitute.org/). Genome data for Laboulbeniomycetes are still lacking.

For highly diverse Sordariomycetes and Leotiomycetes that include over 15,000 described species, 100 genomes are far from enough for investigating their biological and genetic diversity. With the high-throughput next-generation sequencers, exponential growth of the genome data for these fungi in the coming decades is expected. However, downstream bioinformatic analyses, including genome assembly and gene annotation, will likely be a bottleneck and a challenge.

B. Environmental Study

Traditional approaches to the systematics and taxonomy of fungi to a large extent rely on the presence of distinctive morphological characters of reproductive structures or on culture studies in the laboratory. However, many fungi do not produce detectable reproductive morphological structures, and the majority of fungal lineages defy attempts at keeping them in cultures. Significant methodological advancements in DNA extraction and next-generation sequencing have become a useful tool in the characterization of the diversity of environmental or otherwise cryptic fungi. Conversely, so-called invisible fungal lineages identified from environmental samples provide important insight into the ecological and biological diversity of so-called visible fungi, which often are herbarium collections lacking ecological characters (Wang et al. 2011). Fungi in both Sordariomycetes and Leotiomycetes have been shown in many environmental studies with samples from soil and from roots or leaves of different plants (e.g., Arnold et al. 2007; Arnold and Lutzoni 2007; Herrera et al. 2010; Higgins et al. 2011; Napoli et al. 2010; Seephueak et al. 2010; Soca-Chafre et al. 2011; U'Ren et al. 2010). Interestingly, in many cases of environmental study, more species of Sordaromycetes than Leotiomycetes were recovered from samples when **culturing** was used to isolate fungi from the samples (e.g., Gallery et al. 2007; Higgins et al. 2011), but with **direct PCR and cloning approaches or high-throughput sequencing techniques,** more species of Leotiomycetes were discovered compared with the culturing isolation method, especially from plant materials (e.g., Hartmann et al. 2009; Morris et al. 2008; O'Brien et al.

2005). A possible explanation is that many leotiomycetous fungi are likely strict endophytes and cannot be cultured easily in laboratory conditions. Considering endophytic species, sordariomycetous endophytes are more often found in **tropical forests**, and leotiomycetous endophytes can be detected in **temperate and boreal forests** with comparatively less frequency (Arnold and Lutzoni 2007). Sordariomycetes also show a higher endophytic diversity than Leotiomycetes in **flowering plants and lichens**, while Leotiomycetes are more common in **conifers and dead plant tissues** (U'Ren et al. 2010).

Within the class Sordariomycetes, the orders Sordariales, Xylariales, and Hypocreales are often reported from environmental samples, while species from the orders Helotiales and Rhytismatales are the most often encountered Leotiomycetes.

But the availability of reference sequences in GenBank or International Nucleotide Sequence Database (INSD) databases for annotated species in different groups of fungi is also a restrictive factor for identifying unknown fungi from environmental samples (Brock et al. 2009), especially when pragmatic attempts to characterize the diversity of these so-called invisible fungi from environmental samples have relied on operational taxonomic units (OTUs) that have been delimited as discrete units of sequences that share, for example, 95–97 % pairwise similarity (Buée et al. 2009; Jumpponen and Jones 2009; O'Brien et al. 2005). As for almost all environmental studies targeting fungal diversity, the ITS regions of rDNA are standard genetic markers. Recently, an automatic alignment program called SATé was developed for dealing with large data sets (Liu et al. 2009), and phylogenetic analysis with data from environmental samples and annotated specimens may provide profound insights about fungal ecology and diversity (Wang et al. 2011).

VII. Culture and Maintenance

Most saprotrophic and necrotropic species of Sordariomycetes and Leotiomycetes are easily cultured on **synthetic media**. The production of ascomata and conidia (sporulation) is sometimes enhanced by adding **natural substrates** to synthetic agar, such as sterilized host plant tissue. For example, **carnation leaf** is often used to enhance conidium production of ***Fusarium***. However, biotrophic species usually require more elaborate nutritional formulations. No member of Laboulbeniales or Meliolales has been cultured. Furthermore, spores including conidia and ascospores of many fungi in Leotiomycetes do not germinate and grow in culture using standard techniques and media, and this may be due to strict parasitic/endophytic stages in their life histories (e.g., Higgins et al. 2011).

Formulations of a number of general and selective media for **phytopathogenic fungi** are available in Singleton et al. (1992). For methods used with **insect-associated groups** see Benjamin et al. (2000). The preservation of fungal cultures in a metabolically inactive state in liquid nitrogen has been used to minimize mutation. With the increased availability of ultra-low-temperature freezers, more laboratories store their cultures in glycerol solution under −80 °C. Other methods include lyophilization or storage in sterile soil, in sterile distilled water, or on oil-covered agar slants. Protocols of these techniques are described in Smith and Onions (1983) and Singleton et al. (1992).

VIII. Conclusion

Recent phylogenetic studies using DNA sequences and ultrastructural data support a close evolutionary relationship between the perithecial Sordariomycetes and apothecial Leotiomycetes. Extant members of these two highly diverse classes likely evolved from a common ancestor that was a **nonlichenized saprotroph with inoperculate, unitunicate asci**.

During the past two decades, rapid development in molecular phylogeny and genome sequencing has had a great impact on fungal classification. However, progress in these innovations is not well balanced across the fungal kingdom owing to some historic and technical issues. Sordariomycetes and Leotiomycetes present good examples showing how such

imbalances would affect current classification of higher ascomycetes. While ordinal-level classification of Sordariomycetes is well supported by multilocus sequence data, the classification and molecular phylogeny at the higher taxonomic levels for Leotiomycetes have not yet reached a common ground. Nevertheless, molecular data suggest that ecology played important roles in the recent evolution of Leotiomycetes.

A synapomorphy of Sordariomycetes is the perithecial ascoma, which is postulated to be evolved from the apothecium of ancestral Pezizomycotina. However, taxa in a number of unrelated lineages of Sordariomycetes have lost ostioles, which is usually associated with the loss of forcible discharge of ascospores (Malloch 1981; Suh and Blackwell 1999; Zhang et al. 2006). Recent multilocus phylogenetic analyses strongly support the monophyly of three subclasses and most orders in Sordariomycetes. Xylariomycetidae and some taxa of Sordariomycetidae have dark perithecia, amyloid asci, true paraphyses, and periphysate ostioles, which likely are plesiomorphies of Sordariomycetes. Typically, Hypocreomycetidae have light-colored perithecia, nonamyloid apical rings when apical rings are present, and the absence of true paraphyses. The majority of members of Sordariomycetes are **terrestrial**, and life in aquatic habitats is considered a derived character for the class (Samuels and Blackwell 2001; Zhang et al. 2006). Halosphaeriaceae, Sordariales, Diaporthales, Xylariales, and Magnaporthales contain **freshwater** species, while most **marine** species are classified in Halosphaeriaceae, Koralionastelales, and Lulworthiales. Most of these fungi break down lignin and cellulose from plant debris in intertidal and subtidal zones, very rarely in the deep sea. All the major lineages in Sordariomycetes contain aquatic species (Schoch et al. 2007; Shearer 1993; Spatafora et al. 1998). Therefore, the move to aquatic environments occurred multiple times in the class. Xylariomycetidae comprises mostly saprobes, which are also abundant in the other two subclasses. Therefore, the **saprotrophic nutritional mode** may be the ancestral state of Sordariomycetes. Most mycoparasites and insect associates are derived from Hypocreomycetidae, and Sordariomycetidae is rich in coprophilous taxa.

Ascospore wall ornamentation has been used to delimit many fungal genera, but phylogenetic studies on Melanosporales and Sordariales show that this character is prone to convergence. **Ecological features**, such as the root vs. shoot/leaf association, have been recognized as informative characters at the generic level for Sordariomycetes and Leotiomycetes (Zhang et al. 2011).

Acknowledgements We are grateful to Dr. Yu-Ming Ju for photographs of *Xylaria globosa* and Dr. Guohong Cai for photographs of *Anisogramma anomala*.

References

Abbott SP, Sigler L, Currah RS (1998) *Microascus brevicaulis* sp. nov., the teleomorph of *Scopulariopsis brevicaulis*, supports placement of *Scopulariopsis* with the Microascaceae. Mycologia 90:297–302

Acero FJ, Gonzlez J, Schez-Ballesteros J, Rubio V, Checa J, Bills G, Salazar O, Platas G, Pelez F (2004) Molecular phylogenetic studies on the Diatrypaceae based on rDNA-ITS sequences. Mycologia 96:249–259

Aguileta G, Hood ME, Refregier G, Giraud T (2009) Genome evolution in plant pathogenic and symbiotic fungi. Adv Bot Res 49:151–193

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory mycology. Wiley, New York

Amano K (1986) Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies, Tokyo

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88:541–549

Arnold AE, Henk DA, Eells RL, Lutzoni F, Vilgalys R (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99:185–206

Baral HO (1987) Der Apikalapparat der Helotiales. Eine lichtmikroskopische Studies über Arten mit Amyloidring. Z Mykol 53:119–135

Barr ME (1976) Perspectives in the Ascomycotina. Mem N Y Bot Gard 28:1–8

Barr ME (1977) *Magnaporthe*, *Telimenella* and *Hyponectria* (Physosporellaceae). Mycologia 69:952–966. doi:10.2307/3758778

Barr ME (1978) The Diaporthales in North America. Mycol Mem 7:1–232

Barr ME (1990) Prodromus to nonlichenized, pyrenomycetous members of Class Hymenoascomycetes. Mycotaxon 39:43–184

Bellemère A (1994) Asci and ascospores. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, pp 111–126

Benjamin RK, Blackwell M, Humber RA, Jones KG, Klepzig KA, Lichtwardt RW, Malloch D, Noda H, Roeper RA, Spatafora JW, Weir A (2000) The search for diversity of insect and other arthropod-associated fungi. In: Mueller GA, Bills G, Foster MS (eds) Measuring and monitoring biological diversity: standard methods for fungi. Smithsonian Institution, Washington, DC

Blackwell M (1994) Minute mycological mysteries: the influence of insects on the lives of fungi. Mycologia 86:1–17

Blackwell M, Mallach D (1989) *Pyxidiophora*: life histories and arthropod associations of two species. Can J Bot 67:2552–2562

Blackwell M, Hibbett DS, Taylor JW, Spatafora JW (2006) Research coordination network: a phylogeny for kingdom fungi (deep Hypha). Mycologia 98:829–837

Brock PM, Döring H, Bidartondo MI (2009) How to know unknown fungi: the role of a herbarium. New Phytol 181:719–724

Buée M et al (2009) 454 pyrosequencing analyses of forest soils reveal an unexpected high fungal diversity. New Phytol 184:449–456

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163

Bunyard BA, Wang Z, Malloch D, Clayden S, Voitk A (2008) New North American records for *Ascocoryne turficola* (Ascomycota: Helotiales). Fungi 1:23–31

Cain RF, Weresub LK (1957) Studies of coprophilous ascomycetes. V. *Sphaeronaemella fimicola*. Can J Bot 35:119–131

Campbell J, Volkmann-Kohlmeyer B, Gräfenhan T, Spatafora JW, Kohlmeyer J (2005) A re-evaluation of Lulworthiales: relationships based on 18S and 28S rDNA. Mycol Res 109:556–568

Campbell J, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B (2009) Koralionastetales, a new order of marine Ascomycota in the Sordariomycetes. Mycol Res 113:373–380. doi:10.1016/j.mycres.2008.11.013

Carpenter SE (1988) Leotiales, a name to replace Helotiales (Ascomycotina). Mycologia 80:127–130

Carroll G (1988) Fungal endophytes in stems and leaves – from latent pathogen to mutualistic symbiont. Ecology 69:2–9. doi:10.2307/1943154

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia 94:1017–1031

Castlebury LA, Rossman AY, Sung G-H, Hyten AS, Spatafora JW (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. Mycol Res 108:864–872

Celio GJ, Padamsee M, Dentinger BTM, Bauer R, McLaughlin DJ (2006) Assembling the fungal tree of life: constructing the structural and biochemical database. Mycologia 98:850–859. doi:10.3852/mycologia.98.6.850

Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003) *Hypocrea*/*Trichoderma*: species with conidiophore elongations and green conidia. Mycologia 95:1100–1140

Couch BC, Fudal I, Lebrun MH, Tharreau D, Valent B, van Kim P, Notteghem JL, Kohn LM (2005) Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. Genetics 170:613–630

de Hoog GS, Göttlich E, Platas G, Genilloud O, Leotta G, van Brummelen J (2005) Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. Stud Mycol 51:33–76

Dennis RWG (1978) British Ascomycetes. J Gramer, Leher, pp 1–455

Eriksson O (1983) Outline of the Ascomycetes – 1983. Syst Ascomyc 2:1–37

Eriksson OE (1999) Outline of Ascomycota. Myconet 3:1–88

Eriksson OE (2005) Outline of Ascomycota. Myconet 11:1–113

Eriksson OE (2006) Outline of Ascomycota. Myconet 12:1–82

Eriksson OE, Winka K (1997) Supraordinal taxa of Ascomycota. Myconet 1:1–16

Fernndez F, Huhndorf S (2005) New species of *Chaetosphaeria, Melanopsammella* and *Tainosphaeria* gen. nov. from the Americas. Fungal Divers 18:15–57

Fillinger S, Amselem J, Artiguenave F, Billaut A, Choquer M, Couloux A, Cuomo C, Dickman MB, Fournier E, Gioti A, Giraud C, Kodira C, Kohn L, Legeai F, Levis C, Mauceli E, Pommier C, Pradier JM, Quevillon E, Rollins J, Ségurens B, Simon A, Viaud M, Weissenbach J, Wincker P, Lebrun M-H (2007) The genome projects of the plant pathogenic fungi *Botrytis cinerea* and *Sclerotinia sclerotiorum*. In: Clément C, Conreux A, Jeandet P (eds) Macromolecules of grape and wines. Lavoisier, Paris, pp 125–133

Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol Biol 6:99. doi:10.1186/1471-2148-6-99

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev

P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metzenberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CP, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Gallery RE, Dalling JW, Arnold AE (2007) Diversity, host affinity, and distribution of seed-infecting fungi: a case study with *Cecropia*. Ecology 88:582–588

Gams W (2000) *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Stud Mycol 45:187–199

Geiser DM, Gueidan C, Miadlikowska J, Lutzoni F, Kauff F, Hofstetter V, Fraker E, Schoch CL, Tibell L, Untereiner WA, Aptroot A (2006) Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98:1053–1064

Gernandt DS, Platt JL, Stone JK, Spatafora JW, Holst-Jensen A, Hamelin RC, Kohn LM (2001) Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. Mycologia 93:915–933

Glawe D, Rogers J (1982) Observations on the anamorphs of six species of *Diatrype* and *Diatrypella*. Can J Bot 60:245–251

Grünig CR, Queloz V, Duò A, Sieber TN, Holdenrieder O (2008) Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex in tree roots – classification, population biology and ecology. Botany 86:1355–1369

Grünig CR, Queloz V, Duò A, Sieber TN (2009) Phylogeny of *Phaeomollisia piceae* gen. sp. nov.: a dark, septate, conifer-needle endophyte and its relationships to *Phialocephala* and *Acephala*. Mycol Res 113:207–221

Gugnani HC, Talwar RS, Njoku-Obi AN, Kodilinye HC (1976) Mycotic keratitis in Nigeria. A study of 21 cases. Br J Ophthalmol 60:607–613

Hambleton S, Sigler L (2005) *Meliniomyces,* a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (=*Hymenoscyphus ericae)* Leotiomycetes. Stud Mycol 53:1–27

Hansen K, Pfister DH (2006) Systematics of the Pezizomycetes – the operculate discomycetes. Mycologia 98:1029–1040

Hansford CG (1961) The Meliolineae. Syd Ann Mycol II Beihefte 2:1–806

Hansford CG (1963) Iconographia meliolinearum. Syd Ann Mycol II Beihefte 5:1–285

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent pant symbionts. Nat Rev Microbiol 2:43–56

Hartmann M, Lee S, Hallam SJ, Mohn WW (2009) Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. Environ Microbiol 11:3045–3062

Hawksworth DL (ed) (1995) Ascomycete systematics, problems and perspectives in the nineties. NATO ASI series. Plenum, New York, p 453

Hawksworth DL, Sutton BC, Ainsworth GC (1983) Ainsworth & Bisby's dictionary of the fungi (including the lichens). Commonwealth Mycological Institute, Kew

Herrera P, Suarez JP, Kottke I (2010) Orchids keep the ascomycetes outside: a highly diverse group of ascomycetes colonizing the velamen of epiphytic orchids from a tropical mountain rainforest in Southern Ecuador. Mycology 1:262–268

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James TY, Kirk PM, Lucking R, Lumbsch HT, Lutzoni FL, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljag U, Kurtzman CP, Larsson K-H, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schußler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao Y-J, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111:509–547

Higgins KL, Coley PD, Kursar TA, Arnold AE (2011) Culturing and direct PCR suggest prevalent host generalism among diverse fungal endophytes of tropical forest grasses. Mycologia 103:247–260

Hilber R, Hilber O (2002) The genus *Lasiosphaeria* and allied taxa. Author, Germany, p 9

Hodge K (2003) Clavicipitaceous anamorphs. In: White JJ, Bacon C, Hywel-Jones NL, Spatafora J (eds) Clavicipitalean fungi: evolutionary biology, chemistry, biocontrol, and cultural impacts. Marcel Dekker, New York, pp 75–123

Hosoya T, Sasagawa R, Hosaka K, Gi-Ho S, Hirayama Y, Yamaguchi K, Toyama K, Kakishima M (2010) Molecular phylogenetic studies of Lachnum and its allies based on the Japanese material. Mycoscience 51:170–181

Hsieh H-M, Ju Y-M, Rogers J (2005) Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97:844–865

Hsieh H, Lin C, Fang M, Rogers JD, Fournier J, Lechat C, Ju Y-M (2010) Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. Mol Phyl Evol 54:957–969

Huhndorf SM, Miller AN, Fernandez FA (2004a) Molecular systematics of the Coronophorales and new species of *Bertia*, *Lasiohertia* and *Nitschkia*. Mycol Res 108:1384–1398

Huhndorf SM, Miller AN, Fernandez FA (2004b) Molecular systematics of the Sordariales: the order and the family Lasiosphaeriaceae redefined. Mycologia 96:368–387

Huhndorf SM, Greif M, Mugambi GK, Miller AN (2008) Two new genera in the Magnaporthaceae, a new addition to *Ceratosphaeria* and two new species of *Lentomitella*. Mycologia 100:940–955

Huhtinen S (1989) A monograph of *Hyaloscypha* and allied genera. Karstenia 29:45–252

Inderbitzin P, Lim SR, Volkmann-Kohlmeyer B, Kohlmeyer J, Berbee ML (2004) The phylogenetic position of *Spathulospora* based on DNA sequences from dried herbarium material. Mycol Res 108:737–748

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schußler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822

Johnston RP (1997) Tropical Rhytismatales. In: Hyde KD (ed) Biodiversity of tropical microfungi. Hong Kong University Press, Hong Kong, pp 241–254

Ju YM, Hsieh HM, He XS (2011) *Xylaria coprinicola*, a new species that antagonizes cultivation of *Coprinus comatus* in China. Mycologia 103:424–430. doi:10.3852/10-215

Ju Y-M, Rogers J (1996) A revision of the genus *Hypoxylon*. Mycol Mem 20:1–365

Jumpponen A, Jones KL (2009) Massively parallel 454-sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytol 184:438–448

Kang JC, Hyde KD, Kong RYC (1999) Studies on Amphisphaeriales: the Cainiaceae. Mycol Res 103:1621–1627. doi:10.1017/S0953756299001264

Kang JC, Kong R, Hyde KD (2002) Phylogeny of Amphisphaeriaceae (sensu stricto) and related taxa revisited based on nrDNA sequences. Mycotaxon 81:321–330

Kimbrough JW (1981) The discomycete centrum. In: Reynolds RD (ed) Ascomycete systematics: the Luttrellian concept. Springer, Berlin, pp 92–101

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth & Bisby's dictionary of the fungi, 8th edn. CABI Bioscience, Surrey

Kirk PM, Cannon PF, Minter DW, Stalpers JA (eds) (2008) Ainsworth and Bisby's dictionary of the fungi. CAB International, Wallingford

Kohlmeyer J, Charles TM (1981) Sclerocarps: undescribed propagules in a sand-inhabiting marine fungus. Can J Bot 59:1787–1791

Kohlmeyer J, Volkmann-Kohlmeyer B (2002) Fungi on *Juncus* and *Spartina*: new marine species of *Anthostomella*, with a list of marine fungi known on *Spartina*. Mycol Res 106:365–374

Kohlmeyer J, Spatafora JW, Volkmann-Kohlmeyer B (2000) Lulworthiales, a new order of marine Ascomycota. Mycologia 92:453–458

Korf RP (1973) Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The fungi: an advanced treatise, vol IVA. Academic Press, New York, pp 249–319

Krause RA, Webster RK (1972) The morphology, taxonomy, and sexuality of the rice stem rot fungus, *Magnaporthe salvinii* (*Leptosphaeria salvinii*). Mycologia 64:103–114. doi:10.2307/3758018

Landvik S, Kristiansen R, Schumacher T (1998) Phylogenetic and structural studies in the Thelebolaceae (Ascomycota). Mycoscience 39:49–56

Lantz H, Johnston PR, Park D, Minter DW (2010) Molecular phylogeny reveals a core clade of Rhytismatales. Mycologia 103:57–74

Lantz H, Johnston PR, Park D, Minter DW (2011) Molecular phylogeny reveals a core clade of Rhytismatales. Mycologia 103:57–74. doi:10.3852/10-060

Leal J, Prieto A, Bernabe M, Hawksworth DL (2010) An assessment of fungal wall heteromannans as a phylogenetically informative character in ascomycetes. FEMS Microbiol Rev 34:986–1014. doi:10.1111/j.1574-6976.2010.00225.x

Liu YJ, Hall BD (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II, phylogeny. Proc Natl Acad Sci U S A 101:4507–4512. doi:10.1073/pnas.0400938101

Liu K, Raghavan S, Nelesen S, Linder CR, Warnow T (2009) Rapid and accurate large-scale coestimation of sequence alignments and phylogenetic trees. Science 324:1561–1564

Livsey S, Monter DW (1994) The taxonomy and biology of *Tryblidiopsis pinastri*. Can J Bot 72:549–557

LoBuglio KF, Pfister DH (2010) Placement of *Medeolaria farlowii* in Leotiomycetes and comments on sampling within the class. Mycol Prog 9:361–368

Locquin MV (1984) Classification Générale des Mycota. Mycologie Générale et Structurale. Masson, Paris, pp 169–175

Lord KM, Read ND (2011) Perithecium morphogenesis in *Sordaria macrospora*. Fungal Genet Biol 48:388–399. doi:10.1016/j.fgb.2010.11

Lumbsch HT, Huhndorf SM (2007a) Whatever happened to the pyrenomycetes and loculoascomycetes? Mycol Res 111:1064–1074

Lumbsch HT, Huhndorf SM (2007b) Outline of Ascomycota – 2007. Myconet 13:1–58

Lumbsch HT, Huhndorf SM (2010) Life and earth sciences no. 1 Fieldiana. Myconet 14:1–64

Lumbsch HT, Schmitt L, Lindemuth R, Miller A, Mangold A, Fernandez F, Huhndorf S (2005) Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (Leotiomyceta). Mol Phylogenet Evol 34:512–524. doi:10.1016/j.ympev.2004.11.007

Luttrell ES (1951) Taxonomy of the pyrenomycetes. Univ Missouri Stud 24:1–120

Luttrell ES (1989) Morphology of *Meliola floridensis*. Mycologia 81:192–204

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora JW, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr Y, Vilgalys R (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Am J Bot 91:1446–1480

Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, Di Pietro A, Dufresne M, Freitag M, Grabherr M, Henrissat B, Houterman PM, Kang S, Shim WB, Woloshuk C, Xie X, Xu JR, Antoniw J, Baker SE, Bluhm BH, Breakspear A, Brown DW, Butchko RA, Chapman S, Coulson R, Coutinho PM, Danchin EG, Diener A, Gale LR, Gardiner DM, Goff S, Hammond-Kosack KE, Hilburn K, Hua-Van A, Jonkers W, Kazan K, Kodira CD, Koehrsen M, Kumar L, Lee YH, Li L, Manners JM, Miranda-Saavedra D, Mukherjee M, Park G, Park J, Park SY, Proctor RH, Regev A, Ruiz-Roldan MC, Sain D, Sakthikumar S, Sykes S, Schwartz DC, Turgeon BG, Wapinski I, Yoder O, Young S, Zeng Q, Zhou S, Galagan J, Cuomo CA, Kistler HC, Rep M (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464:367–373. doi:10.1038/nature08850

Malloch D (1981) The plectomycete centrum. In: Reynold DR (ed) Ascomycete systematics. Springer, New York, pp 73–91

McLaughlin DJ, McLaughlin EG, Lemke PA (eds) (2001) The Mycota VIIA, VIIB: systematics and evolution. Springer, Heidelberg

Mengoni T (1986) El aparato apical del asco de *Cyttaria harioti* (Ascomycetes-Cyttariales) con microscopia fotonica y electonica. Bull Soc Argent Bot 24:393–401

Miller AN, Huhndorf SM (2005) Multi-gene phylogenies indicate ascomal wall morphology is a better predictor of phylogenetic relationships than ascospore morphology in the Sordariales (Ascomycota, Fungi). Mol Phylogenet Evol 35:60–75

Minter DW, Cannon PF (1984) Ascospore discharge in some members of the Rhytismataceae. Trans Br Mycol Soc 83:65–92

Morris MH, Pérez-Pérez MA, Smith ME, Bledsoe CS (2008) Multiple species of ectomycorrhizal fungi are frequently detected on individual oak root tips in a tropical cloud forest. Mycorrhiza 18:375–383

Müller E, von Arx J (1973) Pyrenomycetes: Meliolales, Coronophorales, Sphaeriales. In: Ainsworth G, Sparrow F, Sussman A (eds) The fungi IV. Academic Press, New York, pp 87–132

Nakagiri A (1984) Two new species of *Lulworthia* and evaluation of genera-delimiting characters between *Lulworthia* and *Lindra* (Halosphaeriaceae). Trans Mycol Soc Jpn 25:377–388

Nakagiri A, Tubaki K (1983) *Lindra obtusa*, a new marine ascomycete and its *Anguillospora* anamorph. Mycologia 75:487–497

Nannfeldt JA (1932) Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Reg Soc Sci Ups IV 8:1–368

Napoli C, Mello A, Borra A, Vizzini A, Sourzat P, Bonfante P (2010) *Tuber melanosporum*, when dominant, affects fungal dynamics in truffle grounds. New Phytol 185:237–247

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71:5544–5550

O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers HJ, Summerbell RC, Robert VARG, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM (2010) Internet-accessible DNA sequence database for identifying Fusaria from human and animal infections. J Clin Microbiol 48:3708–3718. doi:10.1128/JCM. 00989-10

Ohenoja E, Wang Z, Townsend JP, Mitchel D, Votik A (2010) Northern species of earth tongue genus *Thuemenidium* revisited, considering morphology, ecology and molecular phylogeny. Mycologia 102:1089–1095

Okada G, Seifert KA, Takematsu A, Yamaoka Y, Miyazaki S, Tubaki K (1998) A molecular phylogenetic reappraisal of the taxonomy of the *Graphium* complex based on 18S rDNA sequences. Can J Bot 76:1495–1506

Pelaez F, Gonzalez V, Platas G, Sanchez-Ballesteros J, Rubio V (2008) Molecular phylogenetic studies within the Xylariaceae based on ribosomal DNA sequences. Fungal Divers 31:111–134

Peterson KR, Pfister DH (2010) Phylogeny of *Cyttaria* inferred from nuclear and mitochondrial sequence and morphological data. Mycologia 102:1398–1416

Peterson KR, Pfister DH, Bell CD (2010) Cophylogeny and biogeography of the fungal parasite *Cyttaria* and its host Nothogagus, southern beech. Mycologia 102:1417–1425

Pfister DH, Kimbrough JW (2001) Discomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota VIIA, VIIB: systematics and evolution. Springer, Berlin, pp 257–281

Queloz V, Sieber TN, Holdenrieder O, McDonald BA, Grünig CR (2011) No biogeographical pattern for a root-associated fungal species complex. Global Ecol Biogeogr 20:160–169

Raja H, Shearer CA (2008) Freshwater ascomycetes: new and noteworthy species from aquatic habitats in Florida. Mycologia 100:467–489. doi:10.3852/07-167R

Read ND, Beckett A (1996) Ascus and ascospore morphogenesis. Mycol Res 100:1281–1314

Réblov M, Seifert KA (2004) *Cryptadelphia* (Trichosphaeriales), a new genus for holomorphs with *Brachysporium* anamorphs and clarification of the taxonomic status of *Wallrothiella*. Mycologia 96:343–367. doi:10.2307/3762067

Réblov M, Winka K (2000) Phylogeny of *Chaetosphaeria* and its anamorphs based on morphological and molecular data. Mycologia 92:939–954

Réblov M, Gams W, Seifert KA (2011) Monilochaetes and allied genera of the Glomerellales, and a reconsideration of families in the Microascales. Stud Mycol 68:163–191. doi:10.3114/sim.2011.68.07

Robbertse B, Reeves JB, Schoch CL, Spatafora JW (2006) A phylogenomic analysis of the Ascomycota. Fungal Genet Biol 43:715–725. doi:10.1016/j.fgb.2006.05.001

Rogerson CT, Samuels GJ (1989) Boleticolous species of *Hypomyces*. Mycologia 81:413–432

Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798–804

Rossman A, Samuels G, Rogerson C, Lowen R (1999) Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Stud Mycol 42:1–248

Saenz GS, Taylor JW, Gargas A (1994) 18S rRNA gene sequences and supraordinal classification of the Erysiphales. Mycologia 86:212–216

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96:195–206

Samuels GJ, Blackwell M (2001) Pyrenomycetes-fungi with perithecia. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota VII part A. Springer, Berlin, pp 221–255

Samuels GJ, Müller E (1978) Life history studies of Brazilian Ascomycetes 5. Two new species of Ophiostoma and their Sporothrix anamorphs. Sydowia 31:169–179

Samuels GJ, Lu BS, Chaverri P, Candoussau F, Fournier J, Rossman AY (2009) *Cyanonectria*, a new genus for *Nectria cyanostoma* and its *Fusarium* anamorph. Mycol Prog 8:49–58. doi:10.1007/s11557-008-0577-x

Snchez-Ballesteros J, Gonzlez V, Salazar O, Acero J, Portal M, Julin M, Rubio V, Bills G, Polishook J, Platas G, Mochales S, Pelez F (2000) Phylogenetic study of *Hypoxylon* and related genera based on ribosomal ITS sequences. Mycologia 92:964–977

Sati SC, Belwall M (2005) Aquatic hyphomycetes as endophytes of riparian plant roots. Mycologia 97:45–49

Schoch CL, Sung GH, Volkmann-Kohlmeyer B, Kohlmeyer J, Spatafora JW (2007) Marine fungal lineages in the Hypocreomycetidae. Mycol Res 111:154–162

Schoch CL, Sung G-H, López-Girldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Andrie R, Trippe K, Ciuffetti L, Amtoft A, Fraker E, Hodkinson BP, Bonito G, Lutzoni F, Groenewald JZ, Arzanlou M, de Hoog S, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh S-O, Blackwell M, Hillis DM, Griffith G, Castlebury L, Rossman A, Lumbsch HT, Lücking R, Diederich P, Ertz D, Geiser D, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Rogers J, Shoemaker R, Sugiyama J, Summerbell R, Untereiner W, Johnston P, Stenroos S, Zuccaro A, Dyer P, Crittenden P, Trappe JM, Spatafora JW (2009a) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Schoch CL, Wang Z, Townsend JP, Spatafora JW (2009b) Geoglossomycetes cl. nov., Geoglossales ord. nov. and taxa above class rank in the Ascomycota tree of life. Persoonia 22:129–138

Schumacher T, Sivertsen S (1987) *Sarcoleotia globosa* (Sommerf.:Fr.) Korf, taxonomy, ecology and distribution. In: Larsen GA et al (eds) Arctic alpine mycology. Plenum, New York, pp 163–176

Seephueak P, Petcharat V, Phongpaichit S (2010) Fungi associated with leaf litter of *para rubber* (Hevea brasiliensis). Mycology 1:213–227

Seifert KA, Gams W (2001) The taxonomy of anamorphic fungi. In: McLaughlin DJ, McLaughlin EG,

Lemke PA (eds) The Mycota VII part A. Springer, Berlin, pp 307–347

Selosse M, Vohnik M, Chauvet E (2008) Out of the rivers: are some aquatic hyphomycetes plant endophytes? New Phytol 178:3–7

Shearer CJ (1993) The freshwater ascomycetes. Nova Hedwigia 56:1–33

Singleton LL, Mihail JD, Rush CM (eds) (1992) Methods for research on soil-borne phytopathogenic fungi. American Phytopathological Society Press, St. Paul, MN

Smith GJD, Liew ECY, Hyde KD (2003) The Xylariales: a monophyletic order containing 7 families. Fungal Divers 13:175–208

Smith D, Onions HS (1983) The preservation and maintenance of living fungi. Commonwealth Mycological Institute, Kew

Soca-Chafre G, Rivera-Orduna FN, Hidalgo-Lara ME, Hernandez-Rodriguez C, Marsch R, Flores-Cotera LB (2011) Molecular phylogeny and paclitaxel screening of fungal endophytes from *Taxus globosa*. Fungal Biol 115:143–156

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, Ver Loren van Themaat E, Brown JK, Butcher SA, Gurr SJ, Lebrun MH, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O'Connell RJ, Oberhaensli S, Parlange F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristn S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Wessling R, Wicker T, Panstruga R (2010) Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. Science 330:1543–1546

Spatafora JW, Blackwell M (1994) The polyphyletic origins of ophiostomatoid fungi. Mycol Res 98:1–9

Spatafora JW, Volkmann-Kohlmeyer B, Kohlmeyer J (1998) Independent terrestrial origins of the Halosphaeriales (marine Ascomycota). Am J Bot 85:1569–1580

Spatafora JW, Sung GH, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser DM, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL (2006) A five-gene phylogeny of Pezizomycotina. Mycologia 98:1018–1028

Spooner BM (1987) Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. Bibl Mycol 116:1–711

Stenroos S, Laukka T, Huhtinen S, Döbbeler P, Myllys L, Syrjänen K, Hyvönen J (2009) Multiple origins of symbioses between ascomycetes and bryophytes suggested by a five-gene phylogeny. Cladistics 25:1–20

Strobel G, Tomsheck A, Geary B, Spakowicz D, Strobel S, Mattner S, Mann R (2010) Endophyte strain NRRL50072 producing volatile organics is a species of *Ascocoryne*. Mycology 1:187–194

Suh SO, Blackwell M (1999) Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. Mycologia 91:836–848

Summerbell R (2003) *Aspergillus*, *Fusarium*, *Sporothrix*, *Piedraia*, and their relatives. In: Howard DH (ed) Pathogenic fungi in humans and animals. Marcel Dekker, New York, pp 237–498

Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, fungi): identification of localized incongruence using a combinational bootstrap approach. Mol Phyl Evol 44:1204–1222

Sutton BC, Hennebert GL (1995) Interconnections amongst anamorphs and their possible contribution to ascomycetes systematics. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, pp 77–100

Takamatsu S (2004) Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. Mycoscience 45:147–157

Takamasu S, Niinomi S, de Alvarez MGG, Alvarez RE, Havrylenko M, Braun U (2005) *Caespitotheca* gen. nov., an ancestral genus in the Erysiphales. Mycol Res 109:903–911

Takamasu S, Niinomi S, Harada M, Havrylenko M (2010) Molecular phylogenetic analyses reveal a close evolutionary relationship between *Podospheara* (Erysiphales: Erysiphaceae) and its rosaceous hosts. Persoonia 24:38–48

Tang AMC, Jeewon R, Hyde KD (2007) Phylogenetic utility of protein (RPB2, beta-tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of Sordariomycetes (Ascomycota, fungi). Anton Leeuw Int J G Mol Microbiol 91:327–349. doi:10.1007/s10482-006-9120-8

Tavares II (1985) Laboulbeniales. Mycol Mem 9:1–627

Tedersoo L, Pärtel K, Jalrus T, Gates G, Põldmaak K, Tamm H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. Environ Microbiol 11:3166–3178

Thaxter R (1896) Contribution towards a monograph of the Laboulbeniales. Mem Am Acad Arts Sci 12:187–429

Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, McKenzie E, Hyde KD (2009) Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a new species *Ophioceras chiangdaoense* from *Dracaena loureiroi* in Thailand. Fungal Divers 34:157–173

Triebel D, Derek P, Wollweber H, Stadler M (2005) Phylogenetic relationships among *Daldinia*, *Entonaema*, and *Hypoxylon* as inferred from ITS nrDNA analyses of Xylariales. Nova Hedwigia 80:25–43

Upson R, Newsham KK, Bridge PD, Pearce DA, Read DJ (2009) Taxonomic affinities of dark septate root endophytes of Colobauthus quitensis and Deschampsia autarctica, the two native Antarctic vascular plant species. Fungal Ecol 2:184–196

U'Ren JM, Lutzoni F, Miadlkowska J, Arnold AE (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microb Ecol 60:340–353

Verkley GJM (1994) Ultrastructure of the apical apparatus in Leotia lubrica and some Geoglossaceae (Leotiales, Ascomycotina). Persoonia 15:405–430

Verkley GJM (1996) Ultrastructure of the ascus in the genera Lachnum and Trichopeziza (Hyaloscyphaceae, Ascomycotina). Nova Hedwigia 63:215–228

Vijaykrishna D, Mostert L, Jeewon R, Gams W, Hyde KD, Crous PW (2004) *Pleurostomophora*, an anamorph of *Pleurostoma* (Calosphaeriales), a new anamorph genus morphologically similar to *Phialophora*. Stud Mycol 50:387–395

Wanderlei-Silva D, Neto E, Hanlin RT (2003) Molecular systematics of the Phyllachorales (Ascomycota, fungi) based on 18S ribosomal DNA sequences. Brazil Arch Biol Technol 46:315–322

Wang Z, Binder M, Hibbett DS (2002) A new species of *Cudonia* based on morphological and molecular data. Mycologia 94:641–650. doi:10.2307/3761715

Wang Z, Binder M, Hibbett DS (2005) Life history and systematics of the aquatic discomycete Mitrula (Helotiales, Ascomycota) based on cultural, morphological, and molecular studies. Am J Bot 92:1565–1574

Wang Z, Binder M, Schoch CL, Johnston PR, Spatafor JW, Hibbett DS (2006a) Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): a nuclear rDNA phylogeny. Mol Phylogenet Evol 41:295–312

Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS (2006b) Toward a phylogenetic classification of Leotiomycetes based on rDNA data. Mycologia 98:1065–1075

Wang Z, Johnston PR, Yang Z-L, Townsend JP (2009) Evolution of reproductive morphology in leaf endophytes. PLoS One 4:e4246

Wang Z, Nilsson RH, Lopez-Giraldez F, Zhuang WY, Dai YC, Johnston PR, Townsend JP (2011) Tasting soil fungal diversity with earth tongues: phylogenetic test of SATè alignment for environmental ITS data. PLoS One 6:e19039

Weber RWS (2009) Recent developments in the molecular taxonomy of fungi. In: Anke T, Weber D (eds) The Mycota XV: physiology and genetics, 1st edn. Springer, Berlin, pp 1–15

Weir A, Blackwell M (2001) Molecular data support the Laboulbeniales as a separate class of Ascomycota, Laboulbeniomycetes. Mycol Res 105:1182–1190

Weir A, Hammond PM (1997) Laboulbeniales on beetles: host utilization patterns and species richness of the parasites. Biodivers Conserv 6:701–719

Wingfield M, Seifert K, Webber J (1993) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathology. American Phytopathological Society, St. Paul, MN

Xu JR, Zhao XH, Dean RA (2007) From genes to genomes: a new paradigm for studying fungal pathogenesis in *Magnaporthe oryzae*. Adv Genet 57:175–218

Zaffarano P, Duò A, Grünig CR (2010) Characterization of the mating type (MAT) locus in the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex. Fungal Genet Biol 47:761–772

Zhang N, Blackwell M (2001) Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the Diaporthales. Mycologia 93:355–365

Zhang N, Blackwell M (2002) Molecular phylogeny of *Melanospora* and similar pyrenomycetous fungi. Mycol Res 106:148–155

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98:1076–1087. doi:10.3852/mycologia.98.6.1076

Zhang N, Zhao S, Shen Q (2011) A six-gene phylogeny reveals the evolution of mode of infection in the rice blast fungus and allied species. Mycologia 103:1267–1276. doi:10.3852/11-022

Zhuang WY (1988) Studies on some discomycete genera with an ionomidotic reaction: *Ionomidotis*, *Poloniodiscus*, *Cordierites*, *Phyllomyces* and *Ameghiniella*. Mycotaxon 2:261–298

Zhuang WY, Yu ZH, Wu WP, Langue C, Fouret N (2000) Preliminary notes on phylogenetic relationships in the Encoelioideae inferred from 18S rDNA sequences. Mycosystema 19:478–484

4 Pezizomycotina: Lecanoromycetes

Cécile Gueidan[1,2], David J. Hill[3], Jolanta Miadlikowska[4], Francois Lutzoni[4]

CONTENTS

I. Introduction 89
II. Occurrence and Distribution 90
III. Substrate Range and Ecology 91
A. Substrate Range 91
B. Lifestyles 91
C. Mycobiont–Photobiont Associations ... 91
IV. Mycobiont–Photobiont Cellular Contacts 92
V. Morphological and Chemical Features 93
A. Thalli 93
B. Ascomata 93
1. Ascoma Morphology 93
2. Ascoma Development 95
C. Asci 96
1. Ascus Walls 96
2. Ascus Apical Structure 96
3. Dehiscence Mechanisms 97
D. Ascospores 97
E. Interascal Filaments 98
F. Pycnidia 98
G. Asexual Propagules 98
H. Secondary Compounds 99
VI. Origin and Diversification 99
VII. Orders and Classification 100
A. Acarosporales 100
B. Baeomycetales 102
C. Caliciales 103
D. Candelariales 103
E. Lecanorales 104
F. Lecideales 105
G. Ostropales 105
H. Peltigerales 106
I. Pertusariales 107
J. Rhizocarpales 108
K. Sarrameanales 108
L. Teloschistales 109
M. Trapeliales 109
N. Umbilicariales 110
VIII. Conclusion 110
References 111

I. Introduction

Lecanoromycetes (formally named by Eriksson and Winka in 1997) constitutes one of the largest classes of Ascomycota with 14,199 known species (Kirk et al. 2008). The class mainly includes ascomycetes characterized by apothecial ascomata, amyloid asci with a two-layered wall and an apical thickening, and a hamathecium (interascal hyphae) formed with paraphyses or pseudoparaphyses. **This group comprises the largest number of lichen-forming ascomycetes, with approximately 90 % of all known lichen-forming fungi** (Miadlikowska et al. 2006). However, not all members of this class are lichenized; several nonlichenized species are nested within this lineage (Lutzoni et al. 2001, 2004; Schoch et al. 2009). Lecanoromycetes are well known for the broad range of secondary compounds, such as depsidones, terpenoids, and xanthones (Huneck and Yoshimura 1996), that they exclusively produce, in some cases in large quantities out of proportion to the dry weight of their thalli.

In the past, ascoma structure or development and ascus types were used as the main characters in the classification of ascomycete fungi. Nannfeldt (1932) classified the euascomycetes according to their type of ascomatal development: Plectascales, Ascoloculares, and Ascohymeniales, where most Lecanoromycetes

[1]Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
[2]CSIRO, NRCA, Australian National Herbarium, GPO box 1600, Canberra, ACT 2601, Australia; e-mail: Cecile.Gueidan@csiro.au
[3]School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK; e-mail: D.J.Hill@bristol.ac.uk
[4]Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA; e-mail: jolantam@duke.edu; flutzoni@duke.edu

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)

© Springer-Verlag Berlin Heidelberg 2015

belonged. Luttrell (1951, 1955) in his classification system gave more importance to the ascus types and classified the euascomycetes as Bitunicatae (the Ascoloculares) and Unitunicatae (the Ascohymeniales and Plectascales). Unitunicatae were then subdivided according to their ascomata morphology: Plectomycetes, Pyrenomycetes, and Discomycetes, where most Lecanoromycetes belonged (Luttrell 1955). However, subsequent ultrastructural and ontogenetical studies showed that the complexity of these traits had been underestimated and that many taxa did not fit into these broad categories because of intermediate types (e.g., Bellemère and Letrouit-Galinou 1987; Henssen and Jahns 1974; Honegger 1982a). The reliability of these characters for higher-level classification was therefore doubted even before the beginning of the molecular era (e.g., Bellemère 1994; Poelt 1973; Reynolds 1989).

Molecular studies confirmed that classifications based on ascomatal characters often failed to capture monophyletic lineages within the ascomycetes (Berbee 1996; Lindemuth et al. 2001; Lumbsch and Huhndorf 2007a; Lutzoni et al. 2001; Liu and Hall 2004; Spatafora et al. 2006; Schoch et al. 2009). In particular, some groups within Lecanoromycetes (e.g., Ostropales, Caliciales) were shown to include a much larger diversity of ascoma and asci types than expected (Grube et al. 2004; Schmitt et al. 2005; Wedin and Tibell 1997; Wedin et al. 2000a). **Molecular phylogenetic studies also cast doubts on the morphology-based delimitation of orders and families in Lecanoromycetes.** As a result, many families and genera were segregated from the large order Lecanorales, while several additional families were included in Ostropales, the second largest order in Lecanoromycetes (Grube et al. 2004; Hofstetter et al. 2007; Kauff and Lutzoni 2002; Lumbsch et al. 2004; Miadlikowska and Lutzoni 2004; Miadlikowska et al. 2006; Wedin et al. 2005). Lecanoromycetes currently includes 14 orders and 3 subclasses: Acarosporomycetidae, Lecanoromycetidae, and Ostropomycetidae (Gaya et al. 2012; Hodkinson and Lendemer 2011; Lumbsch and Huhndorf 2010; Schmull et al. 2011).

II. Occurrence and Distribution

Lecanoromycetes have a broad distribution and can be found from the tropics to the poles. Their biomass is substantial in boreal to arctic climates, especially in the tundra, where species such as *Cladonia rangiferina* can constitute the main vegetation cover and are the main primary producer and food source for large herbivorous mammals (Brodo et al. 2001). In Antarctica, lichens (mostly from the class Lecanoromycetes) form the most species-diverse group within the vegetation cover, and some of them are found in the coldest and driest habitats of continental Antarctica (Green et al. 1999; Øvstedal and Lewis Smith 2001), where they are the basis of terrestrial life closest to a pole on Earth.

Lecanoromycetes are also very common and diverse in temperate regions, where they have been extensively studied (e.g., Brodo et al. 2001; Clauzade and Roux 1985; McCarthy 2003; Smith et al. 2009). In the tropics, they are thought to be as species diverse as in temperate regions, if not more so (Aptroot and Sipman 1997; Coppins and Wolseley 2002; Lücking 2008). A recent study in Papua New Guinea showed that a single *Elaeocarpus* tree could harbor up to 173 species of lichens, among which approximately 130 species were Lecanoromycetes (Aptroot 2001). Many tropical regions of the world are still mainly understudied, and many new species remain to be discovered (Sipman and Aptroot 2001).

Lecanoromycetes are found in a large number of natural terrestrial habitats, such as woodlands, heathlands, lowland and alpine grasslands, deserts, and arid shrublands. They are also commonly found in urban areas, wastelands, and other anthropogenous habitats (Gilbert 1990; Smith et al. 2010). Although most species occur in terrestrial environments, some species can grow on temporarily submerged substrates, in freshwater (Gilbert

1996; Gilbert and Giavarini 1997; Thüs and Schultz 2009), or in saltwater in the supralittoral zone (Brodo and Sloan 2004; Fletcher 1980).

III. Substrate Range and Ecology

A. Substrate Range

Lecanoromycetes grow predominantly on **tree bark** (corticolous species) and **rocks** (saxicolous species) but are also found on **leaves** (foliicolous species), **soil** (terricolous species), **wood** (lignicolous species), **mosses** (muscicolous species), and **other lichens** (lichenicolous species). Most species tend to be specific to a particular type of substrate, although some can colonize several (e.g., *Parmelia sulcata*). In addition to a natural substrate, some Lecanoromycetes can grow on human-made materials, such as brick, concrete, asphalt, metal, plastic, glass, and rubber (Brodo et al. 2001; Gilbert 1990).

A few species even occur on the bones or shells of land tortoises (Brodo et al. 2001) and indeed on any material with a stable surface exposed for long enough. A few others, mainly from the genera *Aspicilia* s.l. and *Xanthoparmelia*, do not attach to a substrate and occur as an erratic or vagrant (e.g., Pérez 1997; Rosentreter 1993).

B. Lifestyles

In Lecanoromycetes, most lichen-forming (mycobionts) fungi form **mutualistic associations** with one or two photosynthetic partners, either a green alga or a cyanobacterium (photobiont). Mycobionts obtain carbon from their photobiont, in the form of glucose when associated with cyanobacteria or in the form of polyhydric sugar alcohols (polyols) when associated with green algae (Palmqvist et al. 2008). Cyanobacteria found in lichens, such as *Nostoc*, can fix nitrogen and, therefore, can be a source of nitrogen for the mycobiont. The mycobiont protects the photobiont from UV light, temperature extremes, and, to some extent, dessication (Nash 2008). The great majority of lichenized fungi in Lecanoromycetes are obligate mutualists, but a few species of *Stictis* can occur either as lichen symbionts or as nonlichenized saprotrophs, depending on the substrate (Wedin et al. 2004). Some lichenized fungi can also be parasitic on other lichens, either throughout their life (Lawrey and Diederich 2003) or only in the first stage of their development (e.g., *Diploschistes muscorum*) (Friedl 1987). Finally, several lineages within Lecanoromycetes are always nonlichenized, most likely because of secondary losses of lichenization (Baloch et al. 2010; Gueidan et al. 2008; Lutzoni et al. 2001; Schoch et al. 2009). These nonlichenized lineages can have diverse lifestyles, **ranging from saprophytism to parasitism** (Lawrey and Diederich 2003; Sherwood 1977a, b; Sherwood-Pike 1987). They mostly occur on lichens (Lawrey and Diederich 2003) but are also found on diverse substrates such as bark or wood (e.g., Stictidaceae, Odontotremataceae).

C. Mycobiont–Photobiont Associations

Most Lecanoromycetes species associate with a single photobiont, a green alga (chlorobiont) or a cyanobacterium (cyanobiont). Some lichen-forming fungi are associated with both types of photobionts, forming tripartite thalli where the cyanobacterial photobiont is an accessory partner. The cyanobiont is then restricted to special organs of the thallus called cephalodia [although see exceptions in Henskens et al. (2012)], which can be internal or external outgrowths. Cyanobionts fix atmospheric nitrogen, transferring ammonia to the other partners. These tripartite associations are common in Lecanorales (e.g., *Stereocaulon*) and Peltigerales (e.g., *Lobaria*, *Nephroma*, *Peltigera*).

The majority of photobionts belong to Chlorophyta [approximately 90 % according to Tschermak-Woess (1988a); see also Ahmadjian (1967, 1993)]. The green algal genera *Trebouxia*, *Asterochloris* (Trebouxiophyceae), and *Trentepohlia* (Ulvophyceae) are the most common photobionts for Lecanoromycetes.

Other genera of photobionts associated with Lecanoromycetes include *Chlorella* (e.g., in *Pseudocyphellaria*) (Tschermak-Woess 1988b), *Chlorosarcinopsis* (in *Lecidea*) (Plessl 1963), *Coccomyxa* (e.g., in *Icmadophila*, *Peltigera*, *Solorina*) (Jaag 1933), *Dictyochloropsis* (e.g., in *Lobaria*, *Pseudocyphellaria*) (Tschermak-Woess 1984),

Elliptochloris (e.g., in *Baeomyces*) (Tschermak-Woess 1985), *Leptosira* (in *Vezdaea* and *Thrombium*) (Tschermak-Woess 1953; Tschermak-Woess and Poelt 1976), *Myrmecia* (e.g., in *Psora*) (Geitler 1963), and *Phycopeltis* (e.g., in Porinaceae) (Santesson 1952).

Cyanobacterial photobionts are associated with a relatively small number of lichen-forming fungal species (10 %, Tschermak-Woess 1988a; approximately 1,700 species, Rikkinen 2002) and, in Lecanoromycetes, are restricted to Arctomiaceae, Stereocaulaceae, Trapeliaceae, and Peltigerales. **The most common cyanobacterial (primary or accessory) lichen photobionts are *Nostoc* and *Rhizonema*** (Lücking et al. 2009b; Rambold et al. 1998; Tschermak-Woess 1988a). The cyanobacterial genus *Scytonema* was also listed in the past as one of the most common lichen photobionts, but a recent molecular study showed that all lichenized strains of *Scytonema* studied (including those from the Lecanoromycetes genera *Coccocarpia* and *Stereocaulon*) belonged to *Rhizonema*, a new strictly lichenized cyanobacterial lineage genetically distinct from *Scytonema* (Lücking et al. 2009b). The current status of the genus *Scytonema* as a lichen photobiont is therefore in need of determination using molecular data. *Stigonema* and *Gloeocapsa* are also often found in lichens but more often as accessory photobionts (Rambold et al. 1998).

Other cyanobacteria occasionally found as photobionts are *Anabaena* (in *Stereocaulon*) (Duvigneaud 1955), *Calothrix* (in *Coccotrema*, *Stereocaulon*) (Brodo 1973; Lamb 1977), *Dichothrix* (in *Placynthium nigrum*) (Geitler 1934), *Hyphomorpha* (in *Spilonema*) (Henssen 1981), and *Tolypothrix* (in *Hertella*) (Henssen 1985).

Interestingly, patterns of mycobiont–photobiont associations have been observed at a high taxonomic level (Miadlikowska et al. 2006; Rambold et al. 1998). This is somewhat surprising for a symbiotic system that is believed to transmit its photobiont mostly horizonally across generations. However, the contribution of fungal sexual (horizontal transmission) versus asexual (vertical transmission) reproduction to the genetic composition of the mycobiont and photobiont populations has rarely been quantified. Dal Grande et al. (2012) estimated that more than 70 % of lichen thalli across worldwide populations of *Lobaria pulmonaria* were derived from asexual propagules. No recombination was detected for the photobiont, while it was detected in only 7.7 % of the mycobiont individuals. Therefore, vertical transmission of the photobiont through thallus fragments or differentiated asexual propagules might be more prominent than expected in shaping lichen populations and could explain in part the pattern of association observed at a high taxonomic level within Lecanoromycetes.

A population ecology study of the geographically widespread lichen *Cetraria aculeata* revealed that climate and codispersal are the most relevant factors shaping the genetic structure of the photobiont and that rare photobiont switches enabled the mycobiont to achieve a broad geographical distribution crossing bioclimatic zones (Fernandez-Mendoza et al. 2011).

In Lecanoromycetes, the following **patterns of mycobiont–photobiont associations** have been observed (Miadlikowska et al. 2006; Rambold et al. 1998). Certain lineages, such as Parmeliaceae and Teloschistales, are mainly associated with *Trebouxia*, whereas Cladoniaceae, Stereocaulaceae, and Icmadophilaceae are mostly associated with *Asterochloris*. In Peltigerales, *Nostoc* is the main photobiont, and in Ostropales, *Trentepohlia* is the most common photobiont. Although some photobiont associations could potentially be used to classify higher taxa within Lecanoromycetes, current knowledge on the identity of lichen photobionts is still too sparse. They are notoriously difficult to identify when lichenized, or even when cultured, based on morphology only (Friedl and Büdel 2008), and molecular data are currently only available for a limited number of taxa.

IV. Mycobiont–Photobiont Cellular Contacts

The type of cellular contacts between symbionts ranges from appressoria to haustoria in Lecanoromycetes (Honegger 2008), and their structure depends on various factors. The taxonomic affiliation of photobionts plays

a large role, especially its morphology and cell wall composition (Honegger 2008). A correlation has been observed between thallus growth forms and types of cellular contact between symbionts, with simple crustose species tending to have less complex contact structures than do foliose or fruticose species (Honegger 2008). Environmental conditions can also be responsible for variation in the extent of penetration by the fungus (Ben-Shaul et al. 1969; Galun et al. 1970), as well as thallus age (Collins and Farrar 1978; Galun 1988; Geitler 1963). As a result, this character has not been used in the classification of lichen-forming fungi (Poelt 1973).

V. Morphological and Chemical Features

A. Thalli

The lichen thallus is a vegetative structure formed by the fungal hyphae and algal cells. In Lecanoromycetes, thallus structures range from simple to more complex organizations. The simplest thalli are found in leprose (powderlike, Fig. 4.1a) and byssoid (cottonlike) species, which lack differentiation into strata, and are formed of loosely intermingled fungal hyphae and algal cells (Ekman and Tønsberg 2002; Kantvilas 1996; Poelt 1987). The complex thalli (heteromerous) are differentiated into layers (Fig. 4.2): upper cortex, photobiont layer, medulla, and lower cortex.

Two main types of lichen thalli are recognized, mainly for convenience: microlichens and macrolichens. Microlichens can consist of powderlike or cottonlike thalli (**leprose and byssoid** thalli) or corticated granules (**granulose** thalli) or appear as crusts tightly appressed to the substrate and generally lacking rhizines and a lower cortex (**crustose** thalli) (Fig. 4.1b). Crustose thalli are quite diverse, ranging from continuous or slightly cracked crusts to crusts formed by areoles or small lobes often on a fungal hyphal mat (hypothallus). Crustose species generally grow above the substrate, but some have a thallus developing within the superficial layer of the substrate, either in rock (endolithic species such as *Clauzadea immersa*) or in bark (endophloeic species such as *Caloplaca cerinella* or *Lecanora persimilis*).

The largest thalli are generally found in squamulose, foliose, and fruticose lichens (macrolichens). **Squamulose** lichens are composed of scattered or imbricated scalelike lobes. **Foliose** lichens have dorsiventral thalli formed by lobes mostly with a lower cortex, which are often only loosely attached to the substrate, most often by attachment structures such as rhizines (e.g., *Peltigera*) (Fig. 4.1c). Foliose thalli attached to the substrate with a single holdfast are called umbilicate and can be formed either by a single lobe or by several lobes (e.g., *Umbilicaria*) (Hestmark 1997). Lichens with long striplike or cordlike branches that are hanging or standing upward from their substrates are called **fruticose** (e.g., *Usnea*, *Ramalina*) (Fig. 4.1d). Some fruticose lichens (e.g., *Cladonia*) have a mixed thallus formed by a basal squamulose primary thallus and trumpetlike or spikelike structures (podetia) growing upward from the primary thallus and bearing fruiting bodies (secondary thallus).

Thallus growth forms were of prime importance in shaping the first classifications of lichens (Zahlbruckner 1926). With improved microscopical techniques, anatomical and ultrastructural characters became available, and ascomatal characters replaced thallus morphology as the main characters used in classification (e.g., Hafellner 1984; Henssen and Jahns 1974; Luttrell 1951, 1955; Nannfeldt 1932; Poelt 1973). Molecular data confirmed that thallus morphology could not be strictly used for classification purposes because most growth forms were shown to have evolved several times independently in Lecanoromycetes and in lichens in general [e.g., Grube and Arup 2001; Schmitt et al. 2001; Stenroos and DePriest 1998; see also the review in Grube and Hawksworth (2007)].

B. Ascomata

1. Ascoma Morphology

Ascomata (Fig. 4.3) are structures producing spore-bearing cells called asci. They generally consist of a hymenium enveloped by a pro-

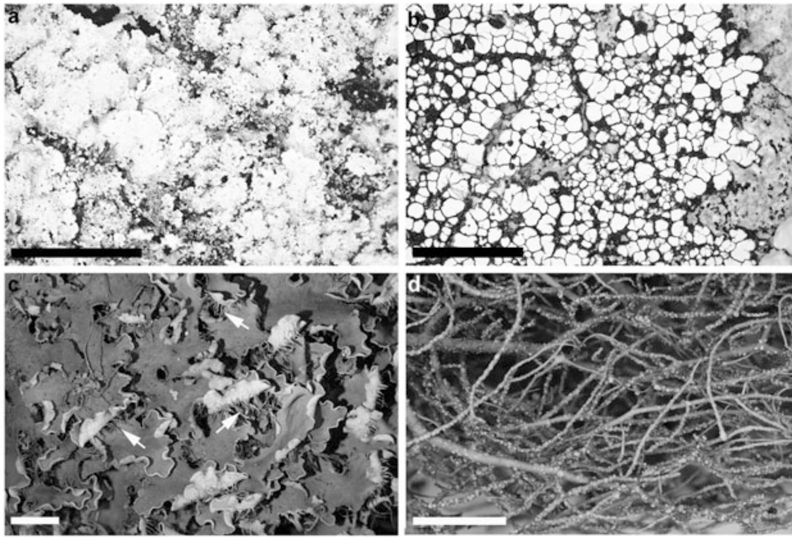


Fig. 4.1 Different types of thallus in Lecanoromycetes. (**a**) Powderlike leprose thallus of *Lepraria membranacea*. (**b**) Crustose areolate thallus of saxicolous species *Rhizocarpon macrosporum*. (**c**) Foliose thallus of *Peltigera rufescens*, with white rhizines visible on lower surface (*white arrows*). (**d**) Cordlike branches of fruticose thallus of *Usnea subfloridana*. Bars = 5 mm

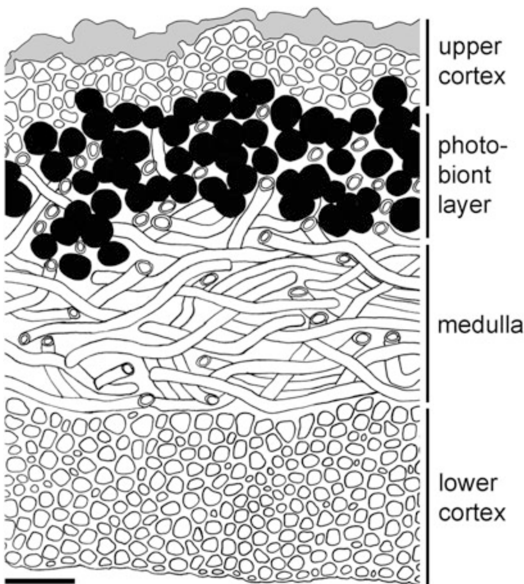


Fig. 4.2 Schematic representation of a cross section in thallus of *Xanthoria parietina*. The thallus is composed of four layers: upper cortex, photobiont layer, medulla, and lower cortex. Bar = 20 μm

tective structure called an excipulum. The hymenium comprises asci and sterile interascal hyphae. There are two main types of ascomata in Lecanoromycetes. The most common type is the **disc-shaped apothecium, where the upper portion of the hymenium is mostly exposed** (Fig. 4.3a). When laterally elongated they are referred to as lirellate apothecia (Fig. 4.3b). In Lecanoromycetes, lirellate apothecia are restricted to Ostropales but can also be found in Arthoniomycetes, a distinct class within Leotiomyceta (Pezizomycotina), which also includes many lichen-forming fungi. The second type of fruiting body in Lecanoromycetes is the **flask-shaped perithecium** (Fig. 4.3c), **where the hymenium is exposed only through an ostiole**. The term perithecium was restricted in the past to species with an ascohymenial development (ascostromatic flask-shaped ascomata were called pseudothecia), but it is now used in a broad sense for all flask-shaped ascomata, regardless of their development type (Kirk et al. 2008). Perithecia are only found in a few lineages in Lecanoromycetes (e.g., Porinaceae, Protothelenellaceae, and Thelenellaceae). Some species have apothecia that can be confused with perithecia because they are immersed in the thallus and the hymenium is exposed only through a small aperture. Called perithecioid apothecia (Fig. 4.3d), these

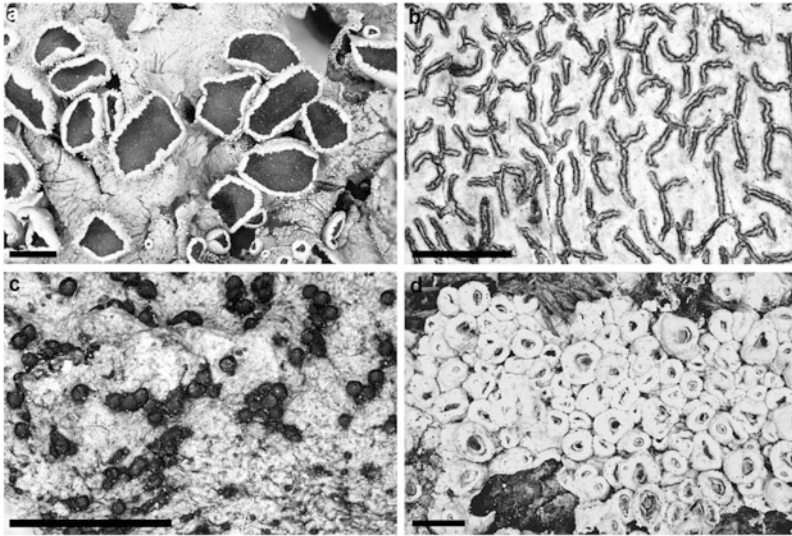


Fig. 4.3 Examples of fruiting bodies in Lecanoromycetes. (**a**) Disc-shaped fruiting bodies (apothecia) in *Xanthoparmelia tinctina*. (**b**) Elongated fruiting bodies (lirellate apothecia) in *Graphis scripta*. (**c**) Flask-shaped fruiting bodies (perithecia) in *Porina guentheri*. (**d**) Enclosed disc-shaped fruiting bodies (perithecioid apothecia) in *Thelotrema lepadinum*. Bars = 2.5 mm

ascomata are frequently found in the subclass Ostropomycetidae.

In early classifications, ascoma morphology was used together with ascus characters to classify ascomycetes (e.g., Luttrell 1955; Nannfeldt 1932). However, molecular phylogenies demonstrated that **some types of ascomata evolved several times independently in Lecanoromycetes** [e.g., Grube et al. 2004; Schmitt et al. 2005, 2009; Wedin and Tibell 1997; see also the review in Grube and Hawksworth (2007)]. In particular, some groups with perithecioid ascomata (e.g., Porinaceae, Protothelenellaceae, and Thelenellaceae) were shown to be nested within the predominantly apothecial subclass Ostropomycetidae (Grube et al. 2004; Schmitt et al. 2005, 2009). In Lecanoromycetes, the convergent evolution of ascoma types also prevents the use of this character for defining orders and families, although some broad trends can be observed (e.g., predominantly lirellate apothecia in Graphidaceae).

In Ostropomycetidae, a correlation between ascoma type and ascoma development (Schmitt et al. 2009) suggested that angiocarpous development was a prerequisite adaptation in lineages in which perithecia have evolved. These perithecioid fruiting ascomata may therefore have a neotenic origin (Grube et al. 2004; Schmitt et al. 2009).

2. Ascoma Development

Types of ascoma development have been used in the past to define higher divisions in filamentous ascomycetes. Originally, two main types of development were distinguished, **ascolocular and ascohymenial** (Nannfeldt 1932). In ascolocular ascomata, the asci develop in the cavity in a preformed stroma, whereas in ascohymenial ascomata, the asci develop in a hymenium not located in a preformed stroma (Kirk et al. 2008). In Lecanoromycetes, ontogenetic studies have helped describe in more detail the development of ascomata (e.g., Henssen 1976; Henssen and Jahns 1974; Letrouit-Galinou and Bellemère 1989). Henssen and Jahns (1974) recognized two main types of development in ascohymenial lichen-forming fungi, depending on the origin of the structure enveloping the ascogonia (or primordium), as well as other more specific types. These development types can lead to apothecia (angiocarps), perithecia (gymnocarps), or perithecioid apothecia (hemiangiocarps).

Letrouit-Galinou and Bellemère (1989) recognized two types of development depending on the differentiation of the excipulum (or parathecium, proper margin). Among the types without any differentiated excipulum are the *Pertusaria* and *Thelotrema* types for perithecioid apothecia and the *Graphis* and *Baeomyces* types for apothecia or lirellate apothecia. Among the types with a differentiated excipulum are the *Aspicilia* and *Gyalecta* types (excipulum reduced to a crown), the *Peltigera*, *Diploicia*, and *Xanthoria* types (typical excipulum), and *Cladonia* and *Parmelia* types (atypical excipulum).

Molecular studies showed that the classification in ascolocular and ascohymenial fungi did not reflect monophyletic groupings (e.g., Berbee 1996; Lindemuth et al. 2001; Lumbsch and Huhndorf 2007a; Lutzoni et al. 2004). As a result, these characters are now rarely mentioned in classification work (e.g., Hibbett et al. 2007). Although the use of ascoma developmental types is inadequate for delimiting higher taxa of Ascomycota, their use at the ordinal and family levels may have some value since they can be characteristic of some groups of Lecanoromycetes (e.g., Parmeliaceae and Agyriaceae) (Henssen et al. 1981; Lumbsch et al. 2001a).

C. Asci

1. Ascus Walls

The first studies of asci with light microscopy (e.g., Chadefaud 1942; Luttrell 1951; Nannfeldt 1932) and electron microscopy resulted in the use of ascus characters in combination with ascomatal characters to establish higher-rank classification systems among filamentous ascomycetes (Barr 1983; Eriksson 1982; Luttrell 1951, 1955; Nannfeldt 1932). Thus, the structure of the ascus wall was one of the main ascus features used in higher classification. Currently, ascus walls are classified in three main types: a thin ascus wall formed of a single layer (**prototunicate ascus**), an ascus wall formed of two layers functioning as a single layer (**unitunicate ascus**), and an ascus wall formed of two layers functioning as two layers (**bitunicate ascus**). Most members of Lecanoromycetes have unitunicate asci (Honegger 1982a; Letrouit-Galinou 1973a).

Among unitunicate ascus types are the *Lecanora*, *Pertusaria*, and *Teloschistes* types from Honegger (1982a, b). Bellemère and Letrouit-Galinou (1987) grouped these three ascus types within the "archeacés" and distinguished the further *Lecidella*, *Catillaria*, *Psora*, *Lecidea*, *Cladonia*, and *Collema* types. Unitunicate asci are also found in *Anzina* (Scheidegger 1985), *Baeomyces* (Bellemère 1977; Honegger 1983), *Dactylospora* (Bellemère and Hafellner 1982), Gyalectaceae (Kauff and Büdel 2005), and *Trapeliopsis* (Bellemère and Letrouit-Galinou 1987).

Functionally bitunicate asci are less common in the Lecanoromycetes. They are found in Collemataceae, Peltigeraceae, and Rhizocarpaceae (Bellemère and Letrouit-Galinou 1987; Honegger 1982a). Prototunicate asci are rather rare in this fungal class and are only found in the families Caliciaceae and Sphaerophoraceae (Wedin and Tibell 1997; Wedin et al. 2000b), in which the released spores form a loose mass.

2. Ascus Apical Structure

With improving microscopy technologies, **the apical structure of asci was shown to be particularly variable within Lecanoromycetes** (e.g., Chadefaud 1973; Chadefaud et al. 1963; Honegger 1978, 1980; Letrouit-Galinou 1973a). Among the variations were the presence or absence of differentiated apical structures (apical thickening, ocular chamber, apical ring, apical nasse, subapical bourrelet) and their reaction to various stains such as iodine (e.g., Chadefaud 1973; Hafellner 1984; Letrouit-Galinou 1973a). Hafellner (1984) was the first to use these characters to systematically revise the classification within Lecanorales s.l. at the family and genus levels. He recircumscribed some species-rich families (e.g., Lecanoraceae and Lecideacae) and described new families (e.g., Catillariaceae and Dactylosporaceae) based on the ascus apical structure (Bellemère and Hafellner 1982; Hafellner 1984). His classification system was broadly accepted (e.g., Eriksson and Hawksworth 1993; Hafellner 1994; Rambold and Triebel 1992), until **molecular data showed that these characters were not as conserved as initially thought and that similar apical structures have evolved several**

times independently in unrelated lineages [Ekman and Wedin 2000; Ekman et al. 2008; Lumbsch et al. 2001b, 2007c; Wedin et al. 2005, 2009; see also the review in Grube and Hawksworth (2007) and Printzen (2010)].

3. Dehiscence Mechanisms

The type of dehiscence (or the process leading to the liberation of ascospores from mature asci) varies greatly in Lecanoromycetes (Bellemère and Letrouit-Galinou 1987; Honegger 1982a). In most members of this class, dehiscence occurs by apical rupture of the ascus, combined with the ejection of the internal part of the ascus wall. When the ejection is not coupled with a sliding between the two layers of the ascus wall, this type of dehiscence is called a **rostrum type or *Lecanora* type** (Bellemère and Letrouit-Galinou 1987; Honegger 1978). It is the most common type of dehiscence in Lecanoromycetes (e.g., Lecanoraceae, Physciaceae, Parmeliaceae), and it has been suggested that it might be the ancestral dehiscence type in this class based on both anatomical data ("type archaeascé") (Chadefaud et al. 1967) and molecular data (Miadlikowska et al. 2006; Wedin et al. 2005).

In *Rhizocarpon*, the internal layer of the ascus wall slides slightly along the external layer (*Rhizocarpon* type or hemifissitunicate type) (Bellemère 1994; Honegger 1980). In *Peltigera*, the sliding of the internal layer along the external layer is more important (*Peltigera* type, fissitunicate type or "Jack in the box") (Bellemère and Letrouit-Galinou 1987; Honegger 1978).

Dehiscence can also occur by apical rupture of the ascus without ejection of the internal part of the ascus wall (e.g., *Trapelia* and *Coccocarpia*) (Bellemère 1994). In *Teloschistes*, the apical pore forms after predehiscent elongation of the ascus wall (***Teloschistes* type or chimney type of dehiscence**) (Bellemère and Letrouit-Galinou 1987; Honegger 1978). In *Pertusaria*, a predehiscent elongation also occurs, but the ascospores are released after bursting or splitting of the ascus tip (Honegger 1982b). Finally, in Caliciaceae and Sphaerophoraceae, ascospores are passively released after deliquescence of prototunicate asci (**evanescent type of dehiscence**) (Wedin and Tibell 1997). Although ascus dehiscence has been used in high-rank classifications (Luttrell 1955), the systematic value of this character has been questioned because dehiscence types more likely correspond to adaptations to various environmental conditions (Bellemère 1994).

D. Ascospores

Ascospore characters, such as septation, pigmentation, size, shape, and number per ascus, were originally used to delimit families or genera in many groups of Lecanoromycetes [e.g., in Graphidaceae (Müller 1880, 1882) or in Acarosporaceae (Zahlbruckner 1907)]. However, many authors have recognized subsequently that classification based on these characters might not reflect evolutionary history because of **convergent evolution in ascospores of lichenized and nonlichenized fungi** (e.g., Poelt 1973; Vainio 1890). Morphologically similar ascospores are indeed often found in distantly related groups, as confirmed by molecular data.

Molecular phylogenetic results have further thrown doubts on the use of ascospores as a main source of characters for classification at the generic level. Several Lecanoromycetes genera that were primarily circumscribed on ascospore characters were shown to be polyphyletic, and **characters such as ascospore septation and color were frequently shown to be homoplasious** (e.g., Ihlen and Ekman 2002; Rivas Plata and Lumbsch 2011; Staiger et al. 2006). Despite this conclusion, when combined with other features, ascospore characters provide important taxonomic information for most groups of lichenized fungi at the species and generic levels (e.g., within Thelotremataceae) (Frisch et al. 2006). Ascospore characters can also, in some cases, be useful at higher taxonomic levels since some broad trends can be observed in some groups. For example, polarilocular ascopores are characteristic of the order Teloschistales and ascospores with

lens-shaped lumina, of the family Graphidaceae s.s. (Poelt 1973).

E. Interascal Filaments

Sterile filaments are usually present in fruiting bodies alongside asci. These interascal filaments constitute the **hamathecium** and are thought to protect the asci or promote their function (Poelt 1973). Historically, two broad categories of interascal filaments were described in ascomycetes depending on their origin in the development of the ascoma. Ascohymenial development leads to the formation of **paraphyses**, whereas ascolocular development forms **paraphysoids or pseudoparaphyses**. These two types of filament may look very similar in mature fruiting bodies and can be confused, so they have been applied only in more recent classification systems of Ascomycota (e.g., Barr 1983). At lower taxonomic levels, hamathecial characters (septation, anastomoses and branching, color, chemistry, and shape of the upper cell) have been used together with other characters to delimitate genera or families in Lecanoromycetes (e.g., Kärnefelt and Thell 1992; Staiger and Kalb 1999; Timdal 1992). The importance of some of these characters has been discussed in light of molecular data (e.g., Rivas Plata and Lumbsch 2011; Staiger et al. 2006). However, a comprehensive reevaluation of the evolution of hamathecial characters and their taxonomic value is still needed.

F. Pycnidia

In lichenized fungi, pycnidia (=conidiomata) are **minute flask-shaped structures located on the surface of, or embedded within, lichen thalli and producing small asexual spores called pycnidiospores** (also called conidiospores or conidia). The role of pycnidia has been insufficiently studied in lichens. Culture studies first suggested that conidiospores might act as asexual dispersal units (Möller 1888), but this possibility was questioned because more recent studies showed that, in Lecanoromycetes, conidiospores germinate only rarely (Ahmadjian 1969; Bailey 1976; Vobis 1977). Microscopical observations revealed that conidiospores most probably act as spermatia since they have been found attached to trichogynes in several species (e.g., Honegger 1984a, b; Jahns 1970).

The systematic value of pycnidial characters was recognized early on (Choisy 1954; Steiner 1901; Zahlbruckner 1903–1907), but their use in systematic studies remains rather limited, mostly because they are difficult to observe. In the 1970s and 1980s, cytological and ontogenetical studies triggered a renewed interest in pycnidial characters, and several types of pycnidia and conidiophores were described in the Lecanoromycetes (e.g., Honegger 1984b; Janex-Favre 1977, 1982; Letrouit-Galinou 1972, 1973b; Letrouit-Galinou and Lallement 1977; Vobis 1980). However, data available on pycnidia remain sparse (Roux et al. 1986), and only relatively few studies have tried to assess their use for classification within Lecanoromycetes (e.g., Krog 1982; Matsumoto and Deguchi 1999; Thell et al. 2002).

G. Asexual Propagules

Lecanoromycetes can disperse asexually by thallus fragmentation aided by undifferentiated or specialized structures. **The two most common types of specialized dispersal structures are isidia** (corticated and more or less cylindrical thallus outgrowths) (Fig. 4.4a) **and soredia** (ecorticated thallus granules produced via openings in the cortex called soralia) (Fig. 4.4b). Such propagules facilitate successful codispersal of both lichen partners, in contrast to fungal dispersal through sexual ascospores, which requires finding appropriate photobionts to reestablish lichen thalli de novo. The taxonomic value of asexual propagules was first recognized by Du Rietz (1924). He described and classified them and introduced the concept of species pairs for species that differ only by their mode of reproduction, either primarily sexual or primarily vegetative. Poelt (1970, 1972) later developed this concept, and since then, it has been debated whether they correspond to conspecific individuals or separate

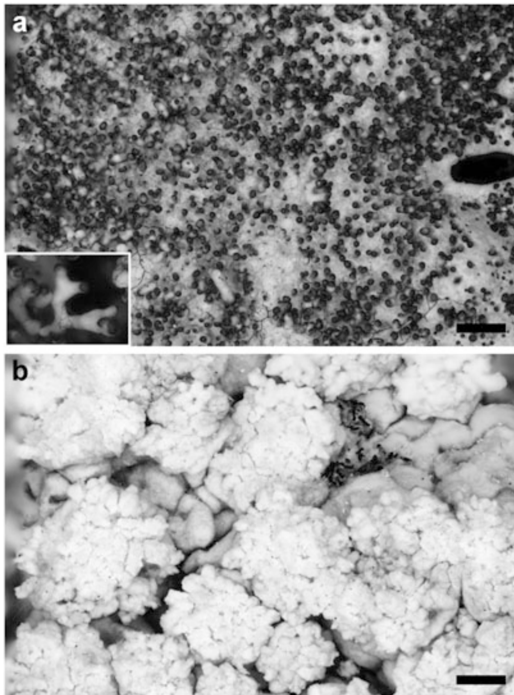


Fig. 4.4 Asexual propagules in Lecanoromycetes. (**a**) Upper surface of thallus of *Parmelina tiliacea* covered with small and easily detached, corticated outgrowths called isidia. In this species, isidia are simple to branched and brown at the tip (*inset*: detail of a long branched isidium). (**b**) Round openings (soralia) in the upper cortex of *Pertusaria flavicans* releasing ecorticated granules called soredia. Bars = 0.5 mm

sister species (e.g., Mattsson and Lumbsch 1989; Tehler 1982). Molecular studies on several species pairs have so far not confirmed them as separate species (e.g., Buschbom and Mueller 2006; Kroken and Taylor 2001; Myllys et al. 2001). The contribution of asexual reproduction to the ecology and evolution of lichens has rarely been addressed (e.g., Buschbom and Barker 2006; Dal Grande et al. 2012; Fedrowitz et al. 2011; Hestmark et al. 2011).

H. Secondary Compounds

Secondary compounds are **insoluble metabolites that are often deposited extracellularly by the fungal partner on the surface of hyphae.** These compounds are very diverse and numerous, with more than 700 described so far from lichens (Elix and Stocker-Wörgötter 2008). Most of these compounds are found exclusively in lichens. They are derived from three main biosynthetic pathways (acetyl-polymalonyl, shikimic, and mevalonic acid pathways) and belong to different chemical groups such as anthraquinones, depsidones, triterpenes, pulvinic acids, and xanthones (Asahina and Shibata 1954; Culberson and Elix 1989; Huneck 2001). Mostly present in the cortex and the medulla, they can help repel herbivores and microorganisms (Lawrey 1986, 1989) but in the cortex also act as light and UV screens (Armaleo et al. 2008; Millot et al. 2012; Solhaug et al. 2003). Armaleo et al. (2011) were the first to identify a gene cluster involved in the biosynthesis of a depside and a depsidone.

Approximately 5,000 lichen species have been investigated for their secondary compounds (Elix and Stocker-Wörgötter 2008). Methods of detection vary from simple spot tests to analysis of extracts with thin-layer chromatography and high-performance liquid chromatography (Huneck and Yoshimura 1996). The presence of secondary compounds has been used at all taxonomic levels, for both classification and species identification. **Most secondary compounds are found widely in Lecanoromycetes and only rarely form synapomorphies for single lineages.** However, the presence of some secondary compounds was shown to be informative at the genus and family levels (Lumbsch 1998a; Schmitt and Lumbsch 2004). The use of chemical characters for species delimitation has been controversial (Lumbsch 1998b). Recent molecular studies show that, depending on the group studied, chemotypes may correspond to separate species (e.g., Tehler and Källersjö 2001) or can represent infraspecific variability (e.g., Leavitt et al. 2011a, b; Nelsen and Gargas 2009; Velmala et al. 2009).

VI. Origin and Diversification

Dating the divergence time of fungi is not an easy task because of the relatively poor fossil record for these organisms and the variable rates of nucleotide substitution across this kingdom (Lumbsch et al. 2008a; Lutzoni and

Pagel 1997; Woolfit and Bromham 2003; Zoller and Lutzoni 2003). As a result, estimates of divergence times in fungi once varied considerably depending on the methods and calibrations used (Berbee and Taylor 1993, 2001; Heckman et al. 2001; Padovan et al. 2005; Taylor and Berbee 2006). With the reinterpretation of fossil data and the development of new phylogenetic methods allowing for rates to vary across lineages, **divergence estimates for the main fungal lineages have now reached a consensus** (Lücking et al. 2009a; Taylor and Berbee 2010), and divergences of more recent lineages have started to be investigated (Amo de Paz et al. 2011; Gueidan et al. 2011).

Soon after the divergence of Pezizomycotina, Leotiomyceta underwent a radiation during which the Lecanoromycetes lineage originated (Gazis et al. 2012; Schoch et al. 2009; Spatafora et al. 2006). So far, all ancestral state reconstruction studies agree that lichenization in Lecanoromycetes evolved at or prior to the onset of the evolution of this fungal class. This acquisition of lichenization has been placed at the base of a lineage including Lecanoromycetes, Eurotiomycetes, and Lichinomycetes (James et al. 2006; Lutzoni et al. 2001), at the base of a lineage including Lecanoromycetes and Lichinomycetes (Gueidan et al. 2008), or at the base of Lecanoromycetes (Schoch et al. 2009). According to Gueidan et al. (2011), Lecanoromycetes diverged from Eurotiomycetes during the late Devonian, around 371 million years ago (mya) (between 322 and 424 mya), and the diversification of extant Lecanoromycetes species (crown group) originated during the Carboniferous, approximately 322 mya (between 269 and 380 mya).

Within Lecanoromycetes, several well-interpreted fossils from amber are available to calibrate the molecular clock. A species of *Anzia* was described from European amber (35–40 mya) (Mägdefrau 1957), two species of *Parmelia* from Dominican amber (15–45 mya) (Poinar et al. 2000), and a species of *Alectoria* from Baltic amber (35–40 mya) (Rikkinen and Poinar 2002). A more recent discovery by Honegger et al. (2013) of exceptionally well-preserved lichen thalli fragments in siltstone of the lower Devonian (415 mya) provided the oldest record of modern lichens.

One of the *Parmelia* fossils and the *Alectoria* fossil were used in a recent study to investigate the origin of Parmeliaceae, one of the largest families within Lecanoromycetes (Amo de Paz et al. 2011). Results show that this family radiated around the Cretaceous–Tertiary boundary (±65 mya), just before a climatic period characterized by temperature and atmospheric CO_2 maxima. Most major parmelioid genera originated during the Eocene and early Oligocene and diversified during the cooler periods of late Oligocene to early Pliocene.

VII. Orders and Classification

A. Acarosporales

Acarosporales (Acarosporomycetidae) (Fig. 4.5) is a small order with a single family, Acarosporaceae, 11 genera, and 183 species (Kirk et al. 2008). They are mostly crustose species with apothecial ascomata, poorly to moderately branched and anastomosed paraphyses, unitunicate polysporous asci, and small hyaline simple ascospores (Hibbett et al. 2007). They occur worldwide and mostly colonize rocks. Traditionally, Acarosporaceae had been placed in the order Lecanorales based on ascus characters (Kirk et al. 2001). However, molecular studies showed that they did not belong to this order but represent instead the **earliest diverging lineage in Lecanoromycetes** (Lumbsch et al. 2007b; Miadlikowska et al. 2006; Reeb et al. 2004; but see Hofstetter et al. 2007). The order Acarosporales was therefore formally described for this family (Hibbett et al. 2007).

Although polyspory is not uncommon in nonlichenized and lichenized ascomycetes, the family Acarosporaceae was originally characterized by its true polyspory (polyspory resulting from a meiosis followed by several mitoses generating more than 100 ascospores, as opposed to polyspory resulting from budding or fragmenting ascospores). However, in Lecanoromycetes, true polyspory is not restricted to the Acarosporaceae. As a result, the circumscription of this family has been variable, with

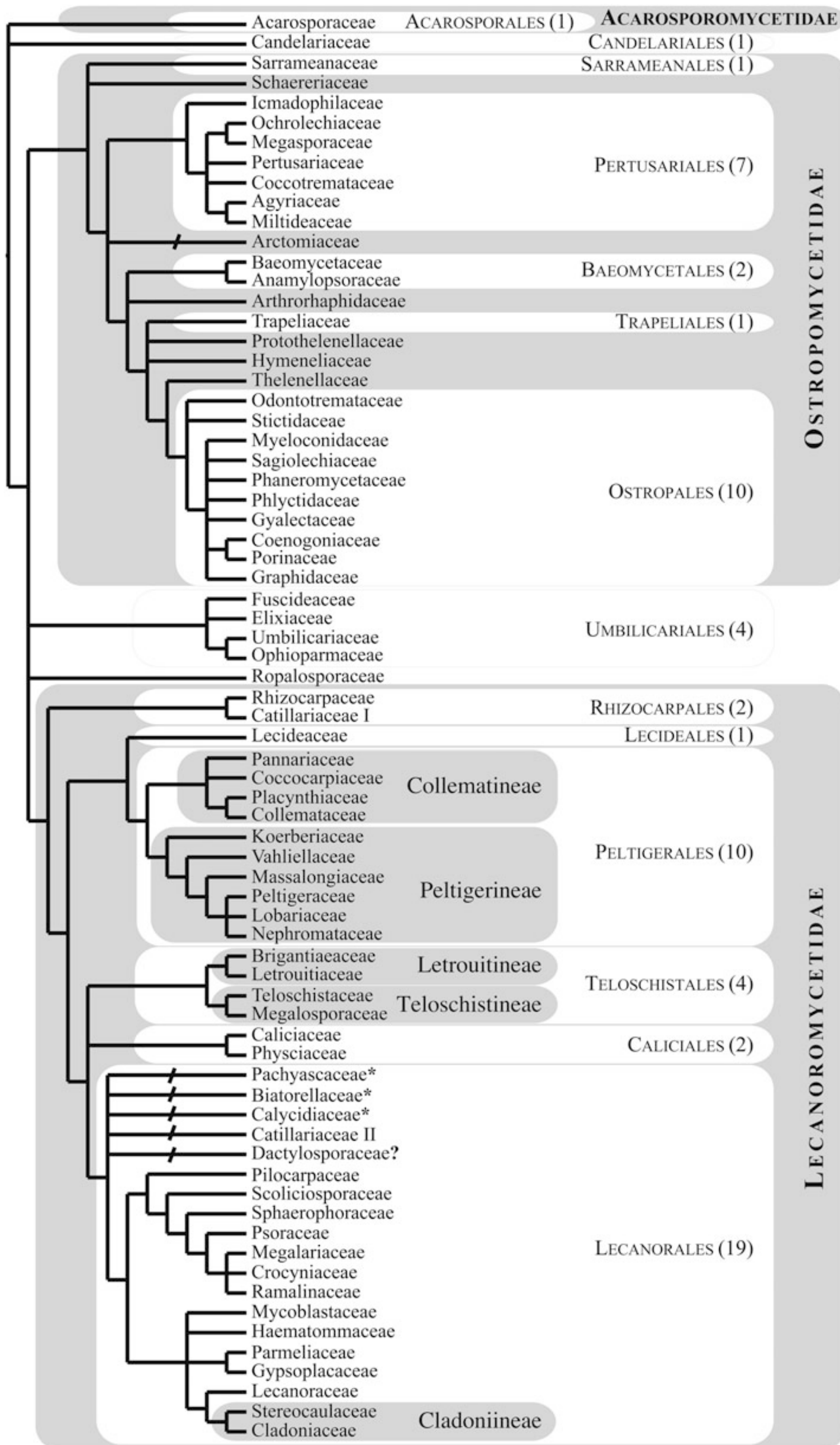


Fig. 4.5 Schematic representation of phylogeny and classification of Lecanoromycetes based on selected published sources (Andersen and Ekman 2005; Arup et al. 2007; Baloch et al. 2010; Bylin et al. 2007; Ekman et al. 2008; Gaya et al. 2012; Hodkinson and Lendemer 2011; Hofstetter et al. 2007; Kirk et al. 2008; Lumbsch

many polysporous genera (e.g., *Maronea*, *Pleopsidium*, and *Thelocarpon*) being successively excluded from, or included in, the family depending on the system adopted for their classification [Golubkova 1988; Hafellner 1995; Magnusson 1936; Zahlbruckner 1907; see the detailed review in Reeb et al. (2004)].

Molecular data have helped resolve the circumscription of Acarosporaceae (Reeb et al. 2004; Wedin et al. 2005), and the genera *Acarospora*, *Glypholecia*, *Pleopsidium*, *Polysporina*, *Sarcogyne*, *Thelocarpella*, and *Timdalia* were confirmed as part of this family, whereas the genera *Biatoridium*, *Maronea*, *Sporastatia*, *Strangospora*, and *Thelocarpon* were excluded. Molecular studies also confirmed the **multiple independent origins of true polyspory in Lecanoromycetes** (Reeb et al. 2004), i.e., Acarosporaceae (with a loss in the two species *Acarospora macrospora* and *Glypholecia scabra*, which have only 30–100 ascospores), *Maronea*, *Sporastatia*, *Strangospora*, and a lineage including *Thelocarpon* and *Biatoridium*.

B. Baeomycetales

The order Baeomycetales (Ostropomycetidae) (Fig. 4.5) currently includes two families, Baeomycetaceae and the monotypic Anamylopsoraceae (Hodkinson and Lendemer 2011; Lumbsch et al. 1995). The family Baeomycetaceae comprises the three genera *Ainoa*, *Baeomyces*, and *Phyllobaeis* (Hibbett et al. 2007; Lumbsch and Huhndorf 2010) and a total of approximately 15 species with crustose to squamulose or foliose thalli, sessile to stipitate apothecia, branched paraphyses, nonamyloid asci or asci with a slightly amyloid apex, and simple to transversally septate hyaline ascospores (Hibbett et al. 2007; Johnston 2001). The species grow on soils, rocks, and bryophytes in moist areas and often are primary colonizers of disturbed substrates (Johnston 2001). First placed in Lecanorales close to Cladoniaceae (Henssen and Jahns 1974; Poelt 1973), this family (including at the time the genera *Baeomyces* and *Icmadophila*) was then transferred to the predominantly nonlichenized order Helotiales (now Leotiales) based on its *Leotia*-type ascus (Chadefaud 1960, 1973; Hafellner 1988; Honegger 1983; Rambold et al. 1993; Tehler 1996).

Early molecular studies suggested that *Baeomyces* might not be related to Leotiales (Platt and Spatafora 1999; Stenroos and DePriest 1998). Later, this genus was shown to form a sister group to the Ostropales s.l., and the ordinal name Baeomycetales was then suggested for this lineage (Kauff and Lutzoni 2002). **Additional molecular data confirmed its phylogenetic placement within Ostropomycetidae** (Lumbsch et al. 2007b; Miadlikowska et al. 2006), and the order Baeomycetales was therefore formally erected for the family Baeomycetaceae (Hibbett et al. 2007).

The delimitation of Baeomycetaceae also underwent some important changes during the last two decades. Some genera previously placed in this family (e.g., *Dibaeis*, *Icmadophila*, *Siphulella*) were segregated into the new family Icmadophilaceae based on morphological evidence (Rambold et al. 1993), and subsequent molecular studies confirmed that these genera are not related to Baeomycetaceae (Platt and Spatafora 1999; Stenroos and DePriest 1998). A new genus (*Phyllobaeis*) was also described for *Baeomyces* species with squamulose primary thalli and brown apothecia (Gierl and Kalb 1993). Finally, molecular data showed that the genus *Ainoa* also belongs to Baeomycetales (Lumbsch et al. 2007b, c). This genus had previously been described to

Fig. 4.5 (continued) and Huhndorf 2010; Lumbsch et al. 2004, 2007b, 2008b; Lutzoni et al. 2004; Miadlikowska et al. 2006; Muggia et al. 2011; Reeb et al. 2004; Rivas Plata et al. 2012; Schmitt et al. 2005, 2012; Schoch et al. 2009; Wedin et al. 2009; Widhelm and Lumbsch 2011; Zhou and Wei 2007). Phylogenetic relationships among taxa (families, orders, and subclasses) were compiled using a "super tree" approach and are shown as resolved if reported with posterior probability ≥95 % or maximum likelihood bootstrap ≥70 % in multiple studies (in most cases) and are not in conflict at the same level of support. The number of recognized families in each order is provided in parentheses after the order name. *Shaded boxes* represent subclasses and suborders. *Oblique bars* across branches indicate families with unknown placement in the Lecanoromycetidae or Ostropomycetidae. *Stars* indicate families for which no DNA sequence is currently available in GenBank. *Question mark* indicates that Dactylosporaceae may be placed outside of Lecanoromycetes (in Eurotiomycetes) (Schoch et al. 2009)

accommodate two morphologically different species of *Trapelia* that did not cluster with *Trapelia* s.s. in a molecular phylogenetic study (Lumbsch et al. 2001b).

The circumscription of the order Baeomycetales is likely to undergo more changes in the future. A morphological study of the family Anamylopsoraceae shows that this monotypic family shares several characters with Baeomycetaceae, such as the ascocarp ontogeny, stipitate ascogonia, annular exciple, and conidiophore type (Lumbsch et al. 1995). However, these two families also differ in other characters, such as the ascus type. Anamylopsoraceae had been placed tentatively in Agyriineae and Agyriales (Lumbsch et al. 1995, 2001a, respectively), but a transfer to Baeomycetales was recently suggested (Hodkinson and Lendemer 2011; Lumbsch et al. 2007b). A comprehensive phylogenetic analysis is needed to determine whether this family belongs to Baeomycetales.

C. Caliciales

The order Caliciales (Lecanoromycetidae) (Fig. 4.5) was **in the past recognized as a phenotypically well-delimited group that included species with mazaedium-forming ascomata and passive ascospore dispersal** (e.g., Zahlbruckner 1903–1907; see detailed review in Tibell 1984) and was erected as an order by Bessey (1907). However, this order underwent drastic changes in circumscription after a first extensive revision by Tibell (1984). Based on a careful morphological study, Tibell excluded several families and genera from Caliciales s.s. (e.g., Sphaerophoraceae and *Chaenotheca*) and restricted the order to the three families Caliciaceae, Mycocaliciaceae, and Sphinctrinaceae (Tibell 1984, 1996). More recently, a study using molecular data demonstrated that **Mycocaliciaceae and Sphinctrinaceae belong in fact to Eurotiomycetes** and that only the family Caliciaceae was strongly supported as part of the order Lecanorales (Wedin and Tibell 1997).

Subsequent studies with broader taxon samplings showed that Caliciaceae were in fact closely related to Physciaceae, in the order Lecanorales (Wedin et al. 2000a, 2005). Both Physciaceae and Caliciaceae were later tentatively placed in Teloschistales as part of the suborder Physciineae (Miadlikowska et al. 2006). In a more recent molecular study focusing on Teloschistales s.l., Gaya et al. (2012) demonstrated phylogenetic instability for relationships among Physciineae, Teloschistineae, and Lecanorales, where the two suborders did not always form a monophyletic group. To ensure the classification was resilient to the various resolution of these three clades, Gaya et al. (2012) elevated Physciineae to the ordinal level by resurrecting the order Caliciales and restricted Teloschistales to Brigantiaeaceae, Letrouitiaceae, Megalosporaceae, and Teloschistaceae. **In this new phylogenetic context, Caliciales includes two families: Caliciaceae** (with two subfamilies Calicioidea and Buellioidea) **and Physciaceae.**

D. Candelariales

The order Candelariales (Candelariomycetidae?) (Fig. 4.5) **includes a single family, Candelariaceae,** and four genera: *Candelaria*, *Candelariella*, *Candelina*, and *Placomaronea* (Lumbsch and Huhndorf 2010; Miadlikowska et al. 2006; Westberg et al. 2009). It is a small group with approximately 66 lichenized species (Kirk et al. 2008) characterized by yellow to orange thalli (secondary chemistry based on pulvinic acid and derivatives), disciform apothecia, unitunicate asci (often polysporous), mostly unbranched paraphyses, and hyaline mostly simple ascospores (Hibbett et al. 2007; Westberg et al. 2007, 2009). Formerly, the family Candelariaceae had been placed in Lecanorales s.l. (Henssen and Jahns 1974; Poelt 1973). Molecular data showed that this family did not cluster within Lecanorales as currently delimited (Hofstetter et al. 2007; Miadlikowska et al. 2006; Wedin et al. 2005), and so the order Candelariales was erected for this family (Hibbett et al. 2007). Candelariales was considered to be a sister order to Acarosporales (Wedin et al. 2005) or possibly formed the second lineage to diverge within Lecanoromycetes after Acarosporales (Miadlikowska et al. 2006) or perhaps

the first diverging lineage in Lecanoromycetes (Hofstetter et al. 2007). Because of this phylogenetic uncertainty, this order was only tentatively recognized as the subclass Candelariomycetidae by Miadlikowska et al. (2006) and Hofstetter et al. (2007). A morphological and molecular revision of the family showed that the current generic delimitation does not reflect monophyletic groups, and further work will be needed to clarify the generic boundaries (Westberg et al. 2007, 2009).

E. Lecanorales

Lecanorales (Lecanoromycetidae) (Fig. 4.5) is the **largest order in Lecanoromycetes** with 19 families and approximately 250 genera (Lumbsch and Huhndorf 2010). The number of species in this order was estimated to be 5,695 (Kirk et al. 2008). It includes mostly lichenized species with varied thallus types, predominantly apothecial ascomata, usually unbranched paraphyses, mostly thick-walled unitunicate asci (often amyloid), and varied ascospores (Kirk et al. 2008). Traditionally, this apparently heterogenous order included most lichenized apothecial ascomycetes, with the exception of those included in Ostropales (e.g., Henssen and Jahns 1974; Poelt 1973; Rambold and Triebel 1992). A new circumscription of this order based on the ascus apical structure led to the exclusion of several taxa from Lecanorales (e.g., Peltigerales and Teloschistales) (Hafellner 1988). **This circumscription was further narrowed after molecular data were used to test the relationships among the main Lecanorales taxa.** This circumscription was confirmed, and the trend continued with the advent of molecular phylogenetics, resulting in the following orders currently being recognized outside the Lecanorales: Acarosporales, Caliciales (with the Physciaceae), Candelariellales, Lecideales, Peltigerales, Pertusariales, Teloschistales, and Umbilicariales (e.g., Gaya et al. 2012; Miadlikowska and Lutzoni 2004; Miadlikowska et al. 2006; Reeb et al. 2004; Schmull et al. 2011; Wedin et al. 2005). Among the remaining 19 families in Lecanorales are the broadly distributed and well-known species-rich Cladoniaceae, Lecanoraceae, Parmeliaceae, and Ramalinaceae (Lumbsch and Huhndorf 2010).

Parmeliaceae is the largest family within Lecanorales, with approximately 2,500 species and 88 genera (Kirk et al. 2008). Earlier, many groups within Parmeliaceae had been tentatively segregated from this family based on morphological, anatomical, and chemical characters (e.g., Alectoriaceae, Anziaceae, Hypogymniaceae, and Usneaceae). But most of these segregate families were not confirmed by molecular phylogenies (e.g., Arup et al. 2007; Mattsson and Wedin 1999; Wedin et al. 1999), and a wider circumscription of Parmeliaceae is currently accepted (Eriksson 2006; Kirk et al. 2008; Lumbsch and Huhndorf 2010). This family comprises a large variety of growth forms (e.g., parmeliod, usneoid, or cetrarioid species), which do not define large monophyletic groups within Parmeliaceae but nevertheless were found to be useful in characterizing and identifying smaller clades within the family (Crespo et al. 2007).

The generic concept is a strongly debated area in lichen research (e.g., Elix 1993; Hale 1984a; Nimis 1998). In Parmeliaceae, large genera (e.g., *Parmelia* s.l.) have been split into multiple smaller genera (e.g., Culberson and Culberson 1981; Elix and Hale 1987; Elix et al. 1986; Hale 1974, 1984b). Molecular phylogenetic studies have shown that many of these newly segregated genera are not monophyletic (e.g., Blanco et al. 2004, 2005; Crespo et al. 2010; Nelsen et al. 2011), and the taxonomic importance of some characters traditionally used to classify parmelioid lichens (e.g., chemistry of the cortex, presence or absence of pores and pseudocyphellae) may have been overestimated (Blanco et al. 2006).

Similar problems were found in other families of Lecanorales (e.g, Lecanoraceae, Ramalinaceae). In the predominantly crustose groups of Lecanorales, which were mainly classified based on ascus characters (Hafellner 1984), molecular phylogenetic studies reported that many of these crustose groups (e.g., Bacidiaceae, Lecanoraceae, Micareaceae) were not monophyletic and that the evolution of the ascus was more complex than had been anticipated and of limited value for classification at this taxonomic level (Andersen and Ekman 2004; Ekman 2001; Ekman and Wedin 2000;

Ekman et al. 2008). Because of a similar composite thallus, Cladoniaceae was placed together with Stereocaulaceae and two other families within the suborder Cladoniineae (Lecanorales s.l.) (Poelt 1973). Molecular phylogenies showed that, although the composite growth form evolved several times in Lecanoromycetes (Stenroos and DePriest 1998), Cladoniaceae and Stereocaulaceae do form a sister group to which the suborder Cladoniineae is now restricted (Miadlikowska et al. 2006; Myllys et al. 2005; Wedin et al. 2000b).

F. Lecideales

Lecideales (Lecanoromycetidae) (Fig. 4.5) is an order recently resurrected for a single family, Lecideaceae, now restricted to the genus *Lecidea* s.s. (sensu Hertel) and some species of *Porpidia* (Schmull et al. 2011). In Zahlbruckner's classification system (1903–1907), **Lecideaceae was a large artificial family within the order Lecanorales that included a heterogeneous assemblage of crustose taxa with lecideine or biatorine apothecia** (e.g., *Bacidia*, *Catillaria*, *Toninia*), among which *Lecidea* was one of the largest lichen genera. The delimitation of this poorly studied family was questioned in later taxonomic works and classification systems (Henssen and Jahns 1974; Hertel and Rambold 1985; Poelt 1973; Santesson 1952; Timdal 1987). In his classification of Lecanorales, Hafellner (1984) was the first to attempt to recircumscribe the two families Lecideaceae and Lecanoraceae. Based on ascus characters, he segregated several new families from the Lecideaceae, among which was Porpidiaceae. His system was broadly accepted (e.g., Eriksson and Hawksworth 1993; Hafellner 1994; Rambold and Triebel 1992), although also sometimes criticized (e.g., Timdal 1992).

Molecular phylogenetic studies confirmed the heterogeneity of early circumscriptions of Lecideaceae and Lecanoraceae (Andersen and Ekman 2004, 2005; Buschbom and Mueller 2004; Ekman 2001; Ekman et al. 2008; Schmull et al. 2011). For example, the genus *Bacidia*, included in Lecideaceae in early classifications (Henssen and Jahns 1974; Poelt 1973; Zahlbruckner 1903–1907), was shown to belong to Ramalinaceae, a family classified within Lecanorales (Ekman 2001). Molecular phylogenies also shed a light on Hafellner's classification system (1984). Characters of the ascus tip used by this author to redelimitate genera and families within Lecanorales do not seem to characterize monophyletic entities in Lecideaceae and related taxa (e.g., Porpidiaceae) (Buschbom and Mueller 2004). Some taxa previously attributed to Lecideaceae were shown to belong to different lineages within Lecanoromycetes, and genera within this family were shown to be poorly delimited (Schmull et al. 2011). **The phylogenetic positions of most members of Lecideaceae are still unknown or unsettled** (Miadlikowska et al. 2006; Schmull et al. 2011). However, they were found to form five distinct groups within Lecanoromycetidae, one of which included only saxicolous species belonging to the genera *Lecidea* and *Porpidia*, including the type species *Lecidea fuscoatra*, which led to the resurrection of the order Lecideales s.s. (Schmull et al. 2011). Additional molecular data are greatly needed to further investigate this species-rich and broadly defined lichen group.

G. Ostropales

Ostropales (Ostropomycetidae) (Fig. 4.5) is a **large order of mostly crustose lichenized and nonlichenized species, with high species diversity in the tropics**. It includes approximately 2,750 species (Kirk et al. 2008) currently classified in ten families: Coenogoniaceae, Graphidaceae (including Gomphillaceae and Thelotremataceae), Gyalectaceae, Myeloconidaceae, Odontotremataceae, Phaneromycetaceae, Phlyctidaceae, Porinaceae, Sagiolechiaceae, and Stictidaceae (Baloch et al. 2010; Lumbsch and Huhndorf 2010; Rivas Plata et al. 2012). This order is characterized by ascomata ranging from perithecial to apothecial, with unbranched or anastomosate paraphyses, unitunicate nonamyloid asci, and morphologically variable ascospores (Kirk et al. 2008; Lücking et al. 2004; Lumbsch et al. 2007b).

The circumscription of the order Ostropales has undergone many changes in the past. It was originally described to accommodate the nonlichenized family Ostropaceae (Nannfeldt 1932), now known as Stictidaceae. Gilenstam (1969) was the first to include lichenized taxa within Ostropales. He recognized the close relationship between the lichenized genus *Conotrema* and the nonlichenized genus *Stictis* and attributed *Conotrema* to Ostropales. He also suggested that the lichenized genera *Diploschistes*, *Graphis*, and *Thelotrema* should be transferred to Ostropales because of their close relationship with *Conotrema* (Gilenstam 1969). Henssen and Jahns (1974) considered these genera and further lichenized groups (then included in Asterothyriaceae, Graphidaceae, and Thelotremataceae) as part of Ostropales. Subsequently, in a morphological revision of Ostropalean fungi, Sherwood (1977a, b) restricted Ostropales to *Odontotrema*, *Ramonia*, most current genera of Stictidaceae, and other genera now excluded from Lecanoromycetes. In this classification, many lichenized taxa (e.g., Graphidaceae and Thelotremataceae) were excluded from Ostropales based on differences in ascospore type (Sherwood 1977a, b).

Early molecular phylogenetic studies confirmed the close relationship between *Stictis* and *Conotrema*, and between Stictidaceae and both Graphidaceae and Thelotremataceae (Winka et al. 1998). The two families Coenogoniaceae and Gyalectaceae (Gyalectales) were then shown to be related to Graphidaceae and Thelotremataceae based on molecular data, and a broad delimitation was adopted for Ostropales (Kauff and Lutzoni 2002): Ostropales s.l. with Coenogoniaceae, Graphidaceae [including Thelotremataceae, as shown by Mangold et al. (2008)], Gyalectaceae, Stictidaceae, and Trapeliaceae. Other families were subsequently attributed to Ostropales s.l. based on additional molecular data: Asterothyriaceae and Gomphillaceae (Lücking et al. 2004), Phlyctidaceae and Solorinellaceae (Miadlikowska et al. 2006), the reinstated family Sagiolechiaceae (Baloch et al. 2010), and, more surprisingly, Porinaceae, a family of lichenized perithecioid ascomycetes (Grube et al. 2004). Trapeliaceae (as Agyriaceae) is now excluded from Ostropales s.l. (Grube et al. 2004; Miadlikowska et al. 2006). Although still recently largely debated (Grube et al. 2004; Lücking et al. 2004; Lumbsch et al. 2004; Miadlikowska et al. 2006), the broader delimitation of Ostropales (but without Trapeliaceae) has been accepted in current classification systems (Hibbett et al. 2007; Lumbsch and Huhndorf 2010).

The phylogenetic placement of Ostropales within Lecanoromycetes has also long been unclear due to an unstable backbone of the Lecanoromycetes phylogeny (Lumbsch et al. 2007b). Ostropales has been found to be sister to all other Lecanoromycetes (Grube et al. 2004; Lücking et al. 2004; Lumbsch et al. 2004), sister to Trapeliales and Hymeneliaceae (Kauff and Lutzoni 2002; Miadlikowska and Lutzoni 2004) or to a lineage including Trapeliales and Baeomycetales (Miadlikowska et al. 2006), sister to Fuscideaceae, a family incertae sedis in Lecanoromycetes (Reeb et al. 2004), and sister to a lineage including *Anzina* and *Arthroraphis* (Wedin et al. 2005), although none of these relationships were strongly supported. Schmitt et al. (2005) reported the Thelenellaceae as sister to the Ostropales s.l. with a high posterior probability, supporting the resolution shown in Fig. 4.5. Nevertheless, more loci and broader taxon samplings are needed to establish the sister taxa of Ostropales with high phylogenetic confidence (Lumbsch et al. 2007b).

H. Peltigerales

Peltigerales (Lecanoromycetidae) (Fig. 4.5) is an order of mainly foliose species, with rounded apothecia, unbranched paraphyses, bitunicate asci with fissitunicate dehiscence, and multiseptate ascospores (Honegger 1978; Kirk et al. 2008). They have a worldwide distribution and colonize diverse substrates, mostly in humid habitats. **Most species in this order are associated with cyanobacteria, either as primary or secondary photobionts.** All Lecanoromycetes with cyanobacteria as their primary photobiont belong to this order [with the only exception being Arctomiaceae, which are classified in Ostropomycetidae (Lumbsch

et al. 2005)]. When cyanobacteria occur only as secondary photobionts, the primary photobionts are then green algae from the genera *Coccomyxa*, *Dictyochloropsis*, or *Myrmecia* (Tschermak-Woess 1988a), and the cyanobacterial secondary photobionts are restricted to gall-like structures called cephalodia. Peltigeralean species associated only with a green alga are rare. The most recent common ancestor of Peltigerales was inferred to be associated with a cyanobacterium as its primary photobiont, which means that the **green algal photobionts were most likely acquired secondarily in this order** (Miadlikowska and Lutzoni 2004). Moreover, the anatomically nonlayered gelatinous thalli mostly found in some genera of Collematineae (e.g., *Collema* and *Leptogium*) seem to have evolved from more complex and anatomically layered thalli (Wedin et al. 2009). The phylogenetic relationships, an overview of phenotypic characters, and the major types of ascus structures within Peltigerales are reported in Spribille and Muggia (2013).

Peltigerales currently includes two suborders, Collematineae and Peltigerineae (Miadlikowska and Lutzoni 2004), and ten families (Spribille and Muggia 2013). Collematineae includes four families: Coccocarpiaceae, Collemataceae, Pannariaceae, and Placynthiaceae. Peltigerinae includes six families: Koerberiaceae, Lobariaceae, Massalongiaceae, Nephromataceae, Peltigeraceae, and Vahliellaceae (Miadlikowska and Lutzoni 2004; Muggia et al. 2011; Spribille and Muggia 2013; Wedin et al. 2007, 2011). Previously, peltigeralean lichens had been recognized at either the ordinal level (Peltigerales) (Hafellner 1988; Kirk et al. 2001) or the subordinal level within the order Lecanorales (Peltigerinae) (Eriksson et al. 2003; Henssen and Jahns 1974; Poelt 1973; Rambold and Triebel 1992; Tehler 1996). Despite this ranking inconsistency, all large-scale molecular phylogenetic studies confirmed the placement of this lineage within Lecanoromycetes (e.g., Kauff and Lutzoni 2002; Lutzoni et al. 2001, 2004; Miadlikowska et al. 2006; Wedin and Wiklund 2004). The current recognition of this clade at the ordinal level and the establishment of the **two suborders Peltigerineae and Collematineae** were proposed by Miadlikowska and Lutzoni (2004) and are now largely adopted (Hibbett et al. 2007; Lumbsch and Huhndorf 2007b, 2010).

The number of families within Peltigerales changed greatly over time [see details in Miadlikowska and Lutzoni (2004)]. The two families Lobariaceae and Peltigeraceae have always been included in Peltigerales (Hafellner 1988; Poelt 1973), but Coccocarpiaceae, Collemataceae, and Pannariaceae have sometimes been excluded and transferred to the Lecanorales s.l. or classified as incertae sedis within Lecanorales s.l. (Eriksson et al. 2003; Hafellner 1988; Henssen and Jahns 1974; Kirk et al. 2001; Poelt 1973). Moreover, Nephrotomataceae and Solorinaceae were recognized as separate families from Peltigeraceae by certain authors (Hafellner 1988; Poelt 1973). Molecular phylogenetic studies have confirmed the placement of Coccocarpiaceae, Collemataceae, Pannariaceae, and Placynthiaceae within the Collematineae, and Lobariaceae, Nephromataceae, and Peltigeraceae within the Peltigerineae (Miadlikowska and Lutzoni 2004; Miadlikowska et al. 2006; Wedin and Wiklund 2004; Wedin et al. 2009). The families Massalongiaceae, Vahliellaceae, and Koerberiaceae were more recently described and attributed to Peltigerineae (Spribille and Muggia 2013; Wedin et al. 2007, 2011).

I. Pertusariales

The order Pertusariales (Ostropomycetidae) (Fig. 4.5) mostly comprises crustose species with disciform to poriform apothecia, thick-walled asci, branched paraphysoids, and generally large ascospores (Lumbsch et al. 1994; Schmitt et al. 2006). They have a worldwide distribution and colonize a broad range of habitats and substrates. Earlier, these species were classified in the suborder Pertusarineae within the order Lecanorales s.l. (Henssen and Jahns 1974; Poelt 1973) and later as the order Pertusariales (Hawksworth and Eriksson 1986). Molecular phylogenies revealed that Pertusariales belongs to Ostropomycetidae (Lutzoni et al. 2004; Miadlikowska et al. 2006; Reeb et al. 2004), and this order was accepted in all recent classifications of Ascomycota (Hibbett et al. 2007; Lumbsch and Huhndorf 2010).

A recent molecular study showed that the type species of *Agyrium* did not cluster with other Agyriaceae but nested within Pertusariales (Schmitt et al. 2010). As a result, these authors reduced the order Pertusariales to synonymy with Agyriales based on the priority

principle. The name Agyriales would then be used for a group including a large majority of species traditionally classified in Pertusariales and only a few mostly nonlichenized and poorly known species of *Agyrium*. Hodkinson and Lendemer (2011) proposed that **the name Pertusariales should be retained over Agyriales** because the principle of priority is not mandatory for taxa above the family rank, and because the name Agyriales was most recently misapplied to a monophyletic group, including the family Trapeliaceae, now recognized as Trapeliales.

Pertusariales currently includes seven families: Agyriaceae (currently only represented by its generic type *Agyrium rufum*), Coccotremataceae, Icmadophilaceae, Megasporaceae, Miltideaceae, Ochrolechiaceae, and Pertusariaceae (Hodkinson and Lendemer 2011; Lumbsch and Huhndorf 2010; Schmitt et al. 2010; Widhelm and Lumbsch 2011), but its circumscription has been problematic. Only species from the families Coccotremataceae, Pertusariaceae, and Ochrolechiaceae had traditionally been included in Pertusariineae/Pertusariales (Eriksson and Hawksworth 1986; Henssen 1976; Henssen and Jahns 1974; Poelt 1973). Species from the Coccotremataceae family were segregated from Pertusariaceae based on differences in ascomata structure and ontogeny (David and Hawksworth 1991; Henssen 1976). Because species of Coccotremataceae also differ from those of Pertusariaceae in other aspects (e.g., the ascus structure, the presence of cephalodia), members of Coccotremataceae had previously been excluded from Pertusariales (Lumbsch et al. 1994), but molecular phylogenetic analyses confirmed their placement within this order (Lumbsch et al. 2002). The segregation of *Ochrolechia* from Pertusariaceae was first suggested by Harris (1990). Schmitt et al. (2006) then formally described and redelimited this family to also include the genus *Varicellaria*. Icmadophilaceae had earlier been classified within Baeomycetaceae (Henssen and Jahns 1974; Poelt 1973), but molecular phylogenies supported the placement of this family within Pertusariales (Miadlikowska et al. 2006; Reeb et al. 2004). The family Megasporaceae was erected for *Megaspora verrucosa*, a species previously classified as part of the genus *Aspicilia* (Clauzade and Roux 1984) and placed in Pertusariales (Lumbsch et al. 1994). This species was later shown based on molecular data to be sister to *Aspicilia* and part of Pertusariales (Schmitt et al. 2006). The genera *Aspicilia* and *Lobothallia* were therefore transferred to Megasporaceae, and Aspiciliaceae ined. was regarded as a synonym of this family (Lumbsch and Huhndorf 2010; Schmitt et al. 2006).

J. Rhizocarpales

The order Rhizocarpales (Lecanoromycetidae?) (Fig. 4.5) **includes two families, Rhizocarpaceae and part of the Catillariaceae** (the genus *Sporastatia*), and approximately 489 species (Kirk et al. 2008). They are characterized by crustose areolate thalli, immersed to sessile apothecia, branched and often anastomosed paraphyses, asci with an amyloid apex, and simple to muriform ascospores (Hafellner 1984). The sister relationship between *Sporastatia* (Catillariaceae) and *Rhizocarpon* (Rhizocarpaceae) was first demonstrated in the study by Reeb et al. (2004). Further studies confirmed this result (Buschbom and Mueller 2004; Lutzoni et al. 2004), and the order Rhizocarpales was proposed by Miadlikowska et al. (2006) to accommodate selected taxa from these two families (many members were never subjected to phylogenetic studies). The phylogenetic position of Rhizocarpales as the first split within Lecanoromycetes has rarely been supported. If confirmed, this order should be considered as a member of Lecanoromycetidae.

K. Sarrameanales

The order Sarrameanales (Ostropomycetidae) (Fig. 4.5) includes a single family, Sarrameanaceae, and the two genera *Loxospora* and *Sarrameana*. It is a small family of approximately ten species occurring mostly in cool temperate regions of both Northern and Southern Hemispheres (Kantvilas 2004). They are crustose species with dark lecideine to lecanorine apothecia, simple to sparingly branched paraphyses, asci with an amyloid domelike tholus lacking an ocular chamber, and simple to transversally septate hyaline ascospores. The genus *Loxospora* was segregated from Haematommataceae and placed in Loxosporaceae (Staiger and Kalb 1995), which was later synonymized with Sarrameanaceae (Kantvilas 2004). **Because of a unique combination of morphological characters, the systematic position of the**

genus *Sarrameana* has always been problematic, possibly as related to Fuscideaceae (Eriksson and Hawksworth 1986), Haematommataceae (Hafellner 1984; Vězda and Kantvilas 1988), Lecideaceae (Vězda and James 1973), and Ophioparmaceae (Kantvilas and Vězda 1996). Recent molecular phylogenetic analyses that included several species of *Loxospora* showed that Sarrameanaceae is not related to any of these families (Lumbsch et al. 2007a, b, 2008b). In several studies, the genus *Loxospora* forms the earliest diverging lineage within the subclass Ostropomycetidae (Lumbsch et al. 2007b; Miadlikowska et al. 2006; Schoch et al. 2009). As a result, Hodkinson and Lendemer (2011) erected the new order Sarrameanales for Sarrameanaceae.

L. Teloschistales

Teloschistales (Lecanoromycetidae) (Fig. 4.5), as recently recircumscribed by Gaya et al. (2012), comprises four families classified in the two suborders Teloschistineae (Megalosporaceae and Teloschistaceae) and Letrouitineae (Brigantiaeaceae and Letrouitiaceae). It includes mostly lichenized species with crustose to foliose or fruticose thalli with a yellow to orange color (anthraquinone pigments), apothecial ascomata, unbranched paraphyses, unitunicate asci with an apical thickening, and mostly hyaline polarilocular ascospores (Kärnefelt 1989; Kirk et al. 2008). They are found worldwide and often favor nutrient-rich substrates.

First recognized as a suborder within Lecanorales s.l. (Teloschistineae; Henssen and Jahns 1974) or placed within the suborder Buelliineae (Poelt 1973), the families Letrouitiaceae, Teloschistaceae, and, tentatively, Fuscideaceae were grouped by Eriksson and Hawksworth (1986) in the order Teloschistales, which they formally described. With the advent of molecular phylogenetics, several taxa were added to Teloschistales, namely, Megalosporaceae (Helms et al. 2003; Lutzoni et al. 2004) and both Caliciaceae and Physciaceae (Miadlikowska et al. 2006), which were shown to form a monophyletic group (Helms et al. 2003; Wedin et al. 2000a). As a result, two suborders were recognized within Teloschistales (Miadlikowska et al. 2006): Physciineae (Physciaceae, including Caliciaceae) and Teloschistineae (Letrouitiaceae, Megalosporaceae, and Teloschistaceae). However, the relationship between Physciineae and Teloschistineae never obtained strong support (Miadlikowska et al. 2006).

In a more recent molecular study, Gaya et al. (2012) detected two competing hypotheses for the relationships among the three clades Lecanorales, Physciineae, and Teloschistineae: either Lecanorales is sister to a lineage including Physciineae and Teloschistineae, or Physciineae is sister to a lineage including Lecanorales and Teloschistineae. To avoid this phylogenetic uncertainty contributing to taxonomic instability, Gaya et al. (2012) proposed **to restrict the name Teloschistales to the Teloschistiineae and resurrect the order Caciliales for the Physciineae**. This study also showed that Brigantiaeaceae, a family classified as incertae sedis in Lecanoromycetidae (Lumbsch and Huhndorf 2010) or as part of Lecanorales (Kirk et al. 2008), belongs to Teloschistales and is sister to Letrouitiaceae (Gaya et al. 2012).

M. Trapeliales

Trapeliales (Ostropomycetidae) (Fig. 4.5) currently includes a single family, Trapeliaceae (Hodkinson and Lendemer 2011). This family had been described for the four genera *Orceolina*, *Placopsis*, *Trapelia*, and *Trapeliopsis* (Hertel 1970). Originally, Trapeliaceae was placed in the Agyriineae, a suborder of Lecanorales with a similar ascus structure (Hafellner 1994). In a comprehensive morphological revision of Agyriineae, the placement of this suborder within Lecanorales was questioned (Lumbsch 1997). Early molecular studies showed that Agyriineae were indeed not related to Lecanorales, and Agyriales was resurrected for this group (Lumbsch et al. 2001a). More recent molecular studies showed that the type species of *Agyrium* (*A. rufum*) belongs to Pertusariales (Lumbsch et al. 2007c; Schmitt et al. 2010). **Because no ordinal name was then available for the lineage including all genera of**

Trapeliaceae and other genera previously placed in Agyriaceae, Hodkinson and Lendemer (2011) proposed to erect the new order Trapeliales for them. Previous molecular data had confirmed the placement within Trapeliaceae of the four genera originally included in this family (Lumbsch et al. 2007b, c; Miadlikowska et al. 2006; Poulsen et al. 2001; Schmitt et al. 2003).

In addition, the genera *Aspiciliopsis*, *Placynthiella*, *Ptychographa*, *Rimularia*, and *Xylographa* had also been shown to belong to this family based on molecular data (Lumbsch et al. 2001b; Schmitt et al. 2003). The genera *Amylora*, *Coppinsia*, *Lignoscripta*, and *Sarea* have also been suggested as belonging to Trapeliaceae (Hodkinson and Lendemer 2011), but their phylogenetic placements still need confirmation from molecular phylogenetic studies.

N. Umbilicariales

The order Umbilicariales (Lecanoromycetes incertae sedis) (Fig. 4.5) is an order of approximately 191 species classified in four families: Elixiaceae, Fuscideaceae, Ophioparmaceae [including Rhizoplacopsidaceae, as it is now considered a synonym of Ophioparmaceae (Lumbsch and Huhndorf 2010)], and Umbilicariaceae (Kirk et al. 2008). The delimitation of the order Umbilicariales has changed greatly over the last decade. Diverse lichen families have recently been shown to belong to this order: Elixiaceae (Lumbsch et al. 2007a), Ophioparmaceae and Fuscideaceae (Bylin et al. 2007; Miadlikowska et al. 2006), Rhizoplacopsidaceae (Zhou and Wei 2006), and Ropalosporaceae, although without support for this last family (Bylin et al. 2007). Umbilicariales was formally described in two different publications during the same year (Hibbett et al. 2007; Zhou and Wei 2007), but the authorship was attributed to the earliest one (Zhou and Wei 2007).

Umbilicariaceae, the largest family within this order, includes lichenized foliose umbilicate species, with disciform apothecia, mostly unbranched paraphyses, unitunicate asci with a small amyloid cap, and simple to muriform ascospores (Hibbett et al. 2007; Louwhoff 2009). They mostly grow on rocks at high altitudes or high latitudes (Davydov 2007). They were traditionally placed in Lecanorales s.l. (Henssen and Jahns 1974), sometimes in a separate suborder, Umbilicariineae (Poelt 1973). Early molecular studies showed that Umbilicariaceae was not related to Lecanorales (Kauff and Lutzoni 2002; Lutzoni et al. 2001; Stenroos and DePriest 1998). **They form a well-supported monophyletic group, and although their placement within Lecanoromycetes is not resolved, they do not seem to belong to any of the three currently recognized Lecanoromycetes subclasses Acarosporomycetidae, Lecanoromycetidae, and Ostropomycetidae** (Hofstetter et al. 2007; Lumbsch et al. 2004; Miadlikowska et al. 2006; Reeb et al. 2004; Wedin et al. 2005). Miadlikowska et al. (2006) suggested that this group might be recognized as a separate subclass in the future.

VIII. Conclusion

Morphological, anatomical, and chemical characters have traditionally been used to classify orders, families, and genera within Lecanoromycetes, the class of Ascomycota with the largest number of lichen-forming fungi. In the last two decades, molecular phylogenies showed that traditional classification systems were not always consistent with the evolutionary history of this fungal class, resulting in changes in the delimitation of orders and families. For example, many families were segregated from the large and heterogenous order Lecanorales and raised to the ordinal level. As a result, the classification system of the Lecanoromycetes is now more on par with the overall supraordinal classification of the Fungi (Hibbett et al. 2007) and comprises 14 orders and 3 subclasses. Additional changes are expected in the future when better taxon and gene sampling will be available to resolve phylogenetic relationships within Lecanoromycetes.

Acknowledgments Thanks to Armando Mendez and Andrea Hart (Botany Library, Natural History Museum) for their help in locating and providing the required literature.

References

Ahmadjian V (1967) A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. Phycologia 6:127–160

Ahmadjian V (1969) Lichen synthesis. Österr Bot Z 116:306–311

Ahmadjian V (1993) The lichen photobiont—what can it tell us about lichen systematics? Bryologist 96:310–313

Amo de Paz G, Cubas P, Divakar PK, Lumbsch HT, Crespo A (2011) Origin and diversification of major clades in parmelioid lichens (Parmeliaceae, Ascomycota) during the Paleogene inferred by Bayesian analysis. PLoS One 6:e28161

Andersen HL, Ekman S (2004) Phylogeny of the Micareaceae inferred from nrSSU DNA sequences. Lichenologist 36:27–35

Andersen HL, Ekman S (2005) Disintegration of the Micareaceae (lichenized Ascomycota): a molecular phylogeny based on mitochondrial rDNA sequences. Mycol Res 109:21–30

Aptroot A (2001) Lichenized and saprobic fungal biodiversity of a single *Elaeocarpus* tree in Papua New Guinea, with the report of 200 species of ascomycetes associated with one tree. Fungal Divers 6:1–11

Aptroot A, Sipman HJM (1997) Diversity of lichenized fungi in the tropics. In: Hyde KD (ed) Biodiversity of tropical microfungi. University Press, Hong Kong, pp 93–106

Armaleo D, Zhang Y, Cheung S (2008) Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential. Mycologia 100:565–576

Armaleo D, Sun XM, Culberson C (2011) Insights from the first putative biosynthetic gene cluster for a lichen depside and depsidone. Mycologia 103:741–754

Arup U, Ekman S, Grube M, Mattsson JE, Wedin M (2007) The sister group relation of Parmeliaceae (Lecanorales, Ascomycota). Mycologia 99:42–49

Asahina Y, Shibata S (1954) Chemistry of lichen substances. Japan Society for the Promotion of Science, Ueno, Tokyo, Japan

Bailey RH (1976) Ecological aspects of dispersal and establishment in lichens. In: Brown DH, Hawksworth DL, Bailey RH (eds) Lichenology: progress and problems. Academic, London, pp 215–247

Baloch E, Lücking R, Lumbsch HT, Wedin M (2010) Major clades and phylogenetic relationships between lichenized and non-lichenized lineages in Ostropales (Ascomycota: Lecanoromycetes). Taxon 59:1483–1494

Barr ME (1983) The ascomycete connection. Mycologia 75:1–13

Bellemère A (1977) L'appareil apical de l'asque chez quelques Discomycètes: étude ultrastructurale comparative. Rev Mycol 40:3–19

Bellemère A (1994) Asci and ascospores in ascomycete systematics. In: Hawksworth DL (ed) Ascomycete systematics. Problems and perspectives in the nineties, NATO advanced science institutes series. Plenum Press, New York, pp 111–126

Bellemère A, Hafellner J (1982) L'ultrastructure des asques du genre *Dactylospora* (Discomycètes) et son intérêt taxonomique. Cryptogamie Mycol 3:71–93

Bellemère A, Letrouit-Galinou MA (1987) Differentiation of lichen asci including dehiscence and sporogenesis: an ultrastructural survey. In: Peveling E (ed) Progress and problems in lichenology in the eighties, vol 25, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 137–161

Ben-Shaul Y, Paran N, Galun M (1969) The ultrastructure of the association between phycobiont and mycobiont in three ecotypes of the lichen *Caloplaca aurantia* var. *aurantia*. J Microsc 8:415–422

Berbee ML (1996) Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470

Berbee ML, Taylor JW (1993) Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127

Berbee ML, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol VII, part B. Systematics and evolution. Springer, Berlin, Heidelberg, New York, pp 229–245

Bessey CE (1907) A synopsis of plant phyla. Univ Nebr Stud 7(4):275–373

Blanco O, Crespo A, Elix JA, Hawksworth DL, Lumbsch HT (2004) A molecular phylogeny and a new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (Ascomycota: Lecanorales). Taxon 53:959–975

Blanco O, Crespo A, Divakar PK, Elix JA, Lumbsch HT (2005) Molecular phylogeny of parmotremoid lichens (Ascomycota, Parmeliaceae). Mycologia 97:150–159

Blanco O, Crespo A, Ree RH, Lumbsch HT (2006) Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. Mol Phylogenet Evol 39:52–69

Brodo IM (1973) The lichen genus *Coccotrema* in North America. Bryologist 76:260–270

Brodo IM, Sloan NA (2004) Lichen zonation on coastal rocks in Gwaii Haanas National Park Reserve, Haida Gwaii (Queen Charlotte Islands), British Columbia. Can Field Nat 118:405–424

Brodo IM, Duran Sharnoff S, Sharnoff S (2001) Lichens of North America. Yale University Press, New Haven, London

Buschbom J, Barker D (2006) Evolutionary history of vegetative reproduction in *Porpidia* s.l. (lichen-forming Ascomcota). Syst Biol 55:471–484

Buschbom J, Mueller G (2004) Resolving evolutionary relationships in the lichen-forming genus *Porpidia* and related allies (Porpidiaceae, Ascomycota). Mol Phylogenet Evol 32:66–82

Buschbom J, Mueller GM (2006) Testing "species pair" hypotheses: evolutionary processes in the lichen-forming species complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. Mol Biol Evol 23:574–586

Bylin A, Arnerup J, Högberg N, Thor G (2007) A phylogenetic study of Fuscideaceae using mtSSU rDNA. In: Frisch A, Lange U, Staiger B (eds) Lichenologische Nebenstunden. Contributions to lichen taxonomy and ecology in honour of Klaus Kalb, vol 96, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 49–60

Chadefaud M (1942) Étude d'asques: II. Structure et anatomie comparée de l'appareil apical chez divers Disco- et Pyrénomycètes. Rev Mycol 7:57–88

Chadefaud M (1960) Les végétaux non vasculaires (Cryptogamie). In: Chadefaud M, Emberger L (eds) Traité de botanique systématique. Masson, Paris, pp 524–529, 543–545 and 639–640

Chadefaud M (1973) Les asques et la systematique des Ascomycetes. Bull Soc Mycol France 89:127–170

Chadefaud M, Letrouit-Galinou MA, Favre MC (1963) Sur l'évolution des asques du type archaeascé chez les Discomycètes de l'ordre des Lécanorales. Compt Rend Acad Sci Paris 257:4003–4005

Chadefaud M, Letrouit-Galinou MA, Favre MC (1967) Sur l'origine phylogénétique et l'évolution des ascomycètes des lichens. Bull Soc Bot Fr Mém 115:79–111

Choisy M (1954) Catalogue des lichens de la region lyonnaise. Édition Paul Chevalier, Paris

Clauzade G, Roux C (1984) Les genres *Aspicilia* Massal. et *Bellemerea* Hafellner et Roux. Bull Soc Bot Centre-Ouest 15:127–141

Clauzade G, Roux C (1985) Likenoj de okcidenta Europo. Ilustrita determinlibro. Bull Soc Bot Centre-Ouest 7:1–893

Collins CR, Farrar JF (1978) Structural resistances to mass transfer in the lichen *Xanthoria parietina*. New Phytol 81:71–83

Coppins BJ, Wolseley P (2002) Lichens of tropical forests. In: Watling R, Frankland JC, Ainsworth AM, Isaac S, Robinson CH (eds) Tropical mycology, vol 2, Micromycetes. CABI Publishing, Wallingford, UK, pp 113–131

Crespo A, Lumbsch HT, Mattsson JE, Blanco O, Divakar PK, Articus K, Wiklund E, Bawingan PA, Wedin M (2007) Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear *RPB*1 gene. Mol Phylogenet Evol 44:812–824

Crespo A, Kauff F, Divakar PK, del Prado R, Pérez-Ortega S, de Paz GA, Ferencova Z, Blanco O, Roca-Valiente B, Núñez-Zapata J, Cubas P, Argüello A, Elix JA, Esslinger TL, Hawksworth DL, Millanes AM, Molina MC, Wedin M, Ahti T, Aptroot A, Barreno E, Bungartz F, Calvelo S, Candan M, Cole MJ, Ertz D, Goffinet B, Lindblom L, Lücking R, Lutzoni F, Mattsson JE, Messuti MI, Miadlikowska J, Piercey-Normore MD, Rico VJ, Sipman H, Schmitt I, Spribille T, Thell A, Thor G, Upreti DK, Lumbsch HT (2010) Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. Taxon 59:1735–1753

Culberson WL, Culberson CF (1981) The genera *Cetrariastrum* and *Concamerella* (Parmeliaceae): a chemosynthetic synopsis. Bryologist 84:273–314

Culberson CF, Elix JA (1989) Lichen substances. In: Harborne JB (ed) Methods in plant biochemistry, vol 1, Plant phenolics. Academic, London, San Diego, pp 509–535

Dal Grande F, Widmer I, Wagner HH, Scheidegger C (2012) Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. Mol Ecol 21:3159–3172

David JC, Hawksworth DL (1991) Validation of six family names of lichenized ascomycetes. Systema Ascomycetum 10:13–18

Davydov DA (2007) Approaches to a classification of the family Umbilicariaceae (lichenised Ascomycota) by anatomical and morphological characters. Turczaninowia 10:51–57

Du Rietz GE (1924) Die Soredien und Isidien der Flechten. Svensk Bot Tidskr 18:371–396

Duvigneaud P (1955) Les *Stereocaulon* des hautes montagnes du Kivu. Essai anatomo-systématique. Lejeunia Mem 14:1–144

Ekman S (2001) Molecular phylogeny of the Bacidiaceae (Lecanorales, lichenized Ascomycota). Mycol Res 105:783–797

Ekman S, Tønsberg T (2002) Most species of *Lepraria* and *Leproloma* form a monophyletic group closely related to *Stereocaulon*. Mycol Res 106:1262–1276

Ekman S, Wedin M (2000) The phylogeny of the families Lecanoraceae and Bacidiaceae (lichenized Ascomycota) inferred from nuclear SSU rDNA sequences. Plant Biol 2:350–360

Ekman S, Andersen HL, Wedin M (2008) The limitations of ancestral state reconstruction and the evolution of the ascus in the Lecanorales (lichenized Ascomycota). Syst Biol 57:141–156

Elix JA (1993) Progress in the generic delimitation of *Parmelia* sensu lato lichens (Ascomycotina: Parmeliaceae) and a synoptic key to the Parmeliaceae. Bryologist 96:359–383

Elix JA, Hale ME (1987) *Canomaculina*, *Myelochroa*, *Parmelinella*, *Parmelinopsis* and *Parmotremopsis*, five new genera in the Parmeliaceae (lichenized Ascomycotina). Mycotaxon 29:233–244

Elix JA, Stocker-Wörgötter E (2008) Biochemistry and secondary metabolites. In: Nash TH III (ed) Lichen biology. Cambridge University Press, Cambridge, UK, pp 104–133

Elix JA, Johnston J, Verdon D (1986) *Canoparmelia*, *Paraparmelia* and *Relicinopsis*, three new genera in the Parmeliaceae (lichenized Ascomycotina). Mycotaxon 27:271–282

Eriksson OE (1982) The families of bitunicate Ascomycetes. Opera Bot 60:1–209

Eriksson OE (2006) Outline of Ascomycota—2006. Myconet 12:1–82

Eriksson OE, Hawksworth DL (1986) Outline of the ascomycetes—1986. Systema Ascomycetum 5:185–324

Eriksson OE, Hawksworth DL (1993) Outline of the ascomycetes—1993. Systema Ascomycetum 12:51–257

Eriksson OE, Winka K (1997) Supraordinal taxa of Ascomycota. Myconet 1:1–16

Eriksson OE, Baral HO, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T (2003) Outline of Ascomycota—2003. Myconet 9:1–103

Fedrowitz K, Kaasalainen U, Rikkinen J (2011) Genotype variability of *Nostoc* symbionts associated with three epiphytic *Nephroma* species in a boreal forest landscape. Bryologist 114:220–230

Fernandez-Mendoza F, Domasche S, Garcia MA, Jordan P, Martin MP, Printzen C (2011) Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. Mol Ecol 20:1208–1232

Fletcher A (1980) Marine and maritime lichens of rocky shores: their ecology, physiology and biological interactions. In: Price JH, Irvine DEG, Farnham WF (eds) The shore environment, vol 2, Ecosystems. Systematics Association special volume no. 17B. Academic, London, New York, pp 789–842

Friedl T (1987) Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. Lichenologist 19:183–191

Friedl T, Büdel B (2008) Photobionts. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, UK, pp 7–26

Frisch A, Kalb K, Grube M (2006) Molecular phylogeny of the Thelotremataceae. A study based on Bayesian analysis of mitochondrial 16S rDNA gene data. In: Wirth V (ed) Contributions towards a new systematics of the lichen family Thelotremataceae, vol 92, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 517–539

Galun M (1988) The fungus-alga relation. In: Galun M (ed) CRC handbook of lichenology, vol I. CRC Press, Boca Raton, FL, pp 147–158

Galun M, Paran N, Ben-Shaul Y (1970) An ultrastructural study of the fungus alga association in *Lecanora radiosa* growing under different environmental conditions. J Microsc 8:801–806

Gaya E, Högnabba F, Holguin Á, Molnar K, Fernández-Brime S, Stenroos S, Arup U, Søchting U, van den Boom P, Lücking R, Sipman HJM, Lutzoni F (2012) Implementing a cumulative supermatrix approach for a comprehensive phylogenetic study of the Teloschistales (Pezizomycotina, Ascomycota). Mol Phylogenet Evol 63:374–387

Gazis R, Miadlikowska J, Lutzoni F, Arnold AE, Chaverri P (2012) Culture-based study of endophytes associated with rubber trees in Peru reveals a new class of Pezizomycotina: Xylonomycetes. Mol Phylogenet Evol 65:294–304

Geitler L (1934) Beiträge zur Kenntnis der Flechtensymbiose IV, V. Arch Protistenk 82:51–85

Geitler L (1963) Über Haustorien bei Flechten und über *Myrmecia biatorellae* in *Psora globifera*. Österr Bot Z 110:270–280

Gierl C, Kalb K (1993) Die Flechtengattung *Dibaeis*. Eine Übersicht über die rosafrüchtigen Arten von *Baeomyces* sens. lat. nebst Anmerkungen zu *Phyllobaeis* gen. nov. Herzogia 9:593–645

Gilbert OL (1990) The lichen flora of urban wasteland. Lichenologist 22:87–101

Gilbert OL (1996) The lichen vegetation of chalk and limestone streams in Britain. Lichenologist 28:145–159

Gilbert OL, Giavarini VJ (1997) The lichen vegetation of acid watercourses in England. Lichenologist 29:347–367

Gilenstam G (1969) Studies in the lichen genus *Conotrema*. Arch Bot 7:149–179

Golubkova NS (1988) The lichen family Acarosporaceae in the U.S.S.R. Komarov Botanical Institute, Academy of Sciences of the U.S.S.R., Leningrad

Green TGA, Schroeter B, Sancho LG (1999) Plant life in Antarctica. In: Pugnaire FI, Valladares F (eds) Handbook of functional plant ecology. Marcel Dekker, New York, Basel, pp 495–543

Grube M, Arup U (2001) Molecular and morphological evolution in the Physciaceae (Lecanorales, lichenized Ascomycotina), with special emphasis on the genus *Rinodina*. Lichenologist 33:63–72

Grube M, Hawksworth DL (2007) Trouble with lichen: the re-evaluation and re-interpretation of thallus form and fruit body types in the molecular era. Mycol Res 111:1116–1132

Grube M, Baloch E, Lumbsch HT (2004) The phylogeny of Porinaceae (Ostropomycetidae) suggests a neotenic origin of perithecia in Lecanoromycetes. Mycol Res 108:1111–1118

Gueidan C, Ruibal C, de Hoog GS, Gorbushina AA, Untereiner WA, Lutzoni F (2008) A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. Stud Mycol 61:111–119

Gueidan C, Ruibal C, de Hoog GS, Schneider H (2011) Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. Fungal Biol 115:987–996

Hafellner J (1984) Studien in Richtung einer naturlicheren Gliederung der Sammelfamilien Lecanoraceae und Lecideaceae. In: Hertel H, Oberwinkler F (eds) Beitrage zur Lichenologie. Festscrift J. Poelt, vol 79, Beiheft zur Nova Hedwigia. J. Cramer, Vaduz, pp 241–371

Hafellner J (1988) Principles of classification and main taxonomic groups. In: Galun M (ed) CRC handbook of lichenology, vol III. CRC Press, Boca Raton, FL

Hafellner J (1994) Problems in Lecanorales systematics. In: Hawksworth DL (ed) Ascomycete systematics. Problems and perspectives in the nineties, NATO advanced science institutes series. Plenum, New York, pp 315–320

Hafellner J (1995) Towards a better circumscription of the Acarosporaceae (lichenized Ascomycotina, Lecanorales). Cryptog Bot 5:99–104

Hale ME (1974) *Bulbothrix*, *Parmelina*, *Relicina*, and *Xanthoparmelia*, four new genera in the Parmeliaceae. Phytologia 28:479–490

Hale ME (1984a) An historical review of the genus concept in lichenology. In: Hertel H, Oberwinkler F (eds) Beitrage zur Lichenologie. Festschrift J. Poelt, vol 79, Beiheft zur Nova Hedwigia. Vaduz, J. Cramer, pp 11–23

Hale ME (1984b) *Flavopunctelia*, a new genus in the Parmeliaceae (Ascomycotina). Mycotaxon 20:681–682

Hawksworth DL, Eriksson OE (1986) The names of accepted orders of ascomycetes. Systema Ascomycetum 5:175–184

Harris RC (1990) Some Florida lichens. Author, Bronx, NY

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129–1133

Helms G, Friedl T, Rambold G (2003) Phylogenetic relationships of the Physciaceae inferred from rDNA sequence data and selected phenotypic characters. Mycologia 95:1078–1099

Henskens FL, Green TGA, Wilkins A (2012) Cyanolichens can have both cyanobacteria and green algae in a common layer as major contributors to photosynthesis. Ann Bot 110:555–563

Henssen A (1976) Studies in the developmental morphology of lichenized Ascomycetes. In: Brown DH, Hawksworth DL, Bailey RH (eds) Lichenology: progress and problems. Academic, London, pp 107–138

Henssen A (1981) *Hyphomorpha* als phycobiont in Flechten. Plant Syst Evol 137:139–143

Henssen A (1985) *Hertella*, a new lichen genus in the Peltigerales from the Southern Hemisphere. Mycotaxon 22:381–397

Henssen A, Jahns HM (1974) Lichenes. Georg Thieme, Stuttgart

Henssen A, Keuck G, Renner B, Vobis G (1981) The lecanoralean centrum. In: Reynolds DR (ed) Ascomycete systematics. The Luttrellian concept. Springer, New York, Heidelberg, Berlin, pp 138–234

Hertel H (1970) Trapeliaceae—eine neue Flechtenfamilie. Vortr Gesamtgeb Bot 4:171–185

Hertel H, Rambold G (1985) *Lecidea* sect. *Armeniacae*: lecideoide Arten der Flechtengattungen *Lecanora* und *Tephromela* (Lecanorales). Bot Jarb Syst 107:469–501

Hestmark G (1997) Growth from the centre in an umbilicate lichen. Lichenologist 29:379–383

Hestmark G, Miadlikowska J, Kauff F, Fraker E, Molnar K, Lutzoni F (2011) Single origin and subsequent diversification of central Andean endemic *Umbilicaria* species. Mycologia 103:45–56

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Lumbsch HT, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüßler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111:509–547

Hodkinson BP, Lendemer JC (2011) The orders of Ostropomycetidae (Lecanoromycetes, Ascomycota): recognition of Sarrameanales and Trapeliales with a request to retain Pertusariales over Agyriales. Phytologia 93:407–412

Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F (2007) Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). Mol Phylogenet Evol 44:412–426

Honegger R (1978) The ascus apex in lichenized fungi. I: The *Lecanora*-, *Peltigera* and *Teloschistes*-types. Lichenologist 10:47–67

Honegger R (1980) The ascus apex in lichenized fungi. II: The *Rhizocarpon*-type. Lichenologist 12:157–172

Honegger R (1982a) Ascus structure and function, ascospore delimitation, and phycobiont cell wall types associated with the Lecanorales (lichenized ascomycetes). J Hattori Bot Lab 52:417–429

Honegger R (1982b) The ascus apex in lichenized fungi. III: The *Pertusaria*-type. Lichenologist 14:205–217

Honegger R (1983) The ascus apex in lichenized fungi. IV: *Baeomyces* and *Icmadophila* in comparison with *Cladonia* (Lecanorales) and the non-lichenized *Leotia* (Helotiales). Lichenologist 15:57–71

Honegger R (1984a) Scanning electron microscopy of the contact site of conidia and trichogynes in *Cladonia furcata*. Lichenologist 16:11–19

Honegger R (1984b) Ultrastructural studies on conidiomata, conidiophores, and conidiogenous cells in six lichen-forming ascomycetes. Can J Bot 62:2081–2093

Honegger R (2008) Morphogenesis. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, pp 69–93

Honegger R, Edwards D, Axe L (2013) The earliest records of internally stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh Borderland. New Phytol 197:164–275

Huneck S (2001) New results on the chemistry of lichen substances. In: Fortschritte der Chemie organischer Naturstoffe, vol 81. Springer, Vienna

Huneck S, Yoshimura I (1996) Identification of lichen substances. Springer, Berlin, Heidelberg

Ihlen PG, Ekman S (2002) Outline of phylogeny and character evolution in *Rhizocarpon* (Rhizocarpaceae, lichenized Ascomycota) based on nuclear ITS and mitochondrial SSU ribosomal DNA sequences. Biol J Linn Soc 77:535–546

Jaag O (1933) *Coccomyxa* Schmidle, Monographie einer Algengattung. Beitr Kryptogamenfl Schweiz 8:1–132

Jahns HM (1970) Untersuchungen zur Entwicklungsgeschichte der Cladoniaceen. Nova Hedwigia 20:1–177

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch T, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge K, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Buck WR, Cole MS, Diederich P, Printzen C, Schmitt I, Schultz M, Yahr R, Zavarzin A, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of the fungi using a six-gene phylogeny. Nature 443:818–822

Janex-Favre MC (1977) Le developpement et la structure des pycnides de l'*Umbilicaria cinereorufescens*. Rev Bryol Lichenol 43:1–18

Janex-Favre MC (1982) Le developpement et la structure des pycnides du lichen *Parmelia acetabulum*. Cryptogamie Bryol L 3:337–349

Johnston J (2001) Baeomycetaceae. In: McCarthy PM (ed) Flora of Australia, vol 58A, Lichens 3. ABRS/CSIRO Australia, Melbourne, pp 14–16

Kantvilas G (1996) A new byssoid lichen genus from Tasmania. Lichenologist 28:229–237

Kantvilas G (2004) Sarrameanaceae. In: McCarthy PM, Mallett K (eds) Flora of Australia, vol 56A, Lichens 4. ABRS/CSIRO Australia, Melbourne, pp 74–77

Kantvilas G, Vězda A (1996) The lichen genus *Sarrameana*. Nord J Bot 16:325–333

Kärnefelt I (1989) Morphology and phylogeny in the Teloschistales. Cryptog Bot 1:147–203

Kärnefelt I, Thell A (1992) The evaluation of characters in lichenized families, exemplified with the alectorioid and some parmelioid genera. Plant Syst Evol 180:181–204

Kauff F, Büdel B (2005) Ascoma ontogeny and apothecial anatomy in the Gyalectaceae (Ostropales, Ascomycota) support the re-establishment of the Coenogoniaceae. Bryologist 108:272–281

Kauff F, Lutzoni F (2002) Phylogeny of the Gyalectales and Ostropales (Ascomycota, Fungi): among and within order relationships. Mol Phylogenet Evol 25:138–156

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth & Bisby's dictionary of the fungi, 9th edn. CAB International, Wallingford, UK

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Ainsworth & Bisby's dictionary of the fungi, 10th edn. CAB International, Wallingford, UK

Krog H (1982) Evolutionary trends in foliose and fruticose lichens of the Parmeliaceae. J Hattori Bot Lab 52:303–311

Kroken S, Taylor JW (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. Mycologia 93:38–53

Lamb IM (1977) A conspectus of the lichen genus *Stereocaulon* (Schreb.) Hoffm. J Hattori Bot Lab 43:191–355

Lawrey JD (1986) Biological role of lichen substances. Bryologist 89:111–122

Lawrey JD (1989) Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. Bryologist 92:326–328

Lawrey JD, Diederich P (2003) Lichenicolous fungi: interactions, evolution, and biodiversity. Bryologist 106:81–120

Leavitt SD, Johnson LA, Goward T, St Clair LL (2011a) Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (Parmeliaceae) in North America. Mol Phylogenet Evol 60:317–332

Leavitt SD, Johnson LA, St Clair LL (2011b) Species delimitation and evolution in morphologically

and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (Parmeliaceae, Ascomycota) in western North America. Am J Bot 98:175–188

Letrouit-Galinou MA (1972) Études sur le *Lobaria laetevirens*. II: Le développement des pycnides. Bull Soc Bot Fr 119:477–485

Letrouit-Galinou MA (1973a) Les asques des lichens et le type archaeascé. Bryologist 76:30–47

Letrouit-Galinou MA (1973b) Les pycnospores et les pycnides du *Gyalecta carneolutea* (Turn.) Oliv. Bull Soc Bot Fr 120:373–384

Letrouit-Galinou MA, Bellemère A (1989) Ascomatal development in lichens: a review. Cryptogamie Bryol L 10:189–233

Letrouit-Galinou MA, Lallement R (1977) Le développement des pycnides du discolichen *Buellia canescens* (Dicks.) D.N. Ann Sci Nat Bot Biol 18:119–134

Lindemuth R, Wirtz N, Lumbsch HT (2001) Phylogenetic analysis of nuclear and mitochondrial rDNA sequences supports the view that loculoascomycetes (Ascomycota) are not monophyletic. Mycol Res 105:1176–1181

Liu YJ, Hall BD (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proc Natl Acad Sci U S A 101:4507–4512

Louwhoff SHJJ (2009) Umbilicariaceae. In: McCarthy PM (ed) Flora of Australia, vol 57, Lichens 5. ABRS and CSIRO Publishing, Canberra, Melbourne, pp 553–562

Lücking R (2008) Foliicolous lichenized fungi, Flora Neotropica Monograph 103. Organization for Flora Neotropica and The New York Botanical Garden Press, Bronx, NY

Lücking R, Stuart BL, Lumbsch HT (2004) Phylogenetic relationships of Gomphillaceae and Asterothyriaceae: evidence from a combined Bayesian analysis of nuclear and mitochondrial sequences. Mycologia 96:283–294

Lücking R, Huhndorf S, Pfister DH, Rivas Plata E, Lumbsch HT (2009a) Fungi evolved right on track. Mycologia 101:810–822

Lücking R, Lawrey JD, Sikaroodi M, Gillevet PM, Chaves JL, Sipman HJM, Bungartz F (2009b) Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. Am J Bot 96:1409–1418

Lumbsch HT (1997) Systematic studies in the suborder Agyriineae (Lecanorales). J Hattori Bot Lab 83:1–73

Lumbsch HT (1998a) Taxonomic use of metabolic data in lichen-forming fungi. In: Frisvad JC, Bridge PD, Arora DK (eds) Chemical fungal taxonomy. Marcel Dekker, New York, pp 345–387

Lumbsch HT (1998b) The use of metabolic data in lichenology at the species and subspecific levels. Lichenologist 30:357–367

Lumbsch HT, Huhndorf SM (2007a) Whatever happened to the pyrenomycetes and loculoascomycetes? Mycol Res 111:1064–1074

Lumbsch HT, Huhndorf SM (2007b) Outline of Ascomycota 2007. Myconet 13:1–58

Lumbsch HT, Huhndorf SM (2010) Myconet, vol 14. Part one. Outline of Ascomycota—2009. Part two. Notes on ascomycete systematics. Nos. 4751–5113. Fieldiana Life Earth Sci 1:1–64

Lumbsch HT, Feige GB, Schmitz KE (1994) Systematic studies in the Pertusariales. I: Megasporaceae, a new family of lichenized Ascomycetes. J Hattori Bot Lab 75:295–304

Lumbsch HT, Lunke T, Feige GB, Huneck S (1995) Anamylopsoraceae—a new family of lichenized ascomycetes with stipitate apothecia (Lecanorales: Agyriineae). Plant Syst Evol 198:275–286

Lumbsch HT, Schmitt I, Döring H, Wedin M (2001a) Molecular systematics supports the recognition of an additional order of Ascomycota: the Agyriales (Ascomycota). Mycol Res 105:16–23

Lumbsch HT, Schmitt I, Döring H, Wedin M (2001b) ITS sequence data suggest variability of ascus types and support ontogenetic characters as phylogenetic discriminators in the Agyriales (Ascomycota). Mycol Res 105:265–274

Lumbsch HT, Wirtz N, Lindemuth R, Schmitt I (2002) Higher level phylogenetic relationships of euascomycetes (Pezizomycotina) inferred from a combined analysis of nuclear and mitochondrial sequence data. Mycol Prog 1:57–70

Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M (2004) Supraordinal phylogenetic relationships of Lecanoromycetes based on a Bayesian analysis of combined nuclear and mitochondrial sequences. Mol Phylogenet Evol 31:822–832

Lumbsch HT, Del Prado R, Kantvilas G (2005) *Gregorella*, a new genus to accommodate *Moelleropsis humida* and a molecular phylogeny of Arctomiaceae. Lichenologist 37:291–302

Lumbsch HT, Archer AW, Elix JA (2007a) A new species of *Loxospora* (lichenized Ascomycota: Sarrameanaceae) from Australia. Lichenologist 39:509–517

Lumbsch HT, Schmitt I, Lücking R, Wiklund E, Wedin M (2007b) The phylogenetic placement of Ostropales within Lecanoromycetes (Ascomycota) revisited. Mycol Res 111:257–267

Lumbsch HT, Schmitt I, Mangold A, Wedin M (2007c) Ascus types are phylogenetically misleading in Trapeliaceae and Agyriaceae (Ostropomycetidae, Ascomycota). Mycol Res 111:1133–1141

Lumbsch HT, Hipp AL, Divakar PK, Blanco O, Crespo A (2008a) Accelerated evolutionary rates in tropical and oceanic parmelioid lichens (Ascomycota). BMC Evol Biol 8:257

Lumbsch HT, Nelsen MP, Lücking R (2008b) The phylogenetic position of Haematommataceae

(Lecanorales, Ascomycota), with notes on secondary chemistry and species delimitation. Nova Hedwigia 86:104–114

Luttrell ES (1951) Taxonomy of pyrenomycetes. University of Missouri Studies 24, Curators of the University of Missouri, Columbia, MO, pp 1–120

Luttrell ES (1955) The ascostromatic Ascomycetes. Mycologia 47:511–532

Lutzoni F, Pagel M (1997) Accelerated evolution as a consequence of transitions to mutualism. Proc Natl Acad Sci U S A 94:11422–11427

Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940

Lutzoni F, Kauff F, Cox C, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung GH, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim YW, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Am J Bot 91:1446–1480

Mägdefrau K (1957) Flechten und Moose in baltischen Bernstein. Ber Deut Bot Ges 70:433–435

Magnusson AH (1936) Acarosporaceae und Thelocarpaceae. In: Zahlbruckner A (ed) Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz, band 9. Akademische Verlagsgesellschaft M.B.H., Leipzig, pp 1–318

Mangold A, Martín MP, Lücking R, Lumbsch HT (2008) Molecular phylogeny suggests synonymy of Thelotremataceae within Graphidaceae (Ascomycota: Ostropales). Taxon 57:476–486

Matsumoto T, Deguchi H (1999) Pycnidial structures and genus concept in the Thelotremataceae. Bryologist 102:86–91

Mattsson JE, Lumbsch HT (1989) The use of the species pair concept in lichen taxonomy. Taxon 38:238–241

Mattsson JE, Wedin M (1999) A re-assessment of the family Alectoriaceae. Lichenologist 31:431–440

McCarthy PM (2003) Catalogue of Australian Lichens. Flora of Australia Supplementary Series, 19, Australian Biological Resources Study, Canberra

Miadlikowska J, Lutzoni F (2004) Phylogenetic classification of Peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. Am J Bot 91:449–464

Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, Hafellner J, Reeb V, Hodkinson BP, Kukwa M, Lücking R, Hestmark G, Otalora MG, Rauhut A, Büdel B, Scheidegger C, Timdal E, Stenroos S, Brodo IM, Perlmutter GB, Ertz D, Diederich P, Lendemer JC, May PF, Schoch C, Arnold AE, Gueidan C, Tripp E, Yahr R, Robertson C, Lutzoni F (2006) New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. Mycologia 98:1088–1103

Millot M, Di Meo F, Tomasi S, Boustie J, Trouillas P (2012) Photoprotective capacities of lichen metabolites: a joint theoretical and experimental study. J Photochem Photobiol B 111:17–26

Möller A (1888) Über die sogenannten Spermatien der Ascomyceten. Bot Zeitung 46:421–425

Muggia L, Nelson P, Wheeler T, Yakovchenko S, Tønsberg T, Spribille T (2011) Convergent evolution of a symbiotic duet: the case of the lichen genus *Polychidium* (Peltigerales, Ascomycota). Am J Bot 98:1647–1656

Müller J (1880) Lichenologische Beiträge, X. Flora 63:17–24, 40–45

Müller J (1882) Lichenologische Beiträge, XV. Flora 65:291–306, 313–322, 326–337, 381–386, 397–402

Myllys L, Lohtander K, Tehler A (2001) β-Tubulin, ITS and group I intron sequences challenge the species pair concept in *Physcia aipolia* and *P. caesia*. Mycologia 93:335–343

Myllys L, Hognabba F, Lohtander K, Thell A, Stenroos S, Hyvonen J (2005) Phylogenetic relationships of Stereocaulaceae based on simultaneous analysis of beta-tubulin, GAPDH and SSU rDNA sequences. Taxon 54:605–618

Nannfeldt JA (1932) Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nov Act Reg Soc Sci Nat Cherbourg 3:161–202

Nash TH III (2008) Introduction. In: Nash TH III (ed) Lichen biology. Cambridge University Press, Cambridge, UK, pp 1–8

Nelsen MP, Gargas A (2009) Assessing clonality and chemotype monophyly in *Thamnolia* (Icmadophilaceae). Bryologist 112:42–53

Nelsen MP, Chavez N, Sackett-Hermann E, Thell A, Randlane T, Divakar PK, Rico VJ, Lumbsch HT (2011) The cetrarioid core group revisited (Lecanorales: Parmeliaceae). Lichenologist 43:537–551

Nimis PL (1998) A critical appraisal of modern generic concepts in lichenology. Lichenologist 30:427–438

Øvstedal DO, Lewis Smith RI (2001) Lichens of Antarctica and South Georgia: a guide to their identification and ecology. Studies in polar research. Cambridge University Press, Cambridge, UK

Padovan AC, Sanson GF, Brunstein A, Briones MR (2005) Fungi evolution revisited: application of the penalized likelihood method to a Bayesian fungal phylogeny provides a new perspective on phylogenetic relationships and divergence dates of Ascomycota groups. J Mol Evol 60:726–735

Palmqvist K, Dahlman L, Jonsson A, Nash TH III (2008) The carbon economy of lichens. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, UK, pp 182–215

Pérez FL (1997) Geoecology of erratic lichens of *Xanthoparmelia vagans* in an equatorial Andean paramo. Plant Ecol 129:11–28
Platt JL, Spatafora JW (1999) A re-examination of generic concepts of baeomycetoid lichens based on phylogenetic analyses of nuclear SSU and LSU ribosomal DNA. Lichenologist 31:409–418
Plessl A (1963) Über die Beziehungen von Haustorienstypus and Organisationshöhe bei Flechten. Österr Bot Z 110:194–269
Poelt J (1970) Das Konzept der Artenpaare bei den Flechten. Vortr Gesamtgeb Bot 4:187–198
Poelt J (1972) Die taxonomische Behandlung von Artenpaaren bei den Flechten. Bot Not 125:77–81
Poelt J (1973) Systematic evaluation of morphological characters. In: Ahmadjian V, Hale ME (eds) The lichens. Academic, New York, London, pp 91–115, and 599–632
Poelt J (1987) On reductions of morphological structures in lichens. In: Peveling E (ed) Progress and problems in lichenology in the eighties, vol 25, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 35–45
Poinar GO, Peterson EB Jr, Platt JL (2000) Fossil *Parmelia* in new world amber. Lichenologist 32:263–269
Poulsen RS, Schmitt I, Søchting U, Lumbsch HT (2001) Molecular and morphological studies on the subantarctic genus *Orceolina* (Agyriaceae). Lichenologist 33:323–329
Printzen C (2010) Lichen systematics: the role of morphological and molecular data to reconstruct phylogenetic relationships. Prog Bot 71:233–278
Rambold G, Triebel D (1992) The inter-lecanoralean associations, vol 48, Bibliotheca Lichenologica. J Cramer, Berlin
Rambold G, Triebel D, Hertel H (1993) Icmadophilaceae, a new family in the Leotiales. In: Feige GB, Lumbsch HT (eds) Phytochemistry and chemotaxonomy of lichenized Ascomycetes—a festschrift in honour of Siegfried Huneck, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 217–240
Rambold G, Friedl T, Beck A (1998) Photobionts in lichens: possible indicators of phylogenetic relationships? Bryologist 101:392–397
Reeb V, Lutzoni F, Roux C (2004) Contribution of *RPB*2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Mol Phylogenet Evol 32:1036–1060
Reynolds DR (1989) The bitunicate ascus paradigm. Bot Rev 55:1–52
Rikkinen J (2002) Cyanolichens: an evolutionary overview. In: Rain AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Netherlands, pp 31–72
Rikkinen J, Poinar GO (2002) Fossilised *Anzia* (Lecanorales, lichen-forming Ascomycota) from European Tertiary amber. Mycol Res 106:984–990
Rivas Plata E, Lumbsch HT (2011) Parallel evolution and phenotypic disparity in lichenized fungi: a case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). Mol Phylogenet Evol 61:45–63
Rivas Plata E, Lücking R, Lumbsch HT (2012) A new classification for the family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). Fungal Divers 52:107–121
Roux C, Bellemère A, Boissière JC, Esnault J, Janex-Favre MC, Letrouit MA, Wagner J (1986) Les bases de la systématique moderne des lichens. Bull Soc Bot France, Actual Bot 133:7–40
Rosentreter R (1993) Vagrant lichens in North America. Bryologist 96:333–338
Santesson R (1952) Foliicolous lichens. I: A revision of the taxonomy of the obligately foliicolous, lichenized fungi. Symb Bot Upsal 12:1–590
Scheidegger C (1985) Systematische Studien zur Krustenflechte *Anzina carneonivea* (Trapeliaceae, Lecanorales). Nova Hedwigia 41:191–218
Schmitt I, Lumbsch HT (2004) Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. Mol Phylogenet Evol 33:43–55
Schmitt I, Messuti MI, Feige GB, Lumbsch HT (2001) Molecular data support rejection of the generic concept in the Coccotremataceae (Ascomycota). Lichenologist 33:315–321
Schmitt I, Lumbsch HT, Sochting U (2003) Phylogeny of the lichen genus *Placopsis* and its allies based on Bayesian analyses of nuclear and mitochondrial sequences. Mycologia 95:827–835
Schmitt I, Mueller G, Lumbsch HT (2005) Ascoma morphology is homoplaseous and phylogenetically misleading in some pyrenocarpous lichens. Mycologia 97:362–374
Schmitt I, Yamamoto Y, Lumbsch HT (2006) Phylogeny of Pertusariales (Ascomycotina): resurrection of Ochrolechiaceae and new circumscription of Megasporaceae. J Hattori Bot Lab 100:753–764
Schmitt I, del Prado R, Grube M, Lumbsch HT (2009) Repeated evolution of closed fruiting bodies is linked to ascoma development in the largest group of lichenized fungi (Lecanoromycetes, Ascomycota). Mol Phylogenet Evol 52:34–44
Schmitt I, Frankhauser JD, Sweeney K, Spribille T, Kalb K, Lumbsch HT (2010) Gyalectoid *Pertusaria* species form a sister-clade to *Coccotrema* (Ostropomycetidae, Ascomycota) and comprise the new lichen genus *Gyalectaria*. Mycology 1:75–83
Schmitt I, Otte J, Parnmen S, Sadowska-Des AD, Lücking R, Lumbsch HT (2012) A new circumscription of the genus *Varicellaria* (Pertusariales, Ascomycota). MycoKeys 4:23–26
Schmull M, Miadlikowska J, Pelzer M, Stocker-Wörgötter E, Hofstetter V, Fraker E, Hodkinson BP, Reeb V, Kukwa M, Lumbsch HT, Kauff F, Lutzoni F (2011) Phylogenetic affiliations of members of

the heterogeneous lichen-forming fungi of the genus *Lecidea* sensu Zahlbruckner (Lecanoromycetes, Ascomycota). Mycologia 103:983–1003

Schoch C, Sung GH, López-Giráldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny B, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, De Hoog GS, Crous PW, Hewitt D, Pfister D, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman A, Lumbsch HT, Lücking R, Büdel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker R, Sugiyama J, Summerbell RC, Untereiner WA, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Sherwood MA (1977a) The ostropalean fungi. Mycotaxon 5:1–277

Sherwood MA (1977b) The ostropalean fungi. II: *Schizoxylon*, with notes on *Stictis*, *Acarosporina*, *Coccopeziza*, and *Carestiella*. Mycotaxon 6:215–260

Sherwood-Pike MA (1987) The ostropalean fungi. III: The Odontotremataceae. Mycotaxon 28:137–177

Sipman HJM, Aptroot A (2001) Where are the missing lichens? Mycol Res 105:1433–1439

Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW, Wolseley PA (2009) The lichens of Great Britain and Ireland. British Lichen Society, The Natural History Museum, London

Smith RM, Thompson K, Warren PH, Gaston KJ (2010) Urban domestic gardens (XIII): composition of the bryophyte and lichen floras, and determinants of species richness. Biol Conserv 143:873–882

Solhaug KA, Gauslaa Y, Nybakken L, Bilger W (2003) UV-induction of sun-screening pigments in lichens. New Phytol 158:91–100

Spatafora J, Sung GH, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser DM, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL (2006) A five-gene phylogeny of Pezizomycotina. Mycologia 98:1018–1028

Spribille T, Muggia L (2013) Expanded taxon sampling disentangles evolutionary relationships and reveals a new family in Peltigerales (Lecanoromycetidae, Ascomycota). Fungal Divers 58:171–184

Staiger B, Kalb K (1995) *Haematomma*-studien. I. Die Flechtengattung *Haematomma*, vol 59, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 1–198

Staiger B, Kalb K (1999) *Acanthothecis* and other graphidioid lichens with warty periphysoids or paraphysis-tips. Mycotaxon 73:69–134

Staiger B, Kalb K, Grube M (2006) Phylogeny and phenotypic variation in the lichen family Graphidaceae (Ostropomycetidae, Ascomycota). Mycol Res 110:765–772

Steiner J (1901) Über die Function und den systematischen Wert der Pycnoconidien der Flechten. In: Festschrift zur Feier des zweihunderjährigen Bestandes des K.K. Staatsgymnasiums im VIII. Bezirke Wiens, 1901. Kainz & Liebhart, Wien, pp 119–154

Stenroos SK, DePriest PT (1998) SSU rDNA phylogeny of cladoniiform lichens. Am J Bot 85:1548–1559

Taylor JW, Berbee ML (2006) Dating divergences in the Fungal Tree of Life: review and new analyses. Mycologia 98:838–849

Taylor JW, Berbee ML (2010) Dating the molecular clock in fungi—how close are we? Fungal Biol Rev 24:1–16

Tehler A (1982) The species pair concept in lichenology. Taxon 31:708–717

Tehler A (1996) Systematics, phylogeny and classification. In: Nash TH III (ed) Lichen biology. Cambridge University Press, Cambridge, UK, pp 217–239

Tehler A, Källersjö M (2001) *Parmeliopsis ambigua* and *P. hyperopta* (Parmeliaceae): species or chemotypes? Lichenologist 33:403–408

Thell A, Stenroos S, Feuerer T, Kärnefelt I, Myllys L, Hyvönen J (2002) Phylogeny of cetrarioid lichens (Parmeliaceae) inferred from ITS and b-tubulin sequences, morphology, anatomy and secondary chemistry. Myc Prog 1:335–354

Thüs H, Schultz M (2009) Fungi, part 1: Lichens. In: Büdel B, Gärtner G, Krienitz L, Preisig HR, Schagerl M (eds) Freshwater flora of Central Europe. Spektrum Akademischer Verlag, Heidelberg, Germany

Tibell L (1984) A reappraisal of the taxonomy of Caliciales. In: Hertel H, Oberwinkler F (eds) Beitrage zur Lichenologie. Festschrift J. Poelt, vol 79, Beiheft zur Nova Hedwigia. J. Cramer, Vaduz, pp 597–713

Tibell L (1996) Caliciales. In: Flora Neotropica, vol 69. New York Botanical Garden, Bronx, NY

Timdal E (1987) Problems of generic delimitation among squamiform members of the Lecideaceae. In: Peveling E (ed) Progress and problems in lichenology in the eighties, vol 25, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 243–247

Timdal E (1992) A monograph of the genus *Toninia* (Lecideaceae, Ascomycetes). Opera Bot 110:1–137

Tschermak-Woess E (1953) Über wenig bekannte und neue Flechtengonidien III. Die Entwicklungsgeschichte von *Leptosira thrombii* nov. spec., der Gonidie von *Thrombium epigaeum*. Österr Bot Z 100:203–216

Tschermak-Woess E (1984) Über die weite Verbreitung lichenisierter Sippen von *Dictyochloropsis* und die systematische Stellung von *Myrmecia reticulata* (Chlorophyta). Plant Syst Evol 147:299–322

Tschermak-Woess E (1985) *Elliptochloris bilobata* kein ganz seltner Phycobiont. Herzogia 7:105–116

Tschermak-Woess E (1988a) The algal partner. In: Galun M (ed) CRC handbook of lichenology, vol I. CRC Press, Boca Raton, FL, pp 39–92

Tschermak-Woess E (1988b) New and known taxa of *Chlorella* (Chlorophyceae): occurrence as lichen phycobionts and observations on living dictyosomes. Plant Syst Evol 159:123–139

Tschermak-Woess E, Poelt J (1976) *Vezdaea*, a peculiar lichen genus, and its phycobiont. In: Brown DH, Hawksworth DL, Bailey RH (eds) Lichenology: progress and problems. Academic, London, pp 89–105

Vainio EA (1890) Étude sur la classification naturelle et la morphologie des lichens du Brésil. Acta Societatis Pro Fauna et Flora Fennica, vol 7. Héritiers J. Simelius, Helsingfors

Velmala S, Myllys L, Halonen P, Goward T, Ahti T (2009) Molecular data show that *Bryoria fremontii* and *B. tortuosa* (Parmeliaceae) are conspecific. Lichenologist 41:231–242

Vězda A, James PW (1973) *Sarrameana paradoxa* A. Vězda et P. James gen. nov et sp. nova, eine bemerkenswerte Flechte aus Neu-Kaledonien. Preslia 45:305–310

Vězda A, Kantvilas G (1988) *Sarrameana tasmanica*, a new Tasmanian lichen. Lichenologist 20:179–182

Vobis G (1977) Studies on the germination of lichen conidia. Lichenologist 9:131–136

Vobis G (1980) Bau und Entwicklung der Flechten-Pycnidien und ihrer Conidien, vol 14, Bibliotheca Lichenologica. J. Cramer, Vaduz

Wedin M, Tibell L (1997) Phylogeny and evolution of Caliciaceae, Mycocaliciaceae, and Sphinctrinaceae (Ascomycota), with notes on the evolution of the prototunicate ascus. Can J Bot 75:1236–1242

Wedin M, Wiklund E (2004) The phylogenetic relationships of Lecanorales suborder Peltigerineae revisited. Symb Bot Upsal 34:469–475

Wedin M, Döring H, Mattsson JE (1999) A multi-gene study of the phylogenetic relationships of the Parmeliaceae. Mycol Res 103:1185–1192

Wedin M, Döring H, Nordin A, Tibell L (2000a) Small subunit rDNA phylogeny shows the lichen families Caliciaceae and Physciaceae (Lecanorales, Ascomycotina) to form a monophyletic group. Can J Bot 78:246–254

Wedin M, Döring H, Ekman S (2000b) Molecular phylogeny of the lichen families Cladoniaceae, Sphaerophoraceae, and Stereocaulaceae (Lecanorales, Ascomycotina). Lichenologist 32:171–187

Wedin M, Doring H, Gilenstam G (2004) Saprotrophy and lichenization as options for the same fungalspecies on different substrata: environmental plasticity and fungal lifestyles in the *Stictis-Conotrema* complex. New Phytol 164:459–465

Wedin M, Wiklund E, Crewe A, Döring H, Ekman S, Nyberg Å, Schmitt I, Lumbsch HT (2005) Phylogenetic relationships of Lecanoromycetes (Ascomycota) as revealed by analyses of mtSSU and nLSU rDNA sequence data. Myc Res 109:159–172

Wedin M, Jørgensen PM, Wiklund E (2007) Massalongiaceae fam. nov., an overlooked monophyletic group among the cyanobacterial lichens (Peltigerales, Lecanoromycetes, Ascomycota). Lichenologist 39:61–67

Wedin M, Wiklund E, Jørgensen PM, Ekman S (2009) Slippery when wet: phylogeny and character evolution in the gelatinous cyanobacterial lichens (Peltigerales, Ascomycetes). Mol Phylogenet Evol 53:862–871

Wedin M, Jørgensen PM, Ekman S (2011) Vahliellaceae, a new family of cyanobacterial lichens (Peltigerales, Ascomycetes). Lichenologist 43:67–72

Westberg M, Arup U, Kärnefelt I (2007) Phylogenetic studies in the Candelariaceae (lichenized Ascomycota) based on nuclear ITS DNA sequence data. Myc Res 111:1277–1284

Westberg M, Frödén P, Wedin M (2009) A monograph of the genus *Placomaronea* (Ascomycota, Candelariales). Lichenologist 41:513–527

Widhelm T, Lumbsch HT (2011) The phylogenetic placement of Miltideaceae inferred from ribosomal DNA sequence data. In: Bates ST, Bungartz F, Lücking R, Herrera-Campos MA, Zambrano A (eds) Biomonitoring, ecology, and systematics of lichens. Festschrift Thomas H. Nash III, vol 106, Bibliotheca Lichenologica. J. Cramer, Berlin, Stuttgart, pp 365–373

Winka K, Ahlberg C, Eriksson OE (1998) Are there lichenized Ostropales? Lichenologist 30:455–462

Wooolfit M, Bromham L (2003) Inceased rates of sequence evolution in endosymbiotic bacteria and fungi with small effective population size. Mol Biol Evol 20:1545–1555

Zahlbruckner A (1903–1907) Lichenes. B. Spezieller Teil. In: Engler A, Prantl K (eds) Die natürlichen Pflanzenfamilien, vol I(1). Engelmann, Leipzig, pp 49–249

Zahlbruckner A (1926) Lichenes. B. Specieller Teil. In: Engler A (ed) Die Natürlichen Pflanzenfamilien, 8, 2nd edn. Engelmann, Leipzig, pp 61–270

Zhou QM, Wei JC (2006) A new genus and species *Rhizoplacopsis weichingii* in a new family Rhizoplacopsidaceae (Ascomycota). Mycosystema 25:376–385

Zhou QM, Wei JC (2007) A new order Umbilicariales J. C. Wei & Q.M. Zhou (Ascomycota). Mycosystema 26:40–45

Zoller S, Lutzoni F (2003) Slow algae, fast fungi: exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*. Mol Phylogenet Evol 29:629–640

5 Pezizomycotina: Eurotiomycetes

David M. Geiser[1], Katherine F. LoBuglio[2], Cécile Gueidan[3]

CONTENTS

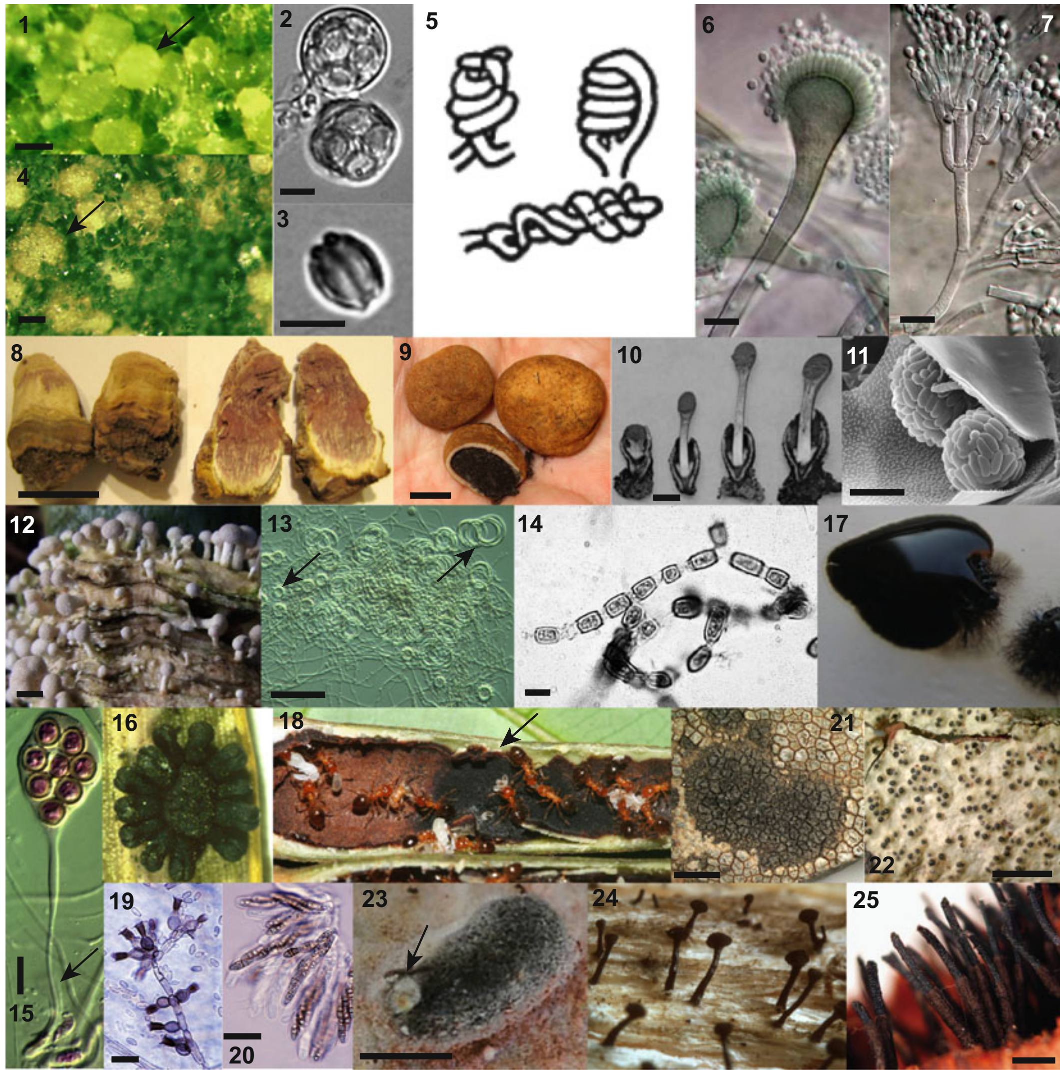
I. **Phylogeny and Taxonomy** 121
A. Eurotiomycetidae 123
1. Eurotiales 123
2. Onygenales 128
3. Arachnomycetales, *Ascosphaera*, and *Eremascus* 129
4. Coryneliales 129
B. Chaetothyriomycetidae 130
1. Chaetothyriales 130
2. Verrucariales 130
3. Pyrenulales 131
4. Celotheliales ad int. (Gueidan et al. 2014) 131
C. Taxa of Uncertain Placement 131
1. Mycocaliciomycetidae/Mycocaliciales. 131
2. Marine Species of *Dactylospora* and *Sclerococcum* 131
3. *Cirrosporium novae-zelandiae* 132
II. **Ecology and Economic Importance** 132
A. Extremophilism 132
B. Animal Pathogens 132
1. Onygenalean Pathogens 132
2. Eurotialean Pathogens 134
C. Plant Pathogens 134
D. Mutualistic Interactions 134
1. Ectomycorrhizae 134
2. Lichens 134
3. Myrmecophytes 135
E. Food Contamination 135
F. Food Production 135
G. Toxic Secondary Metabolites 135
H. Industrial Uses 136
III. **Summary** 136
References 137

[1]Department of Plant Pathology and Environmental Microbiology, 121 Buckhout Laboratory, The Pennsylvania State University, University Park, PA 16802, USA; e-mail: dgeiser@psu.edu
[2]Farlow Herbarium, Harvard University, 22 Divinity Avenue, Cambridge, MA 02138, USA; e-mail: klobuglio@oeb.harvard.edu
[3]CSIRO – National Research Collections Australia, Australia National Herbarium, GPO Box 1600, Canberra, ACT 2601, Australia; e-mail: Cecile.Gueidan@csiro.au

I. Phylogeny and Taxonomy

The phylogenetically informed taxonomic concept of Eurotiomycetes has expanded greatly from the one first proposed by Eriksson (1999) and summarized as "the monophyletic Plectomycetes" by Geiser and LoBuglio (2001), which comprised the orders Eurotiales, Onygenales, and Ascosphaerales. The monophyletic Plectomycetes, consisting mostly of saprotrophs and mammalian pathogens, is strongly associated with the morphological concept of Plectomycetes developed by Nannfeldt (1932) and Luttrell (1951), although **fungi with plectomycetous characters are known to fall in several other non-Eurotiomycete clades (Berbee and Taylor 1992; Geiser et al. 2006).** Key characteristics of monophyletic Plectomycetes include (1) ascomata that are cleistothecial when present, with no ostiolar opening, often borne in some kind of stromatic tissue (Figs. 5.1 and 5.4); (2) spherical asci (Fig. 5.2) with a thin wall that deliquesces at maturity to release oblate to lens-shaped ascospores (Fig. 5.3) free within the ascomata (prototunicate); (3) no distinct hymenial layer within the ascomata; (4) no paraphyses or periphyses; and (5) undifferentiated, hyphal gametangia (Fig. 5.5). However, **early molecular phylogenetic studies revealed that *Capronia pilosella* (Chaetothyriales), a fungus with ascolocular development and bitunicate asci more typical of modern Dothideomycetes, resolved with Eurotialean and Onygenalean fungi** (Berbee 1996; Spatafora et al. 1995). Subsequent phylogenetic results bolstered the connection between Chaetothyriales and Eurotiomycetes (Liu et al. 1999; Lumbsch et al. 2000; Silva-Hanlin and

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015



Figs. 5.1–5.25 Morphological features of Eurotiomycetes. *Bars* represent approximate scale. **Figs. 5.1–5.16** Eurotiomycetidae. **Figs. 5.1–5.10** Eurotiales. **Figs. 5.1–5.8** Aspergillaceae. 1. Cleistothecia (*arrow*); bar = 100 µm. 2. Asci; bar = 5 µm. 3. Ascospore from *Eurotium* (*Aspergillus* section *Aspergillus*) [reproduced from Geiser et al. (2006)]; bar = 5 µm. 4. *Emericella* sexual stage (*Aspergillus* subgenus *Nidulantes*) (*arrow*: hülle cell-encrusted cleistothecium); bar = 100 µm. 5. Gametangial coils typical of Aspergillaceae [reproduced from Benjamin (1955)]. 6. Uniseriate conidiophores of *Aspergillus fumigatus* [reproduced from Samson et al. (2007)]; bar = 10 µm. 7. Terverticillate conidiophores of *Penicillium chrysogenum* [reproduced from Frisvad and Samson (2004)]; bar = 10 µm. 8–10. Stromatic ascomata: 8. *Trichocoma paradoxa*, intact and vertical sections [reproduced from Geiser et al. (2006)]; bar = 5 mm. 9. Stromatic ascomata of *Elaphomyces* sp. (Eurotiales inc. sed.); bar = 1 cm. 10. Stromatic ascomata of *Pseudotulostoma volvata* (Eurotiales inc. sed.): developmental series in cross section [reproduced from Miller et al. (2001)]; bar = 1 cm. **Figs. 5.11–5.14** Onygenales. 11. Spore balls within a ruptured *Ascosphaera* spore cyst [reproduced from Chorbinski and Rypula (2003)]; bar = 10 µm. 12. Mazaedial ascomata of *Onygena* sp. on a sheep's horn (photographer: Ben Mitchell); bar = 5 mm. 13. *Histoplasma capsulata*, featuring yeast phase (*left arrow*) and tightly coiled peridial hyphae (*right arrow*) (photograph courtesy David Malloch); bar = 50 µm. 14. *Coccidioides* arthroconidia (photograph credit: Dr. Hardin, Centers for Disease Control); bar = 5 µm. **Figs. 5.15 and 5.16** *Corynelia uberata* (Coryneliales) [reproduced from Geiser et al. (2006)] (photographs courtesy Peter Johnston). 15. Released unitunicate ascus. *Arrow*: remnant of outer ascus wall; bar = 10 µm. 16. Ascostromatic

Hanlin 1999; Winka et al. 1998), supporting the erection of Chaetothyriomycetes and Chaetothyriomycetidae (Eriksson and Winka 1997; Kirk et al. 2001).

Subsequently, five-locus analyses of Pezizomycotina (Spatafora et al. 2006) and Dothideomycetes (Schoch et al. 2006) provided strong statistical support for the connection between Chaetothyiales and Eurotiomycetes. **In addition, the orders Coryneliales, Pyrenulales, Verrucariales, and Mycocaliciales resolved within a strongly supported clade within which Eurotiales, Onygenales, and Chaetothyriales are derived (Geiser et al. 2006).** Based on these results, three strongly supported monophyletic subclasses were proposed: Eurotiomycetidae (Eurotiales, Onygenales, Arachnomycetales, Coryneliales), Chaetothyriomycetidae (now consisting of Celotheliales ad int., Chaetothyriales, Verrucariales, Pyrenulales), and Mycocaliciomycetidae (Mycocaliciales) (Geiser et al. 2006; Gibas et al. 2002; Gueidan et al. 2014; Hibbett et al. 2007; Spatafora et al. 2006). Mycocaliciomycetidae was included with caution since its inclusion does not receive robust statistical support across all analyses. **Importantly, the classic plectomycetous characters observed in Eurotiales and Onygenales are rarely observed in these newly included orders, so this increased phylogenetic breadth is accompanied by a large increase in morphological as well as ecological diversity (Figs. 5.1–5.25).** Figure 5.26 is a schematic summary phylogeny of Eurotiomycetes that attempts to represent the consensus of molecular phylogenetic studies.

A. Eurotiomycetidae

Key among the evolutionary trends within Eurotiomycetes is that plectomycetous characteristics (Sect. I) are derived within Eurotiales and Onygenales (Fig. 5.26), which comprised the original concept of Eurotiomycetes as suborders Eurotineae and Onygeniniae within Eurotiales (Eriksson and Winka 1997). **Coryneliales, which is basally derived within Eurotiomycetidae, possesses an ascus morphology that is intermediate between the classic plectomycetous characters found in Eurotiales and Onygenales and the ascolocular characters found in Chaetothyriomycetidae and Mycocaliciomycetidae (Geiser et al. 2006).** This morphological trend is accompanied by a transition to primarily saprophytic ecology in Eurotiales and Onygenales, along with some biotrophic associations with plants and animals.

1. Eurotiales

Eurotiales houses some of the most commonly encountered microfungi, including the very important species with *Aspergillus* and *Penicillium* anamorphs. Geiser and LoBuglio (2001) recognized three families in Eurotiales: Elaphomycetaceae, Monascaceae, and Trichocomaceae. This chapter will follow Houbraken and Samson's (2011) proposed segregation of Trichocomaceae into three families, Aspergillaceae, Thermoascaceae, and Trichocomaceae. Monascaceae is absorbed into Aspergillaceae, and Elaphomycetaceae is considered unresolved.

Figs. 5.1–5.25 (Continued) ascomata on *Podocarpus* leaf. **Figs. 5.17–5.23** Chaetothyriomycetidae. **Figs 5.17–5.20** Chaetothyriales. 17. Dimorphic black yeast colony (photograph courtesy Nina Gunde-Cimerman). 18. Cross section of domatium from plant *Leonardoxa africana* ssp. *africana*, revealing its mutualistic plant–ant *Petalomyrmex phylax* tending a black fungus [reproduced from Mayer et al. (2014)]. 19. *Phialophora* anamorph; bar = 2 µm. 20. Bitunicate asci from *Chaetothyrium*. [**Figs. 5.19 and 5.20** reproduced from Geiser et al. (2006); photographs courtesy Wendy Untereiner]; bars = 2 µm. 21. *Placopyrenium canellum* (Verrucariales), a parasitic species growing on the rock lichen *Aspicilia calcarea*; bar = 100 µm. 22. *Pyrenula concatervans* (Pyrenulales) thallus on bark, with visible perithecia; bar = 5 mm. [**Figs. 5.21 and 5.22** reproduced from Geiser et al. (2006); photographs courtesy Cecile Gueidan.] 23. Perithecium of *Celothelium longisporum* (Celotheliales ad int.), showing marginal ostiole (*arrow*); bar = 1 mm. [**Fig. 5.23** reproduced from Gueidan et al. (2014); photograph courtesy André Aptroot.] 24. Mazaedial ascomata of *Mycocalicium* (Mycocaliciales/Mycocaliciomycetidae; photograph courtesy Helge G. Gundersen). 25. Pycnidia of *Cirrosporium novae-zelandiae* (Eurotiomycetes inc. sed.) producing chains of meristematic arthroconidia [reproduced from Réblov and Seifert (2012)]; bar = 1 mm

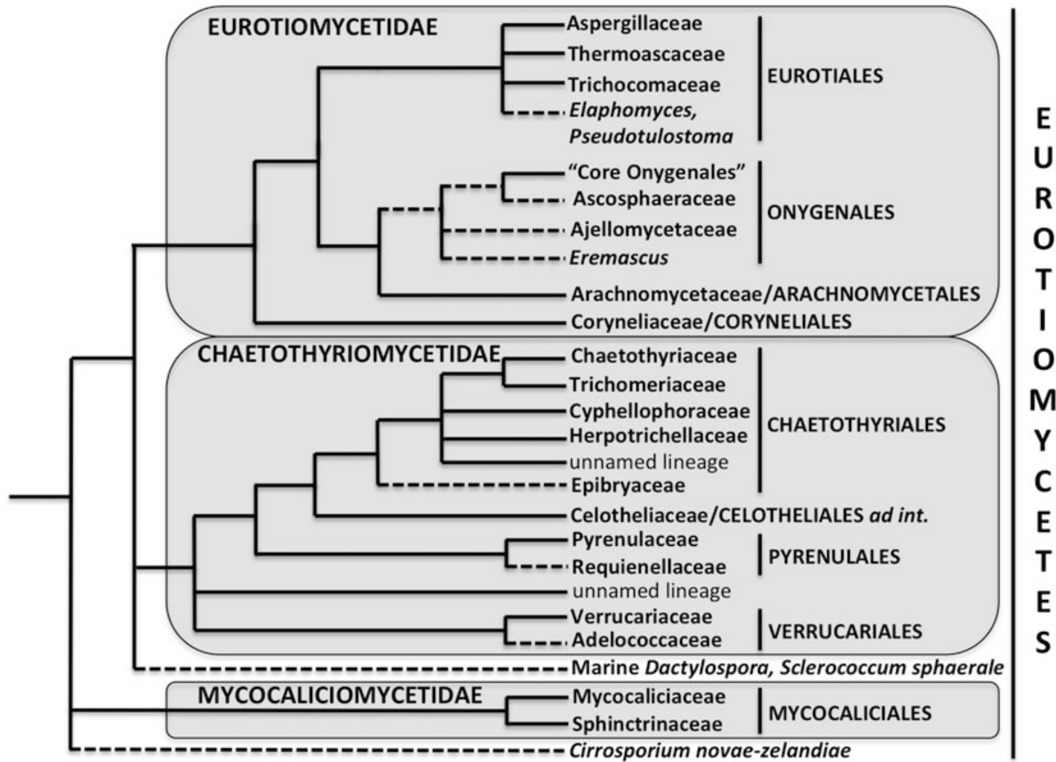


Fig. 5.26 Phylogenetic tree representing current state of knowledge regarding phylogenetic relationships within Eurotiomycetes. Taxa of uncertain or poorly supported phylogenetic position are represented as dashed lines. "Core Onygenales" refers to Onygenaceae, Gymnoascaceae, Arthrodermataceae, and Nannizziopsiaceae (Sect. I.A.2.a). "Unnamed lineages" refer to unnamed lineages 1 and 2 in Gueidan et al. (2014). Phylogenetic positions are inferred based on the studies of Berbee and Taylor (1992), Bowman et al. (1996), del Prado et al. (2006), Diederich et al. (2013), Gargas et al. (1995), Geiser and LoBuglio (2001), Geiser et al. (2006), Gibas et al. (2002), Gueidan et al. (2014), Henkel et al. (2001), Houbraken and Samson (2011), Hyde et al. (2013), Klinger et al. (2013), Réblov and Seifert (2012), Stchigel et al. (2013), Tibell and Wedin (2000), and Untereiner et al. (2002)

a) Aspergillaceae

i. Taxonomy

Houbraken and Samson's (2011) Aspergillaceae encompasses a large majority of *Aspergillus* and *Penicillium* species distributed in 14 strongly supported clades. At least 16 teleomorph genera are represented, some with clear associations to monophyletic groups (e.g., *Eupenicillium, Emericella, Neosartorya, Eurotium*). Houbraken and Samson's (2011) analysis of four protein coding genes resolved monophyletic groups proposed as *Penicillium* s. str. and *Aspergillus* s. str., although only the former received strong statistical support. Most biverticillate *Penicillium* species previously treated as *Penicillium* subgenus *Biverticillium* (Pitt 1979), which were long known to be phylogenetically distinct (Berbee et al. 1995), were segregated into the genus *Talaromyces*. This genus resolves phylogenetically within Trichocomaceae sensu Houbraken and Samson (2011) (Sect. I.A.1.c).

The strongly supported clade corresponding to *Penicillium* s. str. comprises two subclades, proposed as subgenera *Aspergilloides* and *Penicillium* (Houbraken and Samson 2011). Subgenus *Aspergilloides* roughly corresponds to the monoverticillate and biverticillate (with flask-shaped conidiogenous cells) subgenera *Aspergilloides*

and *Furcatum* sensu Pitt (1979) and subgenus *Penicillium* with ter- and quaterverticillate species, mostly representing Pitt's previous concept of subgenus *Penicillium*. **In support of a unitary generic taxonomy, the International Commission on *Penicillium* and *Aspergillus* (ICPA; www.aspergilluspenicillium.org) has recommended use of the name *Penicillium* over the teleomorph names *Eupenicillium* and other genera typified in the *Penicillium* clade.**

The *Aspergillus* s. str. clade, in contrast, receives weak support as a whole but comprises ten well-supported clades. Some of these clades correspond strongly to teleomorph generic concepts, including *Emericella*, *Neosartorya*, and *Eurotium*. As it did with *Penicillium*, **the ICPA has transferred all species within the *Aspergillus* clade into the genus *Aspergillus*,** reviving the very broad generic concept put into place by Raper and Fennell (1965). In a reinterpretation of the Houbraken and Samson (2011) data set, Pitt and Taylor (2014) resolved a tree that showed the ICPA's concept of *Aspergillus* to be paraphyletic, with *Penicillium* derived within it, and recommended generic segregation of clades associated mostly with teleomorph concepts (*Emericella, Neosartorya, Chaetosartorya, Eurotium, Phialosimplex*). **Pitt and Taylor (2014) advocated a narrower concept of *Aspergillus*, provisionally retaining a nonmonophyletic core group of species that includes *A. flavus, A. niger, A. ochraceus*, and *A. terreus*.** This split is bound to have a significant impact due to the importance of these organisms in agriculture, industry, medicine, and basic research, but Pitt and Taylor (2014) argue that the phenotypic differences between these clades are too great to be accommodated in a single genus. Indeed, their conclusion is corroborated by genomic comparisons among *Aspergillus* species: *Emericella nidulans* (*A. nidulans*) and *Neosartorya fumigata* (*A. fumigatus*) have average protein sequence identities similar to those observed between birds and mammals (Fedorova et al. 2008).

ii. Asexual Morphology

A large majority of species in Aspergillaceae produce recognizable *Aspergillus* and *Penicillium* asexual states (Figs. 5.6 and 5.7). ***Aspergillus* and *Penicillium* anamorphs generally produce conidia from phialides on unbranched and branched conidiophores, respectively.** Conidia are borne from the phialides by successive budding, usually forming long chains (Clutterbuck 1969; Mims et al. 1988; Sewall et al. 1990a, b). Conidiophore development is tied closely to the expression of secondary metabolism, with light being a key environmental factor in the regulation of both processes (Bayram et al. 2008; Park and Yu 2012).

There are some interesting exceptions to the *Aspergillus* and *Penicillium* asexual morphology that dominates Aspergillaceae, found mostly in 2 of the 14 clades resolved by Houbraken and Samson (2011). **Derived from within the *Aspergillus* clade is a strongly supported group comprising several canine pathogenic species with highly reduced conidiophores, consisting of simple phialides bearing chains of conidia.** Some of these fungi were previously classified in the genus *Sagenomella* and later segregated into the new genus *Phialosimplex* (Sigler et al. 2010). To accommodate this paraphyletic morphological concept of *Aspergillus* with respect to *Phialosimplex*, the ICPA recommends transfer of these species into *Aspergillus* despite their reduced asexual morphology; Pitt and Taylor's (2014) proposal retains *Phialosimplex*. In addition, a second clade consists of fungi in the genera *Monascus, Xeromyces*, and *Leiothecium*, all of which produce *Chrysosporium*-like thallic anamorphs. Finally, *Dichotomomyces* produces a *Paecilomyces*-like anamorph, despite its clear phylogenetic affinity to *Aspergillus* Section *Fumigati* (Houbraken and Samson 2011).

iii. Sexual Morphology and Self-Fertility

Aspergillaceae produce a wide variety of sexual stages (Geiser 2009). Asci are formed either within a cleistothecium or on an unenclosed mycelium, which in turn may or may not be produced on or within some form of stromatic tissue. **In *Aspergillus* and *Penicillium*, simple cleistothecia represent the most common form,** with walls consisting of flattened cells or hyphal elements (Fig. 5.1). These include the teleomorph generic concepts associated with

Neosartorya, Eurotium, Eupenicillium, and *Fennellia*. More elaborate stromatic forms vary from the production of thick-walled sterile elements, called hülle cells, surrounding the cleistothecium, as seen in *Emericella* (Fig. 5.4), to sclerotiumlike structures that may enclose multiple cleistothecia, as seen under the concepts of *Petromyces* and *Neopetromyces*.

One notable feature of Aspergillaceae is the high frequency of homothallism, **with approximately one-third of the species being self-fertile** (Geiser 2009). Relatively few heterothallic species are known, and the majority of species with no known sexual stage were thought of as largely asexual (Geiser et al. 1996) until fairly recently. The genetic basis of homothallism in Pezizomycotina was first elucidated in a number of Sordariomycetes (Glass and Smith 1994; Pöggeler et al. 1997; Yun et al. 2000) and Dothideomycetes (Yun et al. 1999), but because of a lack of knowledge about mating type in Aspergillaceae, its basis remained undiscovered until complete genome sequences were available. In the canonical situation outside of Aspergillaceae, there is a single *MAT1* mating-type locus, harboring either *MAT1-1* or *MAT1-2* idiomorphic genes in heterothallic species. Homothallics, however, typically possess a combination of genes from both mating-type idiomorphs in the same *MAT1* locus, providing compatibility factors in the same haploid genome and enabling self-fertility. Because these homothallic *MAT1* arrangements tend to be unique to species, they are inferred as having been derived uniquely through convergent evolution (Yun et al. 1999). It turns out that **at least some homothallic Aspergillaceae species deviate from the canonical situation.** The homothallic *Aspergillus* species *A. nidulans* (*Emericella*) and *A. fischeri* (*Neosartorya*) also possess both *MAT1-1* and *MAT1-2* genes in a single haploid genome, but the two idiomorphs are localized on separate chromosomes (Paoletti et al. 2007). In a third homothallic species, *A. alliaceus*, both idiomorphs are colocalized in the same locus (Ramirez-Prado et al. 2008), as typically occurs in other classes of Pezizomycotina. The discovery that species known previously only by their anamorphs, including *Penicillium chrysogenum, Aspergillus fumigatus, A. flavus, A. lentulus, A. oryzae, A. parasiticus*, and *A. terreus*, possess both *MAT1-1* and *MAT1-2* individuals (Dyer and O'Gorman 2012; Henk et al. 2011) enabled the discovery of their heterothallic sexual stages (Arabatzis and Velegraki 2013; Bohm et al. 2013; Horn et al. 2009a, b, 2013; O'Gorman et al. 2009). While sexual stages for a majority of *Aspergillus* and *Penicillium* species remain undiscovered, most are probably heterothallic with cryptic sexual stages. Still, homothallism is unusually common in Aspergillaceae, perhaps under positive selection due to adaptations associated with ascospore-based propagation (Geiser 2009).

b) Thermoascaceae

i. Taxonomy

Thermoascaceae was first proposed to accommodate the thermophilic genera *Thermoascus* and *Dactylomyces,* the latter now recognized as a synonym of the former (Apinis 1967). **Based on its phylogenetic resolution distinct from Aspergillaceae and Trichocomaceae, Houbraken and Samson (2011) defined Thermoascaceae to include *Thermoascus* and *Byssochlamys* species.**

ii. Asexual and Sexual Morphology

Members of Thermoascaceae may produce both blastic and thallic conidia. Blastic conidia are borne from phialides and annellides in branched, *Paecilomyces*-like conidiophores. Thallospores are often thick-walled and produced terminally or intercalary in hyphae. In *Thermoascus,* asci are produced in a firm cleistothecium with a peridium composed of pseudoparenchymatous cells, while in *Byssochlamys* no enclosed cleistothecium is produced. These two genera have similar ascospores that, however, possess rough walls and lack a furrow or slit (Houbraken and Samson 2011).

c) Trichocomaceae

i. Taxonomy

Malloch's (1985) Trichocomaceae approximated the sum of Aspergillaceae, Thermoascaceae, and Trichocomaceae as outlined here. Malloch (1985) recognized two subfamilies

within Trichocomaceae: Dichlaenoideae, which corresponded closely to the current concept of Aspergillaceae, except with the inclusion of *Thermoascus*, and Trichocomoideae, which corresponded closely to the current concept of Trichocomaceae, except with the inclusion of *Byssochlamys*. Malloch defined these two subfamilies based on morphology, physiology, and ecology. Morphologically, Dichlaenoideae were likely to produce true cleistothecia with pseudoparenchymatous walls, often borne in a pseudoparenchymatous stroma, bearing oblate ascospores with a distinct equatorial furrow, and usually associated with starchy or oily substrates. Trichocomoideae produce asci among hyphae that may or may not form an enclosed structure, sometimes borne on some kind of sterile base. Ascospores are spherical to elliptical, usually roughened, and lack an equatorial furrow; these fungi are more likely to be found on woody or cellulosic substrates.

The Trichocomaceae lineage sensu Houbraken and Samson (2011) includes five genera: *Talaromyces*, *Thermomyces*, *Sagenomella*, *Rasamsonia*, and *Trichocoma*. *Rasamsonia* is a new genus in this lineage comprising thermotolerant and thermophilic *Talaromyces* and "*Geosmithia*" species. Interestingly, it is a sister clade to *Trichocoma paradoxa* in Houbraken and Samson's analyses (Houbraken and Samson 2011).

ii. Asexual and Sexual Morphology

Many of the fungi within Trichocomaceae produce biverticillate *Penicillium*-like anamorphs bearing tightly packed, spinulose phialides (formerly *Penicillium* subgenus *Biverticillium*), which distinguishes them from biverticilliate *Penicillium* species that are retained in that genus, in Aspergillaceae. Most are associated phylogenetically with *Talaromyces* (LoBuglio et al. 1993; Pitt 1979) and have been transferred to that genus. *Rasamsonia* species produce conidiophores similar to those of *Talaromyces* but with roughened stipes and asci borne in chains in ascomata with a superficial covering (Houbraken and Samson 2011; Stolk and Samson 1972). *Paecilomyces*-like anamorphs, which are found in all three Eurotialean families and typified in Thermoascaceae (*P. variotti*), are common in Trichocomaceae. **The type genus *Trichocoma* produces large (up to 1 cm), stromatic ascomata shaped like a shaving brush,** with hyphae bearing asci extending from a woodlike base (Fig. 5.8), and a biverticilliate *Penicillium*-like anamorph. *Talaromyces* species produce enclosed ascomata with hyphal peridia.

d) Disputed Families: Monascaceae and Elaphomycetaceae

Geiser and LoBuglio (2001) recognized Monascaceae and Elaphomycetaceae within Eurotiales. We abandon Monascaceae here because three of the four genera in the family, *Monascus, Xeromyces*, and *Leiothecium*, resolve together in a well-supported clade in Houbraken and Samson's (2011) Aspergillaceae; a fourth genus, *Ascorhiza*, remains unresolved.

Elaphomycetaceae encompasses two interesting genera, *Elaphomyces* and *Pseudotulostoma*, that are both ectomycorrhizal (Henkel et al. 2006; Miller and Miller 1984). *Elaphomyces* has large, hypogeous ascomata with a thick, multilayered peridium/stroma (Hawker 1954). **In what appears to be convergent evolution with true truffles, *Elaphomyces* forms spherical, heavily ornamented ascospores produced in a pseudocapillitium.** In *Pseudotulostoma volvata,* a stalked ascoma (mazaedium) reminiscent of a stalked puffball is produced, extending upward from a volvalike structure, which itself is very similar to the hypogeous ascoma produced by *Elaphomyces* (Miller et al. 2001). An anamorph for *Elaphomyces* or *Pseudotulostoma* has not been observed, and *Elaphomyces* grows only very slowly in culture (Miller and Miller 1984).

The lack of resolution in the subordinal ranking and placement of *Elaphomyces* has long been a source of frustration. Trappe (1979) referred to it as "the spurned stepchild of the hypogeous Ascomycota" because of the taxonomic confusion resulting from its weak connections to other orders. **Many authors placed *Elaphomyces* in Plectomycetes because of its irregularly arranged asci and cleistothecial ascomata, but others placed it in Tuberales based on its hypogeous mycorrhizal habit and**

ascospore size and shape. Finally, Trappe (1979) proposed Elaphomycetales as a unique monotypic order. Phylogenetic studies by LoBuglio et al. (1996) and Landvik et al. (1996) demonstrated a strong affinity between *Elaphomyces* and Eurotiales. However, even with the discovery of *Pseudotulostoma*, the phylogenetic status of Elaphomycetaceae remains unclear (Fig. 5.26). Based on sequences of the small subunit (SSU) ribosomal RNA gene, these two genera resolve together basally within the order, but with poor statistical support (Miller et al. 2001). Geiser et al. (2006) resolved a moderately supported clade that included both *Trichocoma* and *Pseudotulostoma*, but this analysis included only DNA sequences from nuclear SSU and large subunit ribosomal RNA (LSU) genes, and *Elaphomyces* was not sampled.

2. Onygenales

Members of Onygenales are also extremely common, known mostly as saprotrophs, particularly in soil and on keratin-rich substrates, and parasites of mammals, causing dermatophytoses and other infections. Currah (1985) recognized four families in Onygenales—Onygenaceae, Arthrodermataceae, Myxotrichaceae, and Gymnoascaceae—but noted morphological and ecological similarities between Myxotrichaceae and some members of Leotiomycetes; this was confirmed by later molecular phylogenetic work resulting in its reclassification (Sugiyama et al. 2002; Wang et al. 2006). Geiser and LoBuglio (2001) followed Currah's (1985) family-level taxonomy; since then, **Arachnomycetaceae/Arachnomycetales [segregated from Gymnoascaceae (Gibas et al. 2002)], Ajellomycetaceae [segregated from Onygenaceae (Untereiner et al. 2004)], and Nannizziopsiaceae [segregated from Onygenaceae (Stchigel et al. 2013)] have been proposed based on phylogenetics.** Much of this segregation reflects the discovery of a deep clade structure within Onygenales identified by Bowman et al. (1996). In the analysis of the nuclear large ribosomal RNA subunit (LSU) gene by Stchigel et al. (2013), Arachnomycetaceae did not resolve with strong statistical support within Onygenales, leading those authors to propose ordinal status as Arachnomycetales. However, no multilocus analysis utilizing a full taxon set of Onygenales has been performed, leaving the relationships between these families, as well as their status as monophyletic groups, unclear. In addition, **the genera *Ascosphaera* and *Eremascus* are poorly resolved in this order** and recognized as members of Ascosphaeraceae and Eremascaceae in the NCBI GenBank taxonomy. Stchigel et al. (2013) resolved Ascosphaeraceae as a family within Onygenales, and *Eremascus* as an outgroup with respect to Onygenales. However, because of the single-locus nature of that analysis, caution is advisable about drawing firm taxonomic conclusions. A stable subordinal taxonomy for this group awaits a thorough multilocus phylogenetic analysis utilizing a rich taxon sample.

a) Core Families of Onygenales

Based on the LSU analysis of Stchigel et al. (2013), there is a well-supported group of clades within Onygenales that represent the core families of this order: Onygenaceae, Gymnoascaceae, Arthrodermataceae, and Nannizziopsiaceae. These families share a range of characteristics outlined in Currah's (1985) treatment of the order. **This includes a wide spectrum of cleistothecial peridium types, including pseudoparenchymatous peridia, peridia composed of wefts of hyphae, and those with a more netlike peridium with and without appendages. Asexual stages are dominated by thallic *Trichophyton*, *Chrysosporium*, *Malbranchea*, and *Microsporum* anamorphs.** *Onygena* is perhaps the most distinctive member of the Onygenaceae because it produces relatively large, stalked mazaedia on keratinous substrates (Fig. 5.12). Members of Onygenaceae characteristically produce pitted ascospores and arthroconidia and aleuroconidia, sometimes both. Arthrodermataceae are distinguished by their smooth ascospores and cleistothecia that bear ossiform (having a bone-like appearance) or derived ossiform cells. Nan-

nizziopsaceae comprises reptile pathogens that produce a skunklike odor (Stchigel et al. 2013). Members of Gymnoascaceae produce a hyphal cleistothecium.

b) Ajellomycetaceae

Based on analyses of the nuclear SSU ribosomal RNA gene, **Bowman et al. (1996) noted that the major human pathogen *Coccidioides immitis* was more closely related to the saprobic fungus *Uncinocarpus reesii* than it was to the pathogens *Histoplasma capsulatum* and *Blastomyces dermatitidis*.** The latter two species, along with *Paracoccidioides brasiliensis*, have since been segregated into the new family Ajellomycetaceae (Untereiner et al. 2004), with *Coccidioides* retained in Onygenaceae. The sexual stage associated with *Histoplasma* and *Blastomyces* is characterized by distinctive, tight peridial coils that extend radially from the cleistothecium (Fig. 5.13; Kwon-Chung 1973). Asci in these taxa are distinguishable from those of other members of Onygenales and Eurotiales by their pyriform shapes; this may be a derived ancestral character connecting this family with Coryneliales (Sect. I.A.3 below).

3. Arachnomycetales, *Ascosphaera*, and *Eremascus*

The phylogenetic distinctiveness of Arachnomycetaceae/Arachnomycetales is well-established as a unique clade (Gibas et al. 2002); its position within or outside of Onygenales remains poorly resolved (Geiser et al. 2006). **Stchigel et al.'s (2013) analysis of LSU sequences resolved an Arachnomycetales clade sister to all Onygenalean families, but the backbone support in this tree was weak.** Geiser et al. (2006) postulated a possible phylogenetic connection between *Ascosphaera* and Arachnomycetaceae, but Stchigel et al.'s (2013) LSU phylogeny showed *Ascosphaera* as sister to the core Onygenales clade and separate from *Arachnomyces*. While the position of this taxon remains poorly resolved, it is generally recognized at the ordinal rank.

Ascosphaera and *Eremascus* are distinctive taxa whose inclusion in the Eurotiales + Onygenales clade is clear, but their exact position remains in doubt. *Ascosphaera* produces a unique double-walled cleistothecium within which ascospores form, and these aggregate into spore balls (Fig. 5.11). *Eremascus* is a xerophilic fungus that produces prototunicate asci naked on a thin mycelium. Berbee and Taylor (1992) first revealed the connection between these taxa and Onygenales. Geiser and LoBuglio (2001) placed both taxa within order Ascosphaerales in the monophyletic Plectomycetes clade because the two genera fell out in a clade distinct from the remainder of Onygenales. The multilocus analysis of Geiser et al. (2006) resolved these genera within Onygenales with strong support, but this conclusion remains in doubt. LSU sequences resolve Ascosphaerales as a basal family within a monophyletic Onygenales and *Eremascus* as an outgroup with respect to Onygenales, Arachnomycetales, and Eurotiales (Stchigel et al. 2013). Klinger et al. (2013) performed a multilocus analysis of *Ascosphaera* species, showing that the genus represents a diverse, monophyletic assemblage. As stated earlier, **hypotheses regarding the supra-generic relationships among these genera with respect to Onygenales and its families remain largely untested.**

4. Coryneliales

Schoch et al. (2009) and Geiser et al. (2006) both resolved a clear sister relationship between Eurotiales + Onygenales and the enigmatic order Coryneliales. **This finding provided an important insight into the evolution of one of the most distinctive characteristics of the Eurotiomycetidae: the globose, evanescent, prototunicate ascus.** Coryneliales are biotrophic associates of woody plants, particularly Podocarpaceae in the Southern Hemisphere, as well as some northern temperate species. These fungi produce ascolocular ascomata similar to the loculoascomycetes that dominate Dothideomycetes (Figs. 5.15 and 5.16). In early developmental studies, Coryneliales was considered an order within Pyrenomycetes because their long-tailed asci terminate in a globose sac that appeared to be unitunicate or prototunicate, having a thin wall that deliquesces at maturity to release the single-celled ascospores in the ascomata. In his landmark

developmental treatise on the taxonomy of Pyrenomycetes, Luttrell (1951) expressed frustration with this taxon, treating it last among the 11 orders described:

The Coryneliales represents a serious obstacle to the system of classification adopted in this review. At this point the correlation between the bitunicate ascus and ascostromatic nature of the ascocarp breaks down completely. Since, however, primary emphasis is placed upon ascus structure, the order is tentatively assigned to the Unitunicatae and included among the Pyrenomycetes.

With its apparently unitunicate asci, Coryneliales defied one of the hallmarks of Luttrell's taxonomic system, the connection between ascostromatic development and the bitunicate, jack-in-the-box ascus. However, more than three decades later, it was discovered that the Corynelialean ascus is not unitunicate. **Using light microscopy, Johnston and Minter (1989) documented the bitunicate nature of asci in Coryneliales (Fig. 5.15), with outer walls that break to release thin-walled, young asci.** This is easy to miss since the bitunicate stage is present only very early in development, before ascospores form. Other than a barely detectable remnant of the outer wall at the base, the mature ascus appears to be unitunicate. Much like members of the core Eurotiomycetidae, the globose apex of the ascus breaks open in an irregular fashion, releasing the ascospores in the ascomatal cavity. This was interpreted as an intermediate form between the bitunicate and prototunicate ascus (Read and Beckett 1996). **Based on phylogenetics, Geiser et al. (2006) postulated that Coryneliales represents a transitional ascomatal form, perhaps between an ancestral ascostromatic bitunicate form retained in Chaetothyriales in Chaetothyriomycetidae and the cleistothecial prototunicate form seen in core Eurotiomycetidae.** This represents just one of several inferred transitions to the prototunicate ascus in the Pezizomycotina (Wedin and Tibell 1997).

B. Chaetothyriomycetidae

Unlike Eurotiomycetidae, few obvious characters dominate the morphologically and ecologically diverse taxa that occupy Chaetothyriomycetidae (Figs. 5.17–5.23). They often produce ostiolate perithecial ascomata with hamathecial elements, and asci that are bitunicate (Fig. 5.20) and either fissitunicate or evanescent (Gueidan et al. 2014). They include diverse mutualists, parasites, and saprotrophs. One common ecological trait observed, primarily in the orders Chaetothyriales and Verrucariales, is an association with extreme xeric habitats, such as high salt and rocky environments. In addition, members of this clade are known to be associated with ants, bryophytes, and deep marine habitats.

1. Chaetothyriales

Geiser et al. (2006) discussed two families within Chaetothyriales; **a multilocus phylogenetic analysis focusing on Chaetothyriomycetidae recognizes five (Chaetothyriaceae, Herpotrichiellaceae, Epibryaceae, Cyphellophoraceae, and Trichomeriaceae)**, with an unnamed family-level clade known only from sequences derived from rock-inhabiting fungi (Gueidan et al. 2014). Chaetothyrialean fungi are diverse saprotrophs and parasites of plants and animals, including humans. Anamorphs vary, including black yeasts and dematiaceous hyphomycete forms that include genera such as *Exophiala* and *Phialophora*. Perithecia have thin walls that are often setose and bear fissitunicate clavate asci. Hamathecial elements include ostiolar periphyses and short apical pseudoparaphyses.

2. Verrucariales

Members of Verrucariales are mostly lichenized. **They are notable among lichens for their ecological diversity, which ranges from xeric terrestrial to freshwater, and even marine habitats, where they are particularly common.** Much of their diversity occurs on rock, particularly in temperate regions (Fig. 5.21). Some Verrucarialean fungi are parasites of lichens, either as free-living or as lichenized forms. Thalli vary tremendously in form and pigmentation (Muggia et al. 2010), with

melanin being common, a character shared with Chaetothyriales (Geiser et al. 2006). Asexual propagules such as isidia and soredia are rare. Ostiolate perithecia may be produced immersed in the thallus or superficially. Asci are bitunicate with either fissitunicate or evanescent release of ascospores. Hamathecial elements are similar to those in Chaetothyriales, with interascal elements absent at maturity (Gueidan et al. 2014). Although phylogenetic studies have started to address generic delimitation within Verrucariaceae, the largest family of Verrucariales (Gueidan et al. 2007, 2009), no molecular data are currently available to confirm the position of a second family, the Adelococcaceae.

3. Pyrenulales

Pyrenulales are also mostly lichenized but usually occur on tree bark (Fig. 5.22), and in tropical regions (Gueidan et al. 2014). Members of this group tend to be associated with algae of the genus *Trentepohlia* (Weerakoon et al. 2012). Thalli are usually thin and immersed or superficial in or on the substrate and lacking in asexual propagules. Unlike most lichen groups, secondary metabolites are uncommon. Perithecia are ostiolar and contain unbranched paraphyses and fissitunicate asci. Many taxa formerly placed in Pyrenulales have been redirected to Dothideomycetes (Geiser et al. 2006; Hyde et al. 2013), and Celotheliaceae was raised to interim ordinal status within Chaetothyriomycetidae (Gueidan et al. 2014; see below). This leaves two families in the order, Requiellaceae and Pyrenulaceae, the latter of which includes the great majority of species and consists of two strongly supported subgroups (Weerakoon et al. 2012).

4. Celotheliales ad int. (Gueidan et al. 2014)

Del Prado et al. (2006) resolved *Celothelium* close to Pyrenulales, leading to its later family-level circumscription (Aptroot et al. 2008). The family Celotheliaceae is a strongly supported monophyletic group within Chaetothyriomycetidae, leading Gueidan et al. (2014) to elevate it ad interim to the ordinal level, together with fungi in the plant pathogenic genera *Phaeomoniella* and *Dolabra*. No anamorph is known for *Celothelium*, so its potential morphological connection to these nonlichenized anamorph taxa currently cannot be investigated. *Celothelium* produces an elongated, carbonized perithecium with a marginal ostiole (Fig. 5.23) bearing filamentous, multiseptate ascospores (Gueidan et al. 2014).

C. Taxa of Uncertain Placement

1. Mycocaliciomycetidae/Mycocaliciales

Mycocaliciales, consisting of families Mycocaliciaceae and Sphinctrinaceae, was proposed as an order distinct from Caliciales (Lecanoromycetes) based on morphology and its distinct SSU sequences, which placed it close to Eurotiales and Onygenales (Gargas et al. 1995; Tibell and Wedin 2000). **These fungi are nonlichenized and occur as parasites or commensals on the thalli of lichens or saprobic fungi.** Mycocaliciales received moderate bootstrap support forming a clade with Eurotiomycetidae and Chaetothyriomycetidae in the analysis of Geiser et al. (2006), and it was later given subclass status in Eurotiomycetes as Mycocaliciomycetidae (Hibbett et al. 2007). Some characters seen with varying frequency in Eurotiomycetidae and Chaetothyriomycetidae [mazaedial ascomata (Fig. 5.24), occasional evanescent asci, their role as parasites or commensals on lichens and other fungi] are sometimes present in Mycocaliciomycetidae. The group is associated with both coelomycetous and hyphomycetous anamorphs.

2. Marine Species of *Dactylospora* and *Sclerococcum*

Among the taxonomically untreated clades identified in the analysis of Gueidan et al. (2014) is a group that includes marine species of *Dactylospora* and *Sclerococcum sphaerale*, an anamorph that occurs on lichens. The clear phylogenetic connection between these taxa and their placement in Eurotiomycetes was

first noted by Diederich et al. (2013). However, these two studies, which utilized different markers and taxon selection, resolve them in slightly different positions within Eurotiomycetes, so their rank status at the ordinal or subclass level remains in question.

3. *Cirrosporium novae-zelandiae*

Cirrosporium novae-zelandiae is an unusual coelomycete that appears to be a Eurotiomycete, grouping in a poorly supported clade with Mycocaliciomycetidae (Réblov and Seifert 2012). **This fungus is highly unusual in that it produces meristem arthroconidia (Hughes 1953), where arthroconidia form in parallel chains from meristematic growth of the conidiophore apex (Fig. 5.25).** While some members of Mycocaliciomycetidae possess coelomycetous anamorphs, the cylindrical pycnidium of *Cirrosporium* is unique, and there is little else to connect these taxa. While the statistical support for the inclusion of *Cirrosporium* in Eurotiomycetes was not significant, the analysis was based on portions of five genes that are useful for resolving relationships at this level. Firm phylogenetic placement of this taxon within Eurotiomycetes may require more sequence data or the inclusion of additional taxa.

II. Ecology and Economic Importance

A. Extremophilism

Eurotiomycetes can degrade a wide variety of organic substrates, sometimes under the most challenging osmotic and temperature conditions. **Some, such as *Aspergillus* species in the *Eurotium* clade, are capable of growing at water activities (a_w) as low as those found in saturated NaCl solutions** (Pitt and Hocking 1997), and species such as *A. fumigatus* (*Neosartorya* clade) grow well at composting temperatures [>50 °C (Raper and Fennell 1965)]. Dimorphic dematiaceous fungi in Chaetothyriales (black yeasts) are abundant in hypersaline waters found in salterns, in NaCl concentrations that may exceed 30 % (Gunde-Cimerman et al. 2000). While saprobic species dominate in Eurotiales, Onygenales, and Chaetothyriales, this metabolic adaptability provides them with some degree of pathogenic potential on plants and animals, usually when the host is weakened by disease or other stress (Gostincar et al. 2011). **Chaetothyriomycetidae includes a very diverse array of inhabitants of rock (Gueidan et al. 2008, 2014), many of which remain unclassified.** This group also includes fungi from deep-sea hydrothermal vents (Le Calvez et al. 2009) and other harsh environments, even isolates capable of resisting conditions simulating space and Martian conditions (Onofri et al. 2008).

B. Animal Pathogens

The Eurotiomycetes include most of the animal pathogenic fungi and occur in Onygenales, Eurotiales, and Chaetothyriales. Most of the mammalian pathogens have a wide host range, and none appear to be obligate pathogens. Disease takes a range of forms from superficial epidermal infections to gravely serious deep mycoses that may invade the respiratory and circulatory systems and disseminate to other parts of the body.

1. Onygenalean Pathogens

Many of the most important fungal pathogens of vertebrates are found in Onygenales. These fungi are often keratinophilic, adapted to degrading keratin, the major protein component of skin, hair, fingernails, feathers, and hooves. This association probably provides these fungi with the opportunity for occasional pathogenicity. **With the exception of the dermatophytes, infections are not believed to be transmitted between individuals; rather, they are caused by fungi normally present in the environment as saprobes.** Some of the most important onygenalean pathogens also tend to be dimorphic, growing as yeasts at 37 °C and as a mycelium at lower temperatures.

a) Histoplasmosis, Blastomycosis, and Paracoccidioidomycosis

The diseases histoplasmosis, blastomycosis, and paracoccidioidomycosis are caused by three dimorphic members of Ajellomycetaceae: *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and species of *Paracoccidioides*. All three usually begin in the respiratory cavity but frequently disseminate to other parts of the body. One of the most serious onygenalean pathogens, ***Histoplasma capsulatum*, is associated with bird and bat excreta and shows some evidence for endemism in the Mississippi and Ohio River valleys of the central United States and all of Central and South America.** The disease it causes, histoplasmosis, is usually marked by a mild lung infection but can disseminate to other organs, where it is often fatal (Kauffman 2007). Histoplasmosis is a significant problem in immune-compromised populations, particularly individuals stricken with HIV/AIDS, presenting in as much as 26 % of AIDS patients in regions where the fungus is endemic (Wheat et al. 1990). Another causal agent of histoplasmosis is *H. duboisii* (previously *H. capsulatum* var. *duboisii*), which is endemic to Central Africa (Loulergue et al. 2007). Blastomycosis is a disease similar to histoplasmosis and is caused by another related fungus, *Blastomyces dermatitidis*. Blastomycosis is often mistaken clinically for cancer or tuberculosis. Paracoccidioidomycosis, caused by *Paracoccidioides brasiliensis*, is yet another similar disease, endemic to South and Central America. This disease also occurs in armadillos in regions where it is endemic (Bagagli et al. 1998).

b) Coccidioidomycosis or "Valley Fever"

Coccidioidomycosis is a disease caused by *Coccidioides immitis* and its cryptic sister species *C. posadasii*. These fungi show a high degree of endemism in the dry valleys extending from the southwestern United States through Central and South America, with *C. immitis* dominating in Central and Southern California and *C. posadasii* extending from Arizona into South America (Fisher et al. 2001). In the southern San Joaquin Valley of California, more than 95 % of individuals showed a positive serum reaction to spherulin, an antigen produced by *C. immitis*. **Valley fever is usually characterized by a mild to moderate respiratory infection, but dissemination can result in far more serious outcomes, including death (Stevens 1995).** Disseminated disease is a risk to both immune-compromised and immune-competent individuals, with differences in dissemination rates associated with ethnicity and gender (Nguyen et al. 2013). The disease is probably contracted by inhaling arthroconidia sent airborne by the disturbance of dry soil, particularly in association with agriculture, but also archeological work, disturbance of soil associated with the excreta and decaying remains of small mammals, earthquakes, and dust storms. **Once inhaled, arthrospores swell in the host to form multinucleate spherules, which then release uninucleate endospores, causing further spread of the infection in the individual (Pappagianis 1988).** No sexual stage has been observed, but population genetic evidence exists for recombination in natural populations (Burt et al. 1996).

c) Dermatophytes

Other Onygenalean fungi tend to exist as weaker pathogens of the skin and nails, termed dermatophytes. Members of Arthrodermataceae with *Microsporum* and *Trichophyton* anamorphs cause tinea infections on the skin, including ringworm and athlete's foot. Onygenalean dermatophytes live in a range of habitats, from mostly soil (geophilic) to mostly animal and human associates (zoophilic or anthropophilic) (Kwon-Chung and Bennett 1992).

d) Chalkbrood of Bees

Several *Ascosphaera* spp. cause chalkbrood disease of bees but probably exist mainly as saprobes on their stored pollen and nectar deposits, as well as on their feces (Skou 1972, 1975). Other *Ascosphaera* spp. can be found infesting the pollen stores and nesting material of bees but are either nonpathogenic or opportunistic pathogens (Klinger et al. 2013). The name chalkbrood describes the disease symptoms; dead larvae are swollen, with spore balls

packed underneath the larval integument. The larval cuticle eventually bursts, releasing the spore balls, which have a chalklike appearance (Skou 1975).

2. Eurotialean Pathogens

A number of *Aspergillus* species are serious human pathogens. **In general, the most common species capable of growing at 37 °C are the most common pathogens, including *A. fumigatus, A. terreus, A. niger, A. ochraceus, and A. nidulans.*** Disease generally starts in the lungs after spores are inhaled. Aspergillosis can vary from an allergic respiratory infection to an invasive disease that produces a fungus ball (aspergilloma) in the lung or disseminate to other parts of the body. Invasive aspergillosis is rare in immune-competent individuals, but it is a serious killer of transplant patients undergoing cytotoxic chemotherapy. ***A. sydowii* has been associated with major diebacks of sea fan corals in the West Indies (Geiser et al. 1998).** *Talaromyces marneffei* is an important disease agent associated with HIV/AIDS in Southeast Asia. This fungus, which is associated with bamboo rats, is unusual for Eurotiales because it is dimorphic; at 37 °C, **vegetative hyphae break up into arthroconidia, which then proliferate in the host as a fission yeast** (Boyce and Andrianopoulos 2013).

C. Plant Pathogens

In comparison to Sordariomycetes and Dothideomycetes, relatively few plant pathogens reside in Eurotiomycetes. **Members of Eurotiales are equipped with the enzymes necessary to degrade complex plant compounds such as cellulose and pectin (Dean and Timberlake 1989) but tend to do so as agents of decay.** Some *Aspergillus* species are considered weak plant pathogens, meaning they are capable of penetrating living plant hosts and occasionally producing rot symptoms. Generally, penetration occurs via wounding by insects or other damaging agents, or in plants that are stressed, but penetration of healthy tissues and systemic infection have been demonstrated (Pitt et al. 1991). However, postharvest infection by *Penicillium* and *Aspergillus* species of a variety of fruits and vegetables is a serious problem. **The greatest agricultural concern regarding Eurotiomycetes involves the production of aflatoxins,** mostly by *A. flavus* and *A. parasiticus* on stored grains and nuts (see Sect. II.G), a process that is frequently initiated by preharvest penetration of the plant by these fungi (Cotty et al. 1994). Members of Coryneliales can be biotrophic parasites of woody hosts, including pines in northern temperate regions and Podocarpaceae in the Southern Hemisphere. Some plant pathogens are also known sporadically in Chaetothyriales and Celotheliales. One of the newly erected families of Chaetothyriaceae, Epibryaceae, consists mostly of species of *Epibryon*, a biotrophic parasite of bryophytes.

D. Mutualistic Interactions

1. Ectomycorrhizae

The Eurotialean genera *Elaphomyces* and *Pseudotulostoma* are known to form ectomycorrhizal associations with trees (Henkel et al. 2006; Miller and Miller 1984; Trappe 1962). Both fungi form an organized mantle on the host root surface and form a Hartig net within the root cortex. *Elaphomyces* is cosmopolitan in temperate forests, forming hypogeous fruiting bodies in association with both conifer and hardwood hosts. *Pseudotulostoma*'s distribution is poorly documented, known only in Guyana and Japan (Asai et al. 2004; Miller et al. 2001).

2. Lichens

Many members of Chaetothyriomycetidae and Mycocaliciomycetidae are lichenized, usually producing small thalli on trees and rocks. Eurotiomycete lichens occur worldwide in many terrestrial, freshwater, and marine habitats and tend to produce relatively few secondary metabolites and rarely produce asexual propagules.

3. Myrmecophytes

Myrmecophytes (known as ant plants) form facultative or obligate mutualisms with ants, producing internal cavities called domatia (Fig. 5.18) that house the insects (Mayer et al. 2014). In addition to mutual benefits of protection and host dispersal occurring between the insect and plant partners, this mutualism includes fungi and possibly bacteria that serve as a food source for the insects and the plants. **The domatial cavities are often lined with a thin black mycelium, which are cultivated by and provide nutrients to the ants (Blatrix et al. 2013; Defossez et al. 2009).** As is the case in fungus-cultivating ambrosia beetles, more than one fungal symbiont may be present, but they are always Chaetothyrialean (Blatrix et al. 2013).

E. Food Contamination

Because they aggressively decay organic material, fungi in Eurotiales are often associated with human and animal foods. **Furthermore, most Eurotiales can tolerate very challenging osmotic conditions, and some are true xerophiles, preferring $a_w < 0.85$** (Pitt 1975)**.** Some genera are capable of growth in $a_w < 0.75$, the equivalent of a saturated NaCl solution. This provides these fungi with the ability to grow in dried and highly concentrated foods, including jams, salted fish, fruitcake, and confectionary (Pitt 1975). These adaptations have been exploited, and certain Eurotialean fungi are used to preserve foods, particularly in the production of cheeses and Asian sauces (see Sect. II.F). On the other hand, this association permits these fungi to contaminate foods, making them unpalatable at least if not toxic through the production of secondary metabolites. In a spoilage mycoflora survey of 294 food samples sent to a British food microbiology laboratory, 68.2 % of the isolates were found to be *Aspergillus* or *Penicillium* (Williams and Bialkowska 1985). Contamination can be problematic even on foods that undergo heat processing because some Eurotiales produce ascospores that are highly heat-resistant.

F. Food Production

Producing cheese from milk is an effective way of **preserving its nutritional properties, and fungi often play a role in the process. *Penicillium roquefortii* and *Penicillium camemberti* are used to make Roquefort-type and camembert-type cheeses, producing unique flavors by oxidizing fatty acids into methyl ketone flavor compounds (Girolami and Knight 1955).** While Western cultures have concentrated on using these fungi on milk protein, in the East they have been used mostly on soy and rice. *Aspergillus oryzae, A. sojae*, and *A. tamarii* have been used for as long as 4,000 years in a number of food fermentation processes, including the production of soy sauce, miso, and sake. Some of these fungi appear to be domesticated forms of wild *Aspergillus* species; ***A. oryzae* is a domesticated version of *A. flavus*, and *A. sojae* is derived from *A. parasiticus* (Kurtzman et al. 1986).** *A. niger* is used to produce organic acids, particularly citric acid that is used as a flavoring agent. The red color of ang-khak (Chinese red yeast rice) is produced traditionally by culturing *Monascus purpureus* on rice. Many mold-ripened sausages in Europe utilize *Penicillium* and *Aspergillus* species, coating them with a white mycelium.

G. Toxic Secondary Metabolites

Members of Eurotiales, particularly Aspergillaceae, are notable among fungi for making an extremely wide variety of toxic secondary metabolites. Two of the most important are the polyketides aflatoxin, produced mostly by *A. flavus* and *A. parasiticus*, and ochratoxin, produced by a variety of *Aspergillus* and *Penicillium* species. Aflatoxins were first discovered after over 100,000 turkeys died from consuming contaminated groundnut meal (Sargeant et al. 1961). Concerns about acute exposure have been raised in recent years after incidents including the discovery of high aflatoxin levels in peanut butter consumed by primary school children in South Africa (South African Medical Research Council 2001), an outbreak of aflatoxicosis in

Kenya associated with stored maize that killed 125 people (Centers for Disease Control and Prevention 2004), and a 2005 veterinary outbreak in the southeastern United States associated with contaminated dog food (Anonymous 2006). In addition to such acute effects following the consumption of high levels of them, aflatoxins are known to be potent carcinogens, particularly of the liver. **Typical of secondary metabolite biosynthesis, all of the enzymatic activities known to be involved directly in aflatoxin biosynthesis are encoded in a large cluster of 25 genes (Brown et al. 1996).** While the aspergilli used in food production, *A. oryzae* and *A. sojae*, have clear connections to aflatoxigenic species, these domesticated variants lack the ability to produce aflatoxins (Sato et al. 2011). Ochratoxin is reasonably anticipated to be a human carcinogen based on animal studies and shows acute effects as well, particularly nephrotoxicity (Pfohl-Leszkowicz and Manderville 2007). In the poultry industry ochratoxin toxicity can have a significant economic impact when susceptible poultry are exposed to ochratoxin-contaminated feed. In human foods, ochratoxin is known to occur in coffee and grapes and is a particular concern because it can accumulate in meats. In addition to these well-studied toxic polyketides, most *Aspergillus* and *Penicillium* species produce a wide variety of diverse secondary metabolites, including terpenes, steroids, and amino-acid-derived compounds (Turner and Aldridge 1983).

H. Industrial Uses

The bright side of the secondary metabolic creativity shown by Eurotialean fungi is the variety of useful compounds produced. These include food additives, antibiotics, and other pharmaceuticals. Perhaps the most celebrated secondary metabolite is the beta-lactam antibiotic penicillin, first discovered by Fleming (1929), who found a contaminant *Penicillium* that showed antibacterial effects on staphylococci. The successful effort during World War II to develop and produce penicillin on an industrial scale resulted in the discovery of a high-yield strain of *Penicillium chrysogenum*, still used today, from a moldy cantaloupe. Additional useful compounds produced by Eurotialean fungi include the echinocandin class of antifungal drugs, cyclic peptides that inhibit the synthesis of cell wall glucans, and the anticholesterolemic drug mevinolin, which acts by inhibiting the HMG-CoA reductase enzyme. Mevinolin is also produced by *Monascus purpureus*, the Eurotialean fungus used to make ang-khak (Endo 1979).

***A. oryzae* and *A. niger* are widely used for the industrial production of extracellular enzymes, including cellulases, amylases, proteases, lipases, and pectinases.** These fungi are well suited to large-scale industrial production because of their highly efficient secretion systems. The enzymes produced include native enzymes produced by *Aspergillus* and heterologous proteins from other organisms. The most widely used industrially produced enzymes are lipases and proteases, as additives to detergents.

III. Summary

Since the advent of fungal molecular systematics, the taxonomy of the fungi discussed here as Eurotiomycetes has changed drastically. First, the morphological concept of "Plectomycetes" proved to be nonmonophyletic, leaving a core clade of orders described as monophyletic Plectomycetes (Geiser and LoBuglio 2001). This large clade of economically important fungi was somewhat morphologically cohesive. However, further advances in molecular systematics brought morphologically and ecologically distinct taxa, particularly fungi with bitunicate/fissitunicate asci, into this phylogenetic realm, resulting in three subclasses, Eurotiomycetidae, Chaetothyriomycetidae, and, eventually, Mycocaliciomycetidae. The inclusion of Coryneliales in Eurotiomycetes helped to bridge the gap in our understanding of the evolutionary relationship between the original concept of Eurotiomycetes and its newer, much broader definition. The recent inclusion of the unusual

coelomycete *Cirrosporium* in Eurotiomycetes suggests that a great deal of undiscovered diversity remains. As more taxa are connected phylogenetically to Eurotiomycetes, we can expect further advances in our understanding of the evolutionary patterns that underlie its considerable morphological and ecological diversity.

References

Anonymous (2006) Diamond Pet Foods narrows recall after aflatoxin tests. J Am Vet Med Assoc 228:490–490

Apinis A (1967) *Dactylomyces* and *Thermoascus*. Trans Br Mycol Soc 50:573–582

Aptroot A, Lücking R, Sipman HJM, Umaña L, Chaves JL (2008) Pyrenocarpous lichens with bitunicate asci: a first assessment of the lichen biodiversity inventory in Costa Rica. In: Wirth D (ed) Bibliotecha Lichenologica, Band 97. J. Cramer, Berlin, pp 1–162

Arabatzis M, Velegraki A (2013) Sexual reproduction in the opportunistic human pathogen *Aspergillus terreus*. Mycologia 105:71–79

Asai I, Sato H, Nara T (2004) *Pseudotulostoma japonicum*, comb. nov. (=*Battarea japonica*), a species of the Eurotiales, Ascomycota. Bull Nat Sci Museum Tokyo Ser B 30:1–7

Bagagli E, Sano A, Coelho K, Alquati S, Miyaji M, De Camargo Z, Gomes G, Franco M, Montenegro M (1998) Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus novemínctus*) captured in an endemic area of paracoccidioidomycosis. Am J Trop Med Hyg 58:505–512

Bayram O, Krappmann S, Ni M, Bok J, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon N, Keller N, Yu J, Braus G (2008) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506

Benjamin CR (1955) Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47:669–687

Berbee ML (1996) Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470

Berbee ML, Taylor JW (1992) Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequence. Mol Biol Evol 9:278–284

Berbee ML, Yoshimura A, Sugiyama J, Taylor JW (1995) Is *Penicillium* monophyletic? An evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S and ITS ribosomal DNA sequence data. Mycologia 87:210–222

Blatrix R, Debaud S, Salas-Lopez A, Born C, Benoit L, McKey D, Atteke C, Djieto-Lordon C (2013) Repeated evolution of fungal cultivar specificity in independently evolved ant-plant-fungus symbioses. PLoS One 8:e68101

Bohm J, Hoff B, O'Gorman C, Wolfers S, Klix V, Binger D, Zadra I, Kurnsteiner H, Poggeler S, Dyer P, Kück U (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. Proc Natl Acad Sci U S A 110:1476–1481

Bowman B, White T, Taylor J (1996) Human pathogenic fungi and their close nonpathogenic relatives. Mol Phylogenet Evol 6:89–96

Boyce K, Andrianopoulos A (2013) Morphogenetic circuitry regulating growth and development in the dimorphic pathogen *Penicillium marneffei*. Eukaryot Cell 12:154–160

Brown D, Yu J, Kelkar H, Fernandes M, Nesbitt T, Keller N, Adams T, Leonard T (1996) Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. Proc Natl Acad Sci U S A 93:1418–1422

Burt A, Carter DA, Koenig GL, White TJ, Taylor JW (1996) Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. Proc Natl Acad Sci U S A 93:770–773

Centers for Disease Control and Prevention (2004) Outbreak of aflatoxin poisoning—Eastern and Central provinces, Kenya, January–July, 2004. Morbidity and Mortality Weekly Report. Atlanta, pp 790–793

Chorbinski P, Rypula K (2003) Studies on the morphology of strains of *Ascosphaera apis* isolated from chalkbrood disease of the honey bees. Electron J Pol Agric Univ 6(5). http://www.ejpau.media.pl/volume6/issue2/veterinary/art-05.html

Clutterbuck AJ (1969) A mutational analysis of conidial development in *Aspergillus nidulans*. Genetics 63:317–327

Cotty P, Bayman P, Egel D, Elias K (1994) Agriculture, aflatoxins and *Aspergillus*. In: Powell K, Renwick A, Peberdy J (eds) FEMS symposium series no. 69. The genus *Aspergillus*. Plenum, New York. pp. 1–27

Currah RS (1985) Taxonomy of the onygenales: arthrodermataceae, gymnoascaceae, myxotrichaceae and onygenaceae. Mycotaxon 24:1–216

Dean R, Timberlake W (1989) Production of cell-wall degrading enzymes by *Aspergillus nidulans*—a model system for fungal pathogenesis of plants. Plant Cell 1:265–273

Defossez E, Selosse M, Dubois M, Mondolot L, Faccio A, Djieto-Lordon C, McKey D, Blatrix R (2009) Ant-plants and fungi: a new three way symbiosis. New Phytol 182:942–949

del Prado RI, Schmitt I, Kautz S, Palice R, Lücking R, Lumbsch HT (2006) Morphological and molecular evidence place the Trypheteliaceae in the Dothideomycetes. Mycol Res 110:511–520

Diederich P, Ertz D, Lawrey J, Sikaroodi M, Untereiner W (2013) Molecular data place the hyphomycetous lichenicolous genus *Sclerococcum* close to *Dacty-*

lospora (Eurotiomycetes) and *S. parmeliae* in *Cladophialophora* (Chaetothyriales). Fungal Divers 58:61–72

Dyer P, O'Gorman C (2012) Sexual development and cryptic sexuality in fungi: insights from *Aspergillus* species. FEMS Microbiol Rev 36:165–192

Endo A (1979) Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J Antibiot 32:852–854

Eriksson OE (1999) Outline of the Ascomycota—1999. Myconet 3:1–88

Eriksson OE, Winka K (1997) Supraordinal taxa of Ascomycota. Myconet 1:1–12

Fedorova N, Khaldi N, Joardar V, Maiti R, Amedeo P, Anderson M, Crabtree J, Silva J, Badger J, Albarraq A, Angiuoli S, Bussey H, Bowyer P, Cotty P, Dyer P, Egan A, Galens K, Fraser-Liggett C, Haas B, Inman J, Kent R, Lemieux S, Malavazi I, Orvis J, Roemer T, Ronning C, Sundaram J, Sutton G, Turner G, Venter J, White O, Whitty B, Youngman P, Wolfe K, Goldman G, Wortman J, Jiang B, Denning D, Nierman W (2008) Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. PLoS Genet 4:e1000046

Fisher MC, Koenig GL, White TJ, San-Blas G, Negroni R, Alvarez IG, Wanke B, Taylor JW (2001) Biogeographic range expansion into South America by *Coccidioides immitis* mirrors New World patterns of human migration. Proc Natl Acad Sci U S A 98:4558–4562

Fleming A (1929) On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 10:226–236

Frisvad JC, Samson RA (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*—a guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol 48:1–174

Gargas A, DePriest PT, Grube M, Tehler A (1995) Multiple origins of lichen symbiosis in fungi suggested by SSU rDNA phylogeny. Science 268:1492–1495

Geiser DM (2009) Sexual structures in *Aspergillus*: morphology, importance and genomics. Med Mycol 47:S21–S26

Geiser DM, LoBuglio KL (2001) The monophyletic Plectomycetes: Ascosphaerales, Onygenales, Eurotiales. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota: systematics and evolution, vol. VIIA. Springer, Berlin, pp 201–220

Geiser DM, Timberlake WE, Arnold ML (1996) Loss of meiosis in *Aspergillus*. Mol Biol Evol 13:809–817

Geiser DM, Taylor JW, Ritchie KB, Smith GW (1998) Cause of sea fan death in the West Indies. Nature 394:137–138

Geiser DM, Gueidan C, Miadlikowska J, Lutzoni F, Kauff F, Hofstetter V, Fraker E, Schoch CL, Tibell L, Untereiner WA, Aptroot A (2006) Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98:1053–1064

Gibas C, Sigler L, Summerbell R, Currah R (2002) Phylogeny of the genus *Arachnomyces* and its anamorphs and the establishment of Arachnomycetales, a new eurotiomycete order in the Ascomycota. Stud Mycol 47:131–139

Girolami R, Knight S (1955) Fatty acid oxidation by *Penicillium roquefortii*. Appl Microbiol 3:264–267

Glass NL, Smith M (1994) Structure and function of a mating-type gene from the homothallic species *Neurospora africana*. Mol Gen Genet 244:401–409

Gostincar C, Grube M, Gunde-Cimerman N (2011) Evolution of fungal pathogens in domestic environments. Fungal Biol 115:1008–1018

Gueidan C, Roux C, Lutzoni F (2007) Using a multigene analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). Mycol Res 111:1147–1170

Gueidan C, Villasenor C, de Hoog G, Gorbushina A, Untereiner W, Lutzoni F (2008) A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. Stud Mycol 61:111–119

Gueidan C, Savić S, Thüs H, Roux C, Keller C, Tibell L, Prieto M, Heiðmarsson S, Breuss O, Orange A, Fröberg L, Wynns AA, Navarro-Rosinés P, Krzewicka B, Pykälä J, Grube M, Lutzoni F (2009) Generic classification of the Verrucariaceae (Ascomycota) based on molecular and morphological evidence: recent progress and remaining challenges. Taxon 58:184–208

Gueidan C, Aptroot A, da Silva Cceres ME, Badali H, Stenroos S (2014) A reappraisal of orders and families within the subclass Chaetothyriomycetidae (Eurotiomycetes, Ascomycota). Mycol Progress 13:1027–1039. doi:10.1007/s11557-014-0990-2

Gunde-Cimerman N, Zalar P, de Hoog S, Plemenitas A (2000) Hypersaline waters in salterns—natural ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32:235–240

Hawker LE (1954) British hypogeous fungi. Phil Trans R Soc Lond Ser B Biol Sci 237:429–546

Henk D, Eagle C, Brown K, Van den Berg M, Dyer P, Peterson S, Fisher M (2011) Speciation despite globally overlapping distributions in *Penicillium chrysogenum*: the population genetics of Alexander Fleming's lucky fungus. Mol Ecol 20:4288–4301

Henkel T, James T, Miller S, Aime M, Miller O (2006) The mycorrhizal status of *Pseudotulostoma volvata* (Elaphomycetaceae, Eurotiales, Ascomycota). Mycorrhiza 16:241–244

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Lumbsch HT, Lutzoni F, Matheny PB, Mclaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM,

Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Horn B, Moore G, Carbone I (2009a) Sexual reproduction in *Aspergillus flavus*. Mycologia 101:423–429

Horn B, Ramirez-Prado J, Carbone I (2009b) Sexual reproduction and recombination in the aflatoxin-producing fungus *Aspergillus parasiticus*. Fungal Genet Biol 46:169–175

Horn B, Olarte R, Peterson S, Carbone I (2013) Sexual reproduction in *Aspergillus tubingensis* from section Nigri. Mycologia 105:1153–1163

Houbraken J, Samson R (2011) Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. Stud Mycol 70:1–51

Hughes SJ (1953) Conidiophores, conidia and classification. Can J Bot 31:577–659

Hyde K, Jones E, Liu J, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous P, Dai D, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey J, Li Y, Liu Y, Lucking R, Monkai J, Muggia L, Nelsen M, Pang K, Phookamsak R, Senanayake I, Shearer C, Suetrong S, Tanaka K, Thambugala K, Wijayawardene N, Wikee S, Wu H, Zhang Y, Aguirre-Hudson B, Alias S, Aptroot A, Bahkali A, Bezerra J, Bhat D, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth D, Hirayama K, De Hoog S, Kang J, Knudsen K, Li W, Li X, Liu Z, Mapook A, McKenzie E, Miller A, Mortimer P, Phillips A, Raja H, Scheuer C, Schumm F, Taylor J, Tian Q, Tibpromma S, Wanasinghe D, Wang Y, Xu J, Yacharoen S, Yan J, Zhang M (2013) Families of Dothideomycetes. Fungal Divers 63:1–313

Johnston PR, Minter DW (1989) Structure and taxonomic significance of the ascus in the Coryneliaceae. Mycol Res 92:422–430

Kauffman C (2007) Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev 20:115–132

Kirk PM, David JC, Stalpers JA (2001) Ainsworth & Bisby's dictionary of the fungi, 9th edn. CAB International, Wallingford, UK

Klinger E, James R, Youssef N, Welker D (2013) A multi-gene phylogeny provides additional insight into the relationships between several *Ascosphaera* species. J Invertebr Pathol 112:41–48

Kurtzman C, Smiley M, Robnett C, Wicklow D (1986) DNA relatedness among wild and domesticated species in the *Aspergillus flavus* group. Mycologia 78:955–959

Kwon-Chung KJ (1973) Studies on *Emmonsiella capsulata* I. Heterothallism and development of the ascocarp. Mycologia 65:109–121

Kwon-Chung KJ, Bennett JE (1992) Medical mycology, 2nd edn. Lea & Febiger, Philadelphia

Landvik S, Shailer NFJ, Eriksson OE (1996) SSU rDNA sequence support for a close relationship between the Elaphomycetales and the Eurotiales and Onygenales. Mycoscience 37:237–241

Le Calvez T, Burgaud G, Mahe S, Barbier G, Vandenkoornhuyse P (2009) Fungal diversity in deep-sea hydrothermal ecosystems. Appl Environ Microbiol 75:6415–6421

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Bio Evol 16:1799–1808

LoBuglio KF, Pitt JI, Taylor JW (1993) Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in Subgenus *Biverticillium*. Mycologia 85:592–604

LoBuglio KF, Berbee ML, Taylor JW (1996) Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr and other mycorrhizal fungi among the ascomycetes. Mol Phylogenet Evol 6:287–294

Loulergue P, Bastides F, Baudouin V, Chandenier J, Mariani-Kurkdijan P, Dupont B, Viard J-P, Dromer F, Lortholary O (2007) Literature review and case histories of *Histoplasma capsulatum* var. *dubosii* infections in HIV-infected patients. Emerg Infect Dis 13:1647–1652

Lumbsch HT, Lindemuth R, Schmitt I (2000) Evolution of filamentous ascomycetes inferred from LSU rDNA sequence data. Plant Biol 5:525–529

Luttrell ES (1951) Taxonomy of the Pyrenomycetes. Univ Missouri Stud Sci Ser 24:1–120

Malloch D (1985) The Trichocomaceae: relationships with other ascomycetes. In: Samson RA, Pitt JI (eds) Advances in *Penicillium* and *Aspergillus* systematics. Plenum, New York, pp 365–382

Mayer V, Frederickson M, McKey D, Blatrix R (2014) Current issues in the evolutionary ecology of ant-plant symbioses. New Phytol 202:749–764

Miller OK, Henkel TW, James TY, Miller SL (2001) *Pseudotulostoma*, a remarkable new volvate genus in the Elaphomycetaceae from Guyana. Mycol Res 105:1268–1272

Miller S, Miller O (1984) Synthesis of *Elaphomyces muricatus* + *Pinus sylvestris* ectomycorrhizae. Can J Bot 62:2363–2369

Mims C, Richardson E, Timberlake W (1988) Ultrastructural analysis of conidiophore development in the fungus *Aspergillus nidulans* using freeze-substitution. Protoplasma 144:132–141

Muggia L, Gueidan C, Grube M (2010) Phylogenetic placement of some morphologically unusual members of Verrucariales. Mycologia 102:835–846

Nannfeldt JA (1932) Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. Nov Act Reg Soc Upsal 8:1–368

Nguyen C, Barker B, Hoover S, Nix D, Ampel N, Frelinger J, Orbach M, Galgiani J (2013) Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of Coccidioidomycosis. Clin Microbiol Rev 26:505–525

O'Gorman C, Fuller H, Dyer P (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457:471–475

Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, Homeck G, de Vera J, Hatton J, Zucconi L (2008) Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. Stud Mycol 61:99–109

Paoletti M, Seymour F, Alcocer M, Kaur N, Caivo A, Archer D, Dyer P (2007) Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. Curr Biol 17:1384–1389

Pappagianis D (1988) Epidemiology of coccidioidomycosis. In: McGinnis MR (ed) Current topics in medical mycology. Springer, Berlin, pp 199–238

Park H, Yu J (2012) Genetic control of asexual sporulation in filamentous fungi. Curr Opin Microbiol 15:669–677

Pfohl-Leszkowicz A, Manderville R (2007) Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res 51:61–99

Pitt JI (1975) Xerophilic fungi and the spoilage of foods of plant origin. In: Duckworth RB (ed) Water relations of foods. Academic, London, pp 273–307

Pitt JI (1979) The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic, London

Pitt JI, Hocking AD (1997) Fungi and food spoilage, 3rd edn. Blackie A&P, London

Pitt JI, Taylor JW (2014) *Aspergillus*, its sexual states and the new International Code of Nomenclature. Mycologia 106(5):1051–1062. doi:10.3852/14-060

Pitt JI, Dyer S, McCammon S (1991) Systemic invastion of developing peanut plants by *Aspergillus flavus*. Lett Appl Microbiol 13:16–20

Poggeler S, Risch S, Kuck U, Osiewacz H (1997) Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics 147:567–580

Ramirez-Prado J, Moore G, Horn B, Carbone I (2008) Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet Biol 45:1292–1299

Raper KB, Fennell DI (1965) The Genus *Aspergillus*. Williams & Wilkins, Baltimore

Read N, Beckett A (1996) Ascus and ascospore morphogenesis. Mycol Res 100:1281–1314

Réblov M, Seifert K (2012) *Cirrosporium novae-zelandiae*, an enigmatic coelomycete with meristem arthroconidia, with ancestors in the Eurotiomycetes. Mycologia 104:1315–1324

Samson RA, Hong S, Peterson SW, Frisvad JC, Varga J (2007) Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. Stud Mycol 59:147–203

Sargeant K, Sheridan A, O'Kelly J (1961) Toxicity associated with certain samples of groundnuts—reply. Nature 192:1096–1097

Sato A, Oshima K, Noguchi H, Ogawa M, Takahashi T, Oguma T, Koyama Y, Itoh T, Hattori M, Hanya Y (2011) Draft genome sequencing and comparative analysis of *Aspergillus sojae* NBRC4239. DNA Res 18:165–176

Schoch CL, Shoemaker R, Seifert K, Hambleton S, Spatafora J, Crous P (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Sewall T, Mims C, Timberlake W (1990a) *abaA* controls phialide differentiation in *Aspergillus nidulans*. Plant Cell 2:731–739

Sewall T, Mims C, Timberlake W (1990b) Conidium differentiation in *Aspergillus nidulans* wild-type and wet-white (*wetA*) mutant strains. Dev Biol 138:499–508

Sigler L, Sutton D, Gibas C, Summerbell R, Noel R, Iwen P (2010) *Phialosimplex*, a new anamorphic genus associated with infections in dogs and having phylogenetic affinity to the Trichocomaceae. Med Mycol 48:335–345

Silva-Hanlin DMW, Hanlin RT (1999) Small subunit ribosomal RNA gene phylogeny of several loculoascomycetes and its taxonomic implications. Mycol Res 103:153–160

Skou JP (1972) Ascosphaerales. Friesia 10:1–24

Skou JP (1975) Two new species of *Ascosphaera*, and notes on the conidial state of *Bettsia alvei*. Friesia 11:62–74

South African Medical Research Council (2001) Aflatoxin in peanut butter (MRC Policy Brief). 2 pp

Spatafora JW, Mitchell TG, Vilgalys R (1995) Analysis of genes coding for small-subunit rRNA sequences in studying phylogenetics of dematiaceous fungal pathogens. J Clin Microbiol 33:1322–1326

Spatafora JW, Schoch CL, Johnson D, Sung G, Hosaka K, O'Rourke B, Serdani M, Spotts R (2006) A five-gene phylogenetic analysis of the Pezizomycotina. Mycologia 98:1018–1028

Stchigel A, Sutton D, Cano-Lira J, Cabanes F, Abarca L, Tintelnot K, Wickes B, Garcia D, Guarro J (2013) Phylogeny of chrysosporia infecting reptiles: proposal of the new family Nannizziopsiaceae and five new species. Persoonia 31:86–100

Stevens D (1995) Coccidiodomycosis. N Engl J Med 332:1077–1082

Stolk AC, Samson RA (1972) The Genus *Talaromyces*—studies on *Talaromyces* and related genera II. Stud Mycol 2:1–65

Sugiyama M, Summerbell RC, Mikawa T (2002) Molecular phylogeny of onygenalean fungi based on small subunit (SSU) and large subunit (LSU) ribosomal DNA sequences. Stud Mycol 47:5–23

Tibell L, Wedin M (2000) Mycocaliciales, a new order for nonlichenized calicioid fungi. Mycologia 92:577–581

Trappe J (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606

Trappe J (1979) Orders, families and genera of hypogeous Ascomycotina (truffles and their relatives). Mycotaxon 9:297–340

Turner WB, Aldridge DC (1983) Fungal metabolites II. Academic, New York

Untereiner WA, Scott JA, Naveau FA, Bachewich J (2002) Phylogeny of *Ajellomyces*, *Polytolypa* and *Spiromastix* (Onygenaceae) inferred from rDNA sequence and non-molecular data. Stud Mycol 47:25–35

Untereiner WA, Scott JA, Naveau FA, Sigler L, Bachewich J, Angus A (2004) The Ajellomycetaceae, a new family of vertebrate-associated Onygenales. Mycologia 96:812–821

Wang Z, Johnston P, Takamatsu S, Spatafora J, Hibbett D (2006) Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. Mycologia 98:1065–1075

Wedin M, Tibell L (1997) Phylogeny and evolution of Caliciaceae, Mycocaliciaceae, and Sphinctrinaceae (Ascomycota), with notes on the evolution of the prototunicate ascus. Can J Bot 75:1236–1242

Weerakoon G, Aptroot A, Lumbsch H, Wolseley P, Wijeyaratne S, Gueidan C (2012) New molecular data on Pyrenulaceae from Sri Lanka reveal two well-supported groups within this family. Lichenologist 44:639–647

Wheat L, Connolly-Stringfield P, Baker R, Curfman M, Eads M, Israel K, Norris S, Webb D, Zeckel M (1990) Disseminated histoplasmosis in the acquired immune deficieny syndrome—clinical findings, diagnosis and treatment, and review of the literature. Medicine 69:361–374

Winka K, Eriksson OE, Bang A (1998) Molecular evidence for recognizing the Chaetothyriales. Mycologia 90:822–830

Williams AP, Bialkowska A (1985) Moulds in mould spoiled foods and food products. Research reports. Leatherhead Food Research Association, Surrey, UK

Yun SH, Berbee ML, Yoder OC, Turgeon BG (1999) Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc Natl Acad Sci U S A 96:5592–5597

Yun SH, Arie T, Kaneko I, Yoder OC, Turgeon BG (2000) Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet Biol 31:7–20

6 Pezizomycotina: Dothideomycetes and Arthoniomycetes

Conrad Schoch[1], Martin Grube[2]

CONTENTS

I.	**Introduction**	143
II.	**Character Evolution: Divergence and Convergence**	144
	A. Morphology and Development	144
	B. Brief Taxonomic History	149
III.	**Ecology and Distribution**	150
	A. Associations with Plants and Plant Debris	150
	B. Aquatic Species	151
	C. Associations with Algae and Other Lichens	152
	D. Rock Environments and Oligotrophism	153
IV.	**Modern Classification and Phylogeny**	153
	A. Dothideomycetes	154
	1. Pleosporomycetidae	155
	2. Dothideomycetidae	158
	3. Incertae Sedis Lineages	160
	B. Arthoniomycetes	163
V.	**Maintenance and Culture**	166
VI.	**Conclusions**	167
	References	168

I. Introduction

The classes Dothideomycetes and Arthoniomycetes were introduced, relying on comparisons of nuclear ribosomal small subunit DNA sequences (Eriksson and Winka 1997), following work by a number of authors (Berbee 1996; Berbee and Taylor 1992; Gargas et al. 1995; Spatafora et al. 1995). Subsequent phylogenetic analyses based on DNA sequences compared from multiple genes indicated support for a **monophyletic clade with lichenized Arthoniomycetes as sister to Dothideomycetes** (Lumbsch et al. 2005; Lutzoni et al. 2004; Spatafora et al. 2006). Following this, a new informal superclass taxon name has been proposed for this monophyletic group of fungi, dothideomyceta, based on combined DNA sequence comparisons from protein coding and ribosomal structural genes (Schoch et al. 2009b, c). Dothideomycetes is by far **the largest class of Ascomycota**, with more than 19,000 species described on morphological comparisons alone (Kirk et al. 2008). Its sister class, Arthoniomycetes, with the single order Arthoniales, is the **largest taxonomic group of primarily lichenized fungi outside of Lecanoromycetes.** Arthoniomycetes comprises 1,500 species and 74 genera, according to the literature (e.g., Kirk et al. 2008), but, like the number for Dothideomycetes, this estimate needs to be updated.

It is clear that the use of DNA and protein sequence comparisons is crucial in the definition of this broad and diverse group of Fungi, and efforts to reconcile morphological and molecular characters continue. However, it remains a monumental task. This is illustrated by the fact that less than 16 % (more than 3,000) of currently accepted species names of Dothideomycetes were deposited under their binomial names in GenBank and the International Nucleotide Sequence Databases (INSD) by the end of 2013. At the same time, for Arthoniomycetes, DNA sequence data are available for less than 200 species, 13 % of proposed species. These numbers clearly underrepresent diversity estimates as phenotypic diversity remains underrepresented in molecular analyses.

Dothideomyceta contains a diverse set of species that subsist as general and specialized

[1]NCBI/NLM/NIH, 45 Center Drive, Bethesda, MD 20892, USA; e-mail: schoch2@ncbi.nlm.nih.gov
[2]Institute of Plant Sciences, Karl-Franzens-University, Holteigasse 6, 8010 Graz, Austria; e-mail: martin.grube@uni-graz.at

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

saprobes in terrestrial and marine ecosystems, plant and animal pathogens, parasites of other fungi, lichens, and mycorrhizal mutualists. A small number of Dothideomycetes are lichenized, while this is the case for the majority of Arthoniomycetes. There are also a considerable number of lichenicolous, fungi, and only a few saprobes in the latter class. Dothideomyceta is also notable for several species that produce biologically active compounds, many of which are known to function in plant pathogenicity. Secondary compound diversity is high in Arthoniomycetes and characterized by a spectrum of compounds apparently correlating with the lichenized lifestyle. Numerous compounds are shared with the unrelated, yet also primarily lichenized, Lecanoromycetes. Among these are diverse orcinol derivates, such as despides and depsidones and other typical lichen compounds (Boustie and Grube 2005), which suggests a parallel chemical evolution in unrelated lichenized lineages. A large body of work on numerous plant pathogenic and other economically important species is available, and molecular-based phylogenetic studies continue to expand the resolution of deep nodes in dothideomyceta while expanding taxon sampling.

II. Character Evolution: Divergence and Convergence

A. Morphology and Development

A more detailed description of morphological features can be found in Barr and Huhndorf (2001). We highlight the most basic concepts here.

Ascomata. The historical concept advocated by Lutrell for loculoascomycetes centered on the designation of a number of morphological types, combined with ascoma structure for five defined orders (Luttrell 1951, 1955). This still influences current taxonomy.

The ascolocular ascomata of Dothideomycetes are formed by vegetative stromatic plectenchyma (tissue formed by hyphae becoming fixed together), giving rise to one or several locules. The reproductive structures developing in the locules are derived from cells before fusion of opposing mating types occurs. In contrast, during ascohymenial development the reproductive structures form in conjunction with growth of the cells after mating fusion.

Flask-shaped, perithecioid ascomata are referred to as pseudothecia when their ascolocular development distinguishes them from the similarly shaped structures with ascohymenial development. A number of lineages in Dothideomycetes also have open, apothecioid ascomata. These include a smaller subset of navicular or boat-shaped structures called hysterothecia, which open with a central suture. Flattened ascomata with a scutate or shieldlike wall of outer cells are referred to as thyriothecia. Cleistothecioid ascomata are enclosed ascomata that often rupture at maturity.

The presence and morphology of types of hamathecial tissues played an important role in delineating orders in Dothideomycetes. Luttrell (1965) used developmental differences to distinguish between seemingly similar hamathecial elements. A representative set of ascomata based on illustrations adapted from von Arx and Müller (1975) are shown in Fig. 6.1. Luttrell's (1973) centrum concepts consisted of a *Pleospora* type (pseudoparaphyses present), a *Dothidea* type (bundles or fascicles of aparaphysate asci), and an *Elsinoë* type (monascous locules), which now constitute the modern orders Pleosporales, Dothideales, and Myriangiales and coincide well with DNA-sequence-based phylogenies (Figs. 6.1a–c and 6.2). The **clearest phylogenetic distinction remains that between the presence and absence of pseudoparaphyses in the centrum**, correlating with the subclasses Pleosporomycetidae and Dothideomycetidae (see section on IV).

The centrum concept was broadly defined as all tissues inside ascomata. This was refined with the introduction of the term hamathecium by Eriksson (1981), which was used to refer only to sterile hyphae or other tissues between the asci.

Pseudoparaphyses are sterile cells extending down from the upper portion of ascomata, initially attached at both ends, although the upper part may become free, while paraphysoids, although similar in appearance, result from interascal tissue stretching during ascomatal development. Pseudoparaphyses can be anastomosing and also show regular septation. Other terms for hamathecial tissues include interascal pseudoparenchyma (tissue that remains unchanged or becomes compressed when the asci develop), periphysoids (short hyphae stretching downward, never reaching the bottom of the ascomal cavity), and periphyses (hyphae in the ostiole).

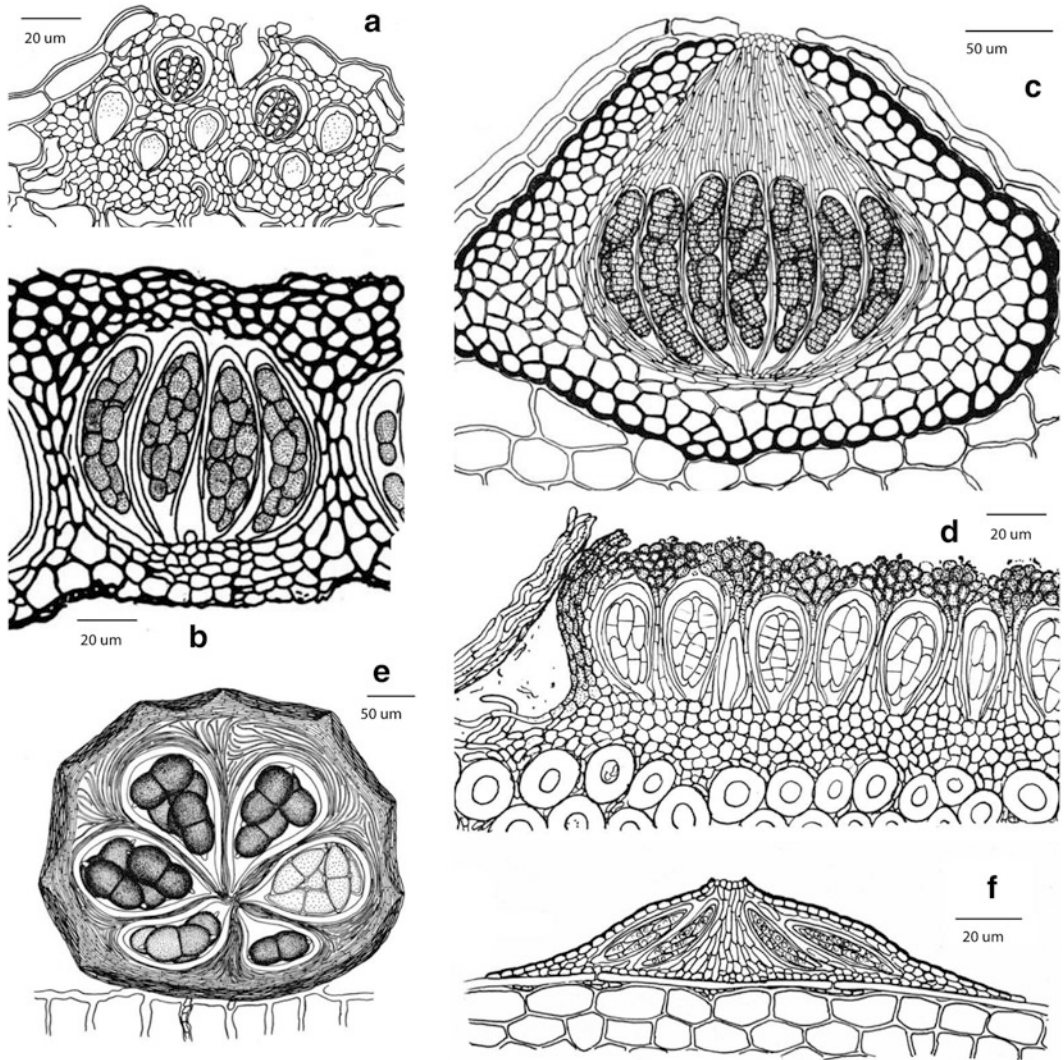


Fig. 6.1 Hamathecial and ascomal variation in dothideomyceta exemplified by selected type species. (**a**) *Elsinoë canavaliae* (Myriangiales, Dothideomycetidae). (**b**) *Dothidea sambuci* (Dothideales, Dothideomycetidae). (**c**) *Pleospora herbarum* (Pleosporales, Pleosporomycetidae). (**d**) *Arthonia dispersa* (Arthoniales, Arthoniomycetes). (**e**) *Zopfia rhizophila* (Pleosporales Pleosporomycetidae). (**f**) *Microthyrium microscopicum* (Dothideomycetes incertae sedis, Microthyriaceae). Adapted from von Arx and Müller (1975), used with permission

Generally, dothideomycete ascomata are closed, perithecioid structures that may sometimes be joined in a large stroma. However, several ascomal variations occur; for example, *Clathrospora heterospora* var. *simmonsii* has the *Pleospora* type of development, but ascogenous hyphae arise from pseudoparaphyses, and an operculate ostiole is formed by the dehiscence of a circular apical cap (Corlett 1967). A number of lineages in Dothideomycetes have open, apothecioid ascomata (see section on Hysteriales and Mytilinidiales and Figs. 6.2 and 6.3). The same general morphology can also be observed in some Arthoniomycetes (Fig. 6.1d). Recent DNA-sequence-based phylogenies have confirmed that these

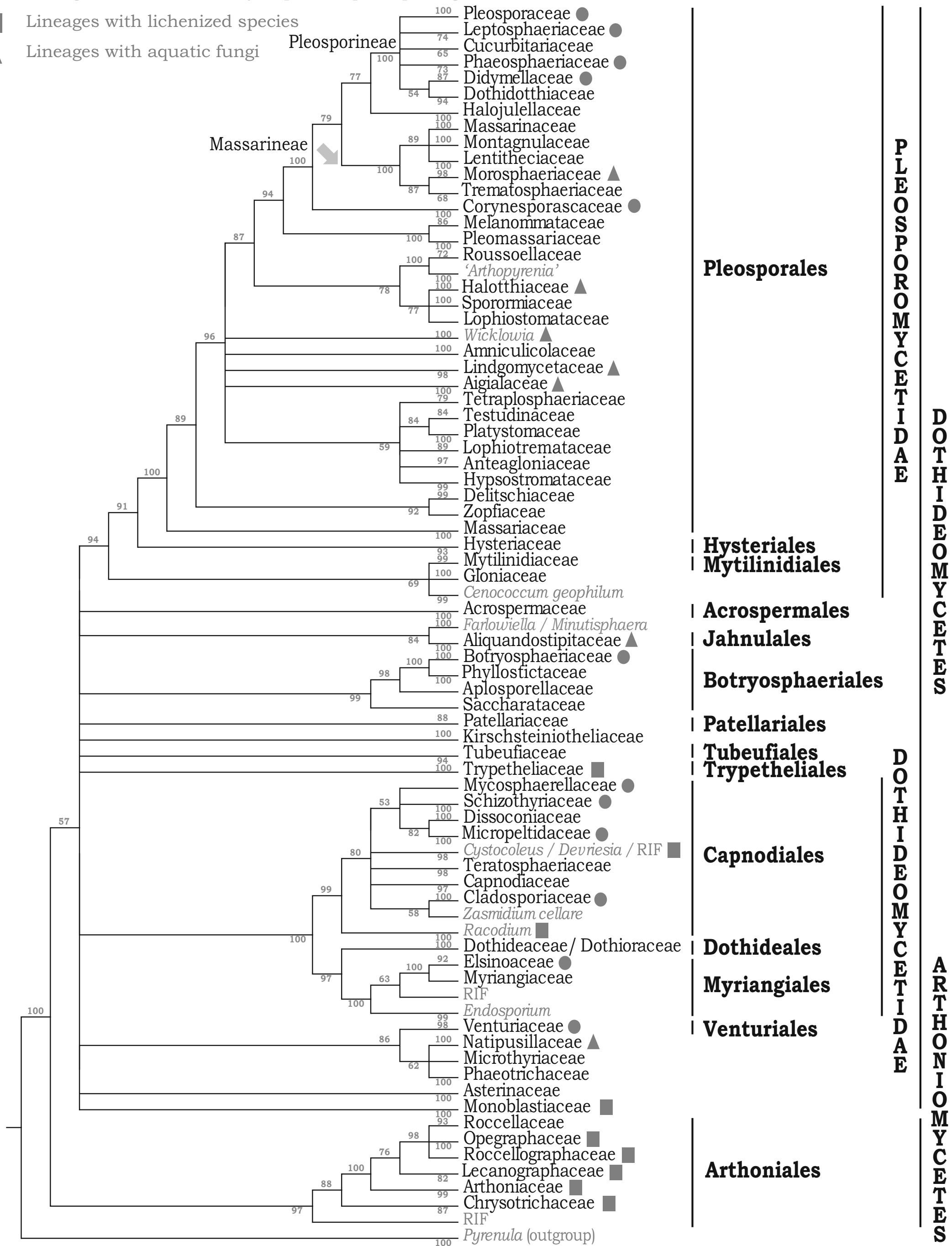


Fig. 6.2 A simplified maximum-likelihood tree obtained from 401 taxa and 5 genes with two *Pyrenula* species as outgroup, showing only relationships recovered in more than 50 % of bootstrap trees. All family-level clades were collapsed, and provisional or uncertain family names are indicated in quotes. RIF denotes "rock-inhabiting fungi"

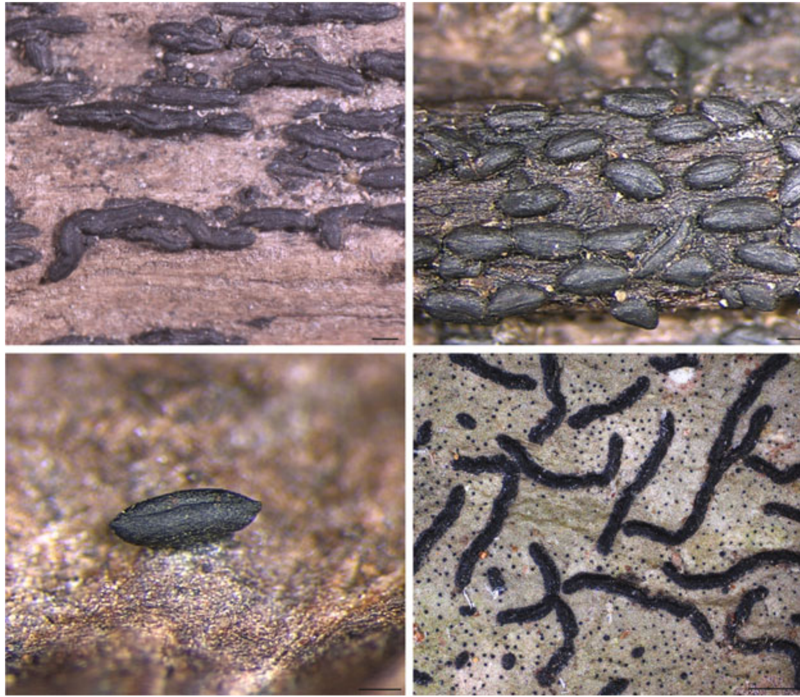


Fig. 6.3 Variety and convergence in hysteriate ascomata. *Top left* to *right*: *Hysterium angustatum* (Hysteriales, Hysteriaceae), *Anteaglonium abbreviatum* (Pleosporales, *Anteagloniaceae*), *Farlowiella carmichaeliana* (Dothideomycetes incertae sedis), *Opegrapha filicina* (Artoniales, Opegraphaceae). Bars = 0.5 mm. Photo credits: *Opegrapha filicina* Walter Obermayer, remainder Sabine Huhndorf

structures are found in several divergent lineages across the two classes. In addition, several unrelated lineages with cleistothecioid ascomata occur, such as several species in Sporormiaceae and Zopfiaceae (Fig. 6.1e). The same situation is also true of species producing thyriothecia in Asterinaceae, Microthyriaceae, and several other families in Dothideomycetes. An example of such a scutate ascoma in *Microthyrium microscopicum* is shown in Fig. 6.1f.

In contrast to the ascolocular ontogeny found in Dothideomycetes, the ascomal development of Arthoniomycetes prompted designation as an "in-between group" or "Zwischengruppe" (Henssen and Jahns 1974; Henssen and Thor 1998). This refers to a **combination of ascolocular and ascohymenial characters**: the asci are bitunicate, whereas a paraphysoid hamathecium develops from generative plectenchyma. With this combination, a plethora of ascomatal types have evolved, ranging from closed perithecialike ascomata (following hemiangiocarpous development), open apothecialike ascomata, and branched and strongly elongate to branched ascomata. Moreover, there are types where asci are more or less scattered over the entire thallus (*Cryptothecia*, *Stirtonia*) and tightly surrounded by networks of hyaline hyphae. An independent reduction of ascomatal structures has occurred in *Arthonia intexta*, a lichenicolous species that forms individual asci in the ascomata of its hosts. An example of an arthoniomycete ascoma is shown in Fig. 6.1d.

Tehler (1990) introduced a detailed terminology to describe the diversity of ascomatal structures in Arthoniomycetes. The main characters to distinguish ascomata were the number of locules in an ascostroma and the number of asci per locule. An additional type has been described since then, with new ascomatal units proliferating from existing ascomata (Henssen and Thor 1998). This latest described mode and the formation of rather diverse ascomata in

Arthoniales might be correlated with the flexible architecture and a prolonged growth of ascogenous hyphae, which also can form anastomoses at their tips with sterile haploid hyphae of the ascomata (Grube and Lücking 2001). Fluorescence microscopy studies of foliicolous species also revealed that after a primary formation of ascogenous hyphae and asci, new ascogenous hyphae can be developed in the older, central parts of ascomata. In other species, the central parts of the ascomata apparently degrade (Sundin and Tehler 1998), while ascus formation proliferates at the periphery of the ascomata.

Asci. The term bitunicate was recognized by Luttrell (1951) after usage in earlier descriptions (quoted in Reynolds 1989). It was applied to asci with two distinct walls where the outer wall separates and ruptures upon expansion of the inner wall to forcibly eject the ascospores. Bitunicate asci are noticeably thick-walled when viewed under a light microscope and appear to consist of outer and inner membranes, the ectotunica and the endotunica. Electron microscopy has revealed that **two, separate, morphologically distinct layers do not strictly occur**. Instead, the ascus wall is comprised of several layered zones characterized by fibrils (Funk and Shoemaker 1967; Furtado and Olive 1971; Reynolds 1971). Although some subsequent authors regarded the use of the term bitunicate as still appropriate despite this information (e.g., Müller 1981), others have pointed out that this also applies to several types of ascostromatic asci and that **a focus on the variable types of dehiscence may be more appropriate** (Reynolds 1989). Eriksson (1981) distinguished between seven types of dehiscence, of which four may apply to the broad concepts of bitunicate asci. A traditional concept of bitunicate asci is that they follow a pattern of **fissitunicate dehiscence.** A schematic drawing based on a selection of Eriksson's concepts is indicated in Fig. 6.4.

In fissitunicate dehiscence, the inner wall extends completely and the rigid outer wall is ruptured, similar to a Jack-in-the-box (Ingold 1933). This occurs in the majority of Dothideomycetes, but variations abound. In rostrate dehiscence, only the apical part of the endotunica separates from the ectotunica, and a part of the endoascus is protruded, but the ecto- and endotunica do not separate. Semifissitunicate dehiscence is an intermediate type between fissitunicate and rostrate dehiscence where the exoascus ruptures by an apical slit and the endoascal layers extend upward.

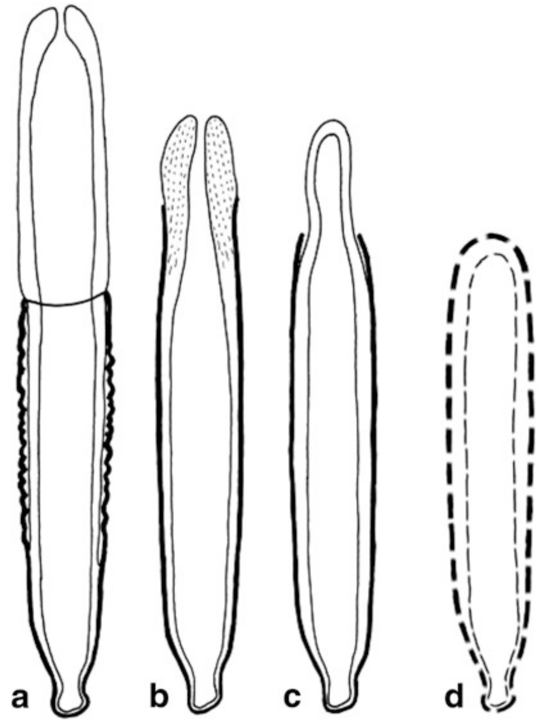


Fig. 6.4 Ascus types of bitunicate ascomycetes after Eriksson (1981) and Bellemère (1994). (**a**) Fissitunicate. (**b**) Semifissitunicate. (**c**) Rostrate. (**d**) Pseudoprototunicate. Adapted with permission from Hofmann (2009)

Members of Arthoniomycetes have dehiscence that tends to be more closely related to what Eriksson defined as either semifissitunicate or rostrate (Eriksson 1981), but rostrate dehiscence is also found in Asterinaceae in Dothideomycetes (Hofmann 2009).

An additional variation occurs in species with enclosed ascomata (cleistothecia), where the asci dissolve at maturity, and is referred to as pseudoprototunicate (Eriksson 1981; Reynolds 1989). This occurs in species of the families Phaeotrichaceae and Zopfiaceae, for example, *Phaeotrichum* and *Zopfia* spp. Passive ascospore release is also known from Arthoniomycetes and found in *Tylophoron* (Lumbsch

et al. 2009), *Tylophorella*, *Sporostigma* (Grube 2001a), and *Wegea*. The latter three genera were classified as members of Arthoniales based on anatomical evidence (e.g., structure of juvenile asci), but their position in Arthoniales still needs to be confirmed by molecular data. An apothecioid species with ostensibly unitunicate asci and explosive spore release, *Catinella olivacea*, was only recently placed in Dothideomycetes (Greif et al. 2007) and hints at more unaccounted variation in ascus dehiscence.

Anamorphs and sterile species. Asexual morphs (anamorphs) play an important role in the life cycle of several Dothideomycetes. It is often the dominant form encountered by pathologists isolating fungi from diseased plant and other tissues, and therefore the species with the largest body of genetic and genomic work are often encountered in their asexual states. Although early mycologists included anamorphic fungi in their classification systems, separate systems of classification were proposed by Fuckel (1870) and Saccardo (1882–1931). The use of DNA-based comparisons have now shown that asexual species can be placed within the current classification system without requiring that sexual and asexual structure be produced in culture. Despite this, these artificial groups still facilitate the identification of species, and many mycologists continue to use terms informally to distinguish between species bearing their asexual spores (conidia) in structures that are enclosed (coelomycetes) (Sutton 1980) or unenclosed (hyphomycetes) (Seifert et al. 2011). The development of conidia was studied in great detail in the previous century, initially with light microscopy and later using time-lapse photography and scanning electron microscopy (Cole and Sampson 1979; Mangenot and Reisinger 1976). In Dothideomycetes, coelomycetes and hyphomycetes occur interchangeably throughout the class while only a small number of asexual forms have been tied to Arthoniomycetes, for example, the coelomycete genus *Briancoppinsia* (Diederich et al. 2011) and the hyphomycete genus *Reichlingia* (Ertz and Tehler 2011). Asexual fungi can also exist as sterile mycelia and produce specific vegetative structures such as thick-walled sclerotia that can survive in soil; one prominent example is the mycorrhizal species *Cenococcum geophilum* (LoBuglio et al. 1996).

The way the nomenclature of these asexual forms has been treated to date will change drastically. The International Code for algae, fungi and plants, which governs fungal nomenclature, underwent expansive changes after the 2011 Melbourne congress (Hawksworth 2011). One of these changes will be to remove the dual system for sexual and asexual morphs that was allowed under previous editions of the code and **only allow a single name**. Attempts are under way to use a selection process that will address the stability of commonly used names (Hawksworth 2012). However, this means that **anamorph and teleomorph generic names will compete to label monophyletic groups** irrespective of the presence of a particular morph (Hawksworth 2012; Wingfield et al. 2012). For instance, *Cladosporium* may likely be an accepted generic name instead of *Davidiella*, and *Bipolaris* could replace *Cochliobolus* (Manamgoda et al. 2012). Whatever the final choices of the relevant nomenclatural bodies, this will result in a huge number of changes affecting nomenclature in Dothideomycetes (and, to a lesser extent, Arthoniomycetes).

B. Brief Taxonomic History

Historically, ascomycete taxonomy relied heavily on morphological characters such as the morphology of the ascus, septation of ascospores, the morphology and development of the ascoma, and the structure and organization of the centrum. Luttrell's proposed class, Loculoascomycetes (Luttrell 1955), was mainly based on the Ascoloculares of Nannfeldt (1932), in which the asci develop in cavities in a preformed stroma, and included lichenized fungi such as the constituent families of the Arthoniomycetes. A major difference was that Luttrell placed more emphasis on ascus structure than Nannfeldt.

The first descriptive taxonomic work in Dothideomycetes is credited to Fries (1818), who circumscribed the genus *Dothidea*. This was done without designating a type species;

only recently could this genus and the resulting taxa be stabilized by conserving *Dothidea sambuci* as the type species and by designating an epitype (Shoemaker 2003; Shoemaker and Hambleton 2005). The first descriptions of a distinctive bitunicate ascus were done in the mid-nineteenth century (Currey 1856; Desmazières 1843; Pringsheim 1858), and by 1887 the asci and the fissitunicate action of ascospore ejection could be summarized (De Bary 1887). However, the common occurrence of bitunicate asci and the correlation between this and other important criteria were not realized until the early twentieth century. The development of the ascoma as an important distinctive feature was used by Nitschke and Fuckel when they applied the family name Dothideaceae (Fuckel 1870) to fungi with asci in locules within a stroma, rather than in a true perithecial wall. This family was raised to ordinal rank by Lindau (1897), but the first close description to the modern class Dothideomycetes was provided by von Höhnel (1909) when he expanded Lindau's concept to include species with monascous and polyascous locules. Nannfeldt (1932) followed these concepts to emphasize the importance of ascomatal development and its correlation with ascus characters. It was one of Nannfeldt's students, Rolf Santesson, who completed the first integration of the separate classification of lichenized fungi in a **combined classification** (Santesson 1952). Santesson regarded lichenized pyrenomycetes as closely related to nonlichenized pyrenomycetes or loculoascomycetes. This meant that **constituents of Arthoniomycetes were classified with other nonlichenized bitunicate species for the first time**. In the ensuing decades, several mycologists produced influential works agreeing or disagreeing with these concepts in varying degrees as they relate to Dothideomycetes and Arthoniomycetes [see Barr and Huhndorf (2001) and Hawksworth (1985) for a more detailed taxonomic history]. With the rise of molecular methods in fungal classification, a first integration of lichenized fungi in a fungal molecular phylogeny was corroborated by Gargas et al. (1995). This unified view of lichenized and nonlichenized fungi was subsequently refined, and support was shown for a separate lineage that included Dothideomycetes and Arthoniomycetes (James et al. 2006; Lutzoni et al. 2001).

III. Ecology and Distribution

A. Associations with Plants and Plant Debris

An important set of species in Dothideomycetes subsist as what can loosely be defined as plant associates, while none of the members of Arthoniomycetes could so far be detected in a similar role. The vast majority of species in Dothideomycetes appear to be encountered as **saprobes** breaking down dead leaves and woody material, but several Dothideomycete species grow in close proximity to living plants, as **endophytes** in leaves, stems, or roots, as **epiphytes** growing on leaf surfaces, or as **parasites**, which includes acting as **pathogens** causing disease. At least one unique lineage in Dothideomycetes, containing the asexual species *Cenococcum geophilum*, occurs in a **mycorrhizal relationship** with a wide range of plant hosts across a broad geographic distribution (LoBuglio et al. 1996). It has only been reported as hyphae, sclerotia, and host-colonized ectomycorrhizal root tips (Tedersoo et al. 2010). Surprisingly, recent phylogenetic results indicate that this lineage is closely related to members of the Gloniaceae, saprobic species often found on wood with no indication of a biotrophic lifestyle to date (Schoch et al. 2009a; Spatafora et al. 2012). This highlights the fact that much of the biology of these organisms remains poorly described. Often species are only reported from one environmental niche, while they may in fact have diverse and complex life cycles that can only be revealed after intensive sampling and analysis. To date, only a small number of species in the dothideomyceta has been studied in this fashion.

The most extensive body of biological knowledge on Dothideomycetes exists on parasitic plant pathogens. Numerous economically important species occur throughout this class, but the most focus has been on species in suborder Pleosporineae. Species in *Alternaria*, *Cochliobo-*

lus, Didymella, Pyrenophora, *Leptosphaeria*, and several other closely related species have been used as models for plant pathogenicity in molecular biology, genetics, and, more recently, in genomics and related fields. Smaller groups of species in Botryosphaeriaceae, Elsinoaceae, Mycosphaerellaceae, and Venturiaceae continue to be studied in more detail.

Most plant-parasitic interactions of disease-causing fungi exist along a spectrum from **necrotrophy** (killing the host cells in advance to use them for energy and nutrients) to **biotrophy** (living inside living plant cells). The majority of plant-parasitic fungi in Dothideomycetes are necrotrophs, with only a few described so far as biotrophs (e.g., Simon et al. 2005). However, several necrotrophs also require periods of biotrophy. The variability of this **hemibiotrophic** lifestyle is exemplified by the causal agent of black leg on *Brassica* species, *Leptosphaeria maculans*, which recently had its genome sequence published (Rouxel et al. 2011). This pathogen sequentially occurs as a saprobe on stem residues, a necrotrophic pathogen on the plant host, and an endophyte without causing any symptoms (Rouxel and Balesdent 2005). During this process, the fungus needs to employ an array of extracellular enzymes to kill and then break down plant material and evade plant defenses. To improve understanding of these interactions, much recent research has focused on a diverse group of molecules called **effectors** that are employed during these processes. Effectors suppress plant recognition systems for pathogens (de Wit et al. 2009). Progress is being made in understanding how these pathogens interact with plants by mining new genome data (Ohm et al. 2012; van de Wouw and Howlett 2011). At the same time, molecular ecological sampling is providing a more complete picture of the diversity, host affinities, and geographic dispersal of fungal endophytes in this group (Higgins et al. 2007; U'Ren et al. 2010).

B. Aquatic Species

In addition to the large group of terrestrial saprobes, Dothideomycetes includes a number of saprobic species on woody debris and mangroves in aquatic (freshwater, marine, and brackish) environments. These species exhibit their main adaptations in the spores they produce, such as the gelatinous sheaths and appendages that help them to adhere to the submerged surfaces of plants and wood. Marine species have been defined as fungi that grow exclusively in marine environments (Kohlmeyer and Kohlmeyer 1979). Sixty-six genera and 110 species are currently listed under Dothideomycete species, which are found predominantly in marine environments (Jones et al. 2009). These species occur in or on macroalgae, sea grasses, and decaying wood, but they are most commonly isolated from intertidal zones and mangroves, where they play an important role in breaking down complex carbohydrates. Several lineages in the Dothideomycetes are dominated by marine or freshwater species, often with a mixture isolated from both environments. The majority of aquatic species tend to reside in the Pleosporomycetidae, with only a few species isolated from within the Dothideomycetidae, but several aquatic lineages still remain unresolved in the latest phylogenetic analyses (Shearer et al. 2009; Suetrong et al. 2009). Shearer et al. (2009) speculated that the absence of pseudoparaphyses outside Pleosporomycetidae may limit survival in habitats with fluctuating water levels because aquatic species often have abundant pseudoparaphyses embedded in gel that could potentially protect them from desiccation during dry conditions. Fungi from aquatic environments continue to be of interest for their ability to produce secondary metabolites (Mayer et al. 2007; Rateb and Ebel 2011).

Several novel families, predominantly containing fungi from aqueous environments, have only recently been described. These include Aigialaceae, Morosphaeriaceae, Lentitheciaceae, Lindgomycetaceae, and Amniculicolaceae, all in Pleosporales (Suetrong et al. 2010). A new order, Jahnulales, has been described to contain the predominantly freshwater Aliquandostipitaceae, but indications are that marine species also form part of this group (Suetrong et al. 2010, 2011).

It is clear that aquatic fungi represent an important part of the poorly documented

diversity in dothideomyceta, and several unclassified lineages require more in-depth documentation.

C. Associations with Algae and Other Lichens

Lichenization appears to be a primary ecological trait in Arthoniomycetes, whereas only a few separate lineages in Dothideomycetes contain lichen-forming fungi. This discrepancy is well reflected by the difference in thallus complexity in both classes. Most lichen-forming species in Dothideomycetes develop only thalli with rather simple organization (Trypetheliaceae, Arthopyreniaceae, Monoblastiaceae). In *Cystocoleus* and *Racodium* (lineages in Capnodiales) **the shape of the microfilamentous thalli is determined by the algal partner** (conglutinate hyphae encage a central algal thread (Muggia et al. 2008)).

In comparison, the **thallus structures in Arthoniomycetes are more diverse.** "Advanced," surface-detached thallus forms are particularly common in Roccellaceae, which includes crustose, placodioid, shrubby, and pendant fruticose thalli (foliose thallus types, which are ubiquitous in Lecanoromycetes, are hardly developed in Arthoniomycetes). Studies of Tehler and coworkers suggest that fruticose thallus growth has evolved repeatedly in Roccellaceae and Opegraphaceae (e.g., Tehler and Irestedt 2007; Ertz and Tehler 2011). *Roccella* and *Dendrographa* seem to represent independent evolutions of fruticose lichens within Roccellaceae, and, similarly, *Ingaderia* and *Pentagenella* in Opegraphaceae.

Representatives of Trentepohliales are the most common photobionts in both Arthoniomycetes and Dothideomycetes. These algal partners usually form three-dimensional filamentous plane and platelike thalli in the free-living stage. In the lichenized stage, the fertile structures of the algae are not developed, and the shape of algal filaments can variably be modified. Filamentous growth of *Trentepohlia* is often strongly modified in lichenized stages, and the filaments are reduced to concatenations of a few algal cells. The growth modification is not so distinct in lichen mycobionts associating with members of the genus *Phycopeltis*. These planar growing algae are particularly common partners of foliicolous species in Arthoniomycetes. According to cell sizes and branching patterns of algal filaments, there seem to be species-specific relations with different species of *Trentepohlia* and *Phycopeltis* (Lücking 1995; Thor 1990), but the taxonomy and phylogenetic relationships of Trentepohliales are still poorly resolved. Chrysotrichaceae and certain species assigned to Arthoniaceae form lichens with coccal green algae. Several *Arthonia* species in Mediterranean habitats are poorly (or temporarily) lichenized. Their hyphae permeate the surface of woody twigs. *Arthonia muscigena* is lichenized and specialized to grow on mosses, whereas *Dawsophila* (Arthoniaceae) is a bryosymbiotic, nonlichenized genus of Arthoniaceae (Grube 1998). These fruit bodies of *Dawsophila* are extremely small and specialized to growing between the leaf lamellae of the moss *Dawsonia* (Polytrichaceae).

Approximately 215 species of Arthoniomycetes (13 %) are known as **lichenicolous** fungi (Lawrey and Diederich 2003), a number that will very likely increase. Most of these lichenicolous Arthoniales are highly specialized on their hosts and represent commensals or locally destructive parasites. Lichenicolous species are often assigned to mainly lichenized genera (such as *Arthonia*, *Enterographa*, *Mazosia*, *Opegrapha*, or *Schismatomma*), suggesting that lichenized habit frequently and independently gave rise to lichenicolous lifestyles in unrelated Arthoniomycetes. However, phylogenetic studies should test relationships more carefully because lichenicolous species do not always form monophyletic groups with seemingly congeneric, lichenized relatives (Ertz and Tehler 2011). Observations suggest that the lichenicolous species in *Arthonia* retained an affinity to the algal symbionts by densely entwining the host's algal cells (Grube and Matzer 1997; M. Grube personal observation). The lichenicolous fungi in Arthoniomycetes apparently share the strategy for obtaining nutrients with their lichenized ancestors but gave up on the formation of their own thallus structure. This may coincide with a switch of the algal partner: many *Arthonia* species infect lichens with coccal, trebouxioid algae.

Approximately 500 species of Dothideomycetes are lichen-forming, and 266 species represent lichenicolous fungi (Lawrey and Diederich 2003). In contrast to the likely singular lichenization transition giving rise to Arthoniomycetes, the **Dothideomycetes may comprise the highest number of transitions to the lichenized lifestyle in a class** (six independent origins) (Nelsen et al. 2009). The lichenicolous life style is found in three orders of Dothideomycetes, one of which also contains lichenized members. According to the different patterns of lichenized and lichenicolous fungi in Dothideomycetes and Arthoniomycetes, the evolution of lichenicolous fungi has different foundations, and a generalized view of their evolution from the lichenized life style is not well supported. This could also be reflected by the differential preferences of lichenicolous fungi to the lichen symbionts. So far, lichen mycoparasitism has been shown microscopically in the pleosporalean genus *Pyrenidium* (De los Ríos and Grube 2000), but further study of more lichenicolous fungi in Dothideales is required to clarify the preference for either of their host symbionts. Other species seem to show preference for the algal partner. Based on ascomatal characters, the lichenicolous genus *Zwackhiomyces* is closely related to *Anisomeridium* (Monoblastiaceae, Dothideomycetes) (M. Grube unpublished observation). Specific histochemical investigation for the lichenicolous fungi revealed clear association with the host's unicellular coccal algae (Grube and Hafellner 1990). Several lichenicolous *Phoma* species were described in the Phaeosphaeriaceae (Lawrey et al. 2012). Like other diverse lineages such as *Lichenoconium* (Lawrey et al. 2011), these species remain outside of current familial and ordinal classification. It should also be studied in this case whether the switch between lichenized and lichenicolous life style coincides with a change of algal associations. Several fungi grow as endophytes inside lichens (endolichenic fungi) that include an important subset of fungi in Dothideomycetes (Arnold et al. 2009; Hofstetter et al. 2007; U'Ren et al. 2010). Similarly, endophytic fungi inside macroalgae include several Dothideomycetes (Zuccaro et al. 2008). These may very likely represent important environments containing undescribed Dothideomycete diversity.

D. Rock Environments and Oligotrophism

Rock-inhabiting fungi (RIF) are **nonlichenized fungi that are adapted to subsistence in extreme conditions on rock surfaces**. These oligotrophic fungi are slow-growing and asexual with poor diagnostic features. RIF often show meristematic or microcolonial growth, and they tend to have high concentrations of melanin in their cell walls. These features generally confer high levels of stress tolerance in fungi and allow them to tolerate desiccation, osmotic pressure and acidity, and poor nutrient availability and to survive high heat in a desiccated stage.

Melanized fungi are also strikingly tolerant to ionizing radiation and have been found in unusual habitats, such as Antarctic dry valleys (Onofri et al. 2007) or damaged nuclear reactors and nuclear reactor cooling water (Zhdanova et al. 2000), where they are able to survive conditions analogous to outer space. This capacity is a side effect of **a perhaps widespread ancient adaptation to the rock habitat**. It is therefore particularly interesting that RIF are found in several unrelated lineages of Dothideomycetes and could represent an ancestral trait; they also form an early diverging group in the Arthoniomycetes (Ruibal et al. 2009). Recent analyses by Gueidan et al. (2011) support a much earlier origin of the dothideomycetan rock-inhabiting lifestyle than can be postulated in Chaetothyriales. The origin of RIF in Dothideomycetes is estimated to date back to 309–420 million years ago in the late Devonian, but it is not possible to fully rule out independent origins of RIF in Arthoniomycetes and Dothideomycetes after a suggested diversification around 292–301 million years ago in the Carboniferous.

IV. Modern Classification and Phylogeny

The earliest phylogenetic analysis performed on a group or subgroup of dothideomyceta

was done on Arthoniales (Tehler 1990). The author used 92 morphological, biological, and other nonmolecular characters to run a cladistic analysis on 20 members of Arthoniales to determine relatedness within this group. A more expansive analysis was run a year later (Reynolds 1991) on a morphological data matrix representing the major ascostromatic and fissitunicate families and orders using 30 characters. This included lichenized families such as Opegraphaceae and Trypetheliaceae. An important conclusion was that loculoascomycetes are not monophyletic, but the author also suggested that the use of molecular data would be crucial in order to elucidate poorly resolved nodes on the tree. This set the stage forthe first phylogenetic studies using DNA sequence comparisons.

A phylogenetic analysis of dothideomyceta is shown in Fig. 6.2. A maximum-likelihood RAxML run was performed on 400 taxa (using *Pyrenula aspistea* and *Pyrenula pseudobufonia* as outgroups). Sequences were taken from GenBank relying extensively on data produced in Schoch et al. (2009a) and Ertz and Tehler (2011). Alignments from five loci (fragments from large and small nuclear rDNA, mitochondrial small subunit rDNA, transcription elongation factor, and the second largest subunit of the RNA polymerase I gene) were individually obtained with SATe (Liu et al. 2009). This was concatenated and analyzed with introns and ambiguous regions excluded, resulting in 6,299 characters of which 56 % were missing. Phylogenetic analysis was done using the "bootstopping" criterion in RAxML (Stamatakis et al. 2008) under the CIPRES v. 3.1 Web portal (Miller et al. 2010). The analysis was performed applying unique model parameters for each gene and codon, and the data set was divided into nine partitions, as previously described in Schoch et al. (2009a). A general time-reversible model approximation (GTRCAT) was applied, and the resulting tree was compressed to indicate major lineages in MEGA (Tamura et al. 2011). The alignment (and complete phylogeny) is deposited in TreeBASE (11726, www.treebase.org).

A. Dothideomycetes

Phylogenetic analyses of single-gene data sets of the nuclear small ribosomal subunit never resulted in unambiguous support for Dothideomycetes as a monophyletic entity with phylogenies indicating the class as a paraphyletic assemblage (Berbee 1996; Berbee and Taylor 1992; Spatafora et al. 1995) or monophyletic (LoBuglio et al. 1996; Reynolds 1998; Winka et al. 1998) but often with poor statistical support. Although the orders Pleosporales and Dothideales were almost always well resolved, their shared monophyly was very dependent on the method of study and taxon sampling. All these studies generally supported the conclusion that loculoascomyetes do not form a monophyletic group, and the subsequent use of additional phylogenetic markers affirmed the preceding small subunit phylogenies, which tentatively supported a monophyletic Dothideomycetes. The second largest subunit of the RNA polymerase gene (*RPB2*) showed strong bootstrap representation for a monophyletic Dothideomycetes (Liu et al. 1999), with similar results using the largest subunit of the RNA polymerase gene (*RPB1*) (Lumbsch et al. 2000). A more complete set of samples found support for a single ancestor to loculoascomycetes in a phylogeny of *RPB2* sequences (Liu and Hall 2004), but this was contrasted by a multigene study that included *RPB2* (Lutzoni et al. 2004). The **lack of reliable morphological characters to indicate ancestral relatedness is challenging** in dothideomyceta (Lumbsch and Huhndorf 2007). The first indications that morphological characters are informative above the level of family were provided by Berbee (1996), who proposed that the presence of pseudoparaphyses was a good monophyletic character to delimit pleosporaceous taxa, and this was generally well supported in subsequent phylogenies (Liew et al. 2000; Silva-Hanlin and Hanlin 1999) despite the fact that *Leptosphaerulina* (lacking pseudoparaphyses) was shown to be in the Pleosporales clade. The **presence or absence of pseudoparaphyses correlated with a molecular derived phylogeny**, which was subsequently confirmed by additional authors (Lumbsch and Lindemuth 2001) and resulted in the designations of two subclasses, Pleosporomycetidae and Dothideomycetidae (Schoch et al. 2006). A large number of new and existing orders containing single families with relatively low coverage in taxon sampling and phylogenetic markers have been proposed elsewhere for Dothideomycetes. We refrain from utilizing

these names here until more analysis is possible (Hyde et al. 2013)

1. Pleosporomycetidae

The subclass Pleosporomycetidae was described (Schoch et al. 2006) based on phylogenetic comparisons of DNA and the presence of a centrum with pseudoparaphyses. This description included only the order Pleosporales and initially a single species outside of this order, *Lophium mytilinum*. Since then, it has been expanded to include additional orders—Hysteriales, Mytilinidiales, and Jahnulales (Schoch et al. 2009a). However, the placement of Jahnulales in Pleosporomycetidae remains tenuous because DNA-based phylogenetic support for this relationship is still ambiguous (Fig. 6.2).

Pleosporales. This is the largest order in Dothideomycetes, consisting of 332 genera and more than 4,700 species (Kirk et al. 2008). The order was recently monographed by Zhang et al. (2012), and readers are referred to that work for a more detailed discussion of the morphology and biology of the order. Ecologically, members subsist as epiphytes, endophytes, or parasites of plants. Several species are saprophytes on dead plant material or dung, and hyperparasites can occur on fungi or insects in addition to a smaller number of lichenized species in Arthopyreniaceae. The concept of Pleosporales has changed since it was first proposed by Luttrell (1955) and subsequently emended and validated by Luttrell and Barr (Barr 1987). The advent of molecular data has shown that a classification based on the distinction of pseudoparaphyses morphology does not agree with DNA-based phylogenies. Therefore, Melanommatales as described by Barr by relying on the shapes of pseudoparaphyses (Barr 1990) are now considered synonymous with Pleosporales, while the type-bearing family Melanommataceae is currently accepted in a more restricted sense as part of Pleosporales (Zhang et al. 2009). In addition, a number of families included in Barr's 1987 description have proven to be only distantly related to this order. Most notably, Botryosphaeriaceae is now placed outside of Pleosporales, as are Hysteriaceae, Venturiaceae, and Tubeufiaceae (Kruys et al. 2006; Schoch et al. 2006, 2009a; Zhang et al. 2009).

Familial designations in Pleosporales traditionally relied on distinctions of the ascomata, size, and shape variations in the ostiole as well as the presence of periphyses and other cells in the centrum. The lifestyles of different species were also considered informative. However, as noted earlier, DNA-based phylogenies exposed several familial concepts that require readjustment because several of these characters are shown to be highly convergent (Schoch et al. 2006). Taxon sampling of DNA data in the order has been greatly expanded in the last few years, and a more complete phylogeny could soon delineate 19 families with the tentative inclusion of an additional 5 families (Zhang et al. 2009). Currently, 28 families are recognized in the order, with several of these taxon concepts still not well supported in multigene phylogenies (Zhang et al. 2012). The most obvious discrepancy is the treatment of Pleomassariaceae, which is accepted in Lumbsch and Huhndorf (2010) but synonymized by Zhang et al. (2009). It is indicated as being separate in Fig. 6.2 and now includes a number of recently described anamorph species with stellate conidia in *Prosthemium* (Tanaka et al. 2010).

In addition, several family-level branches in the Pleosporales phylogeny remain unnamed and await further sampling. One example is the unique branch supporting the anamorphic rubber pathogen *Corynespora cassiicola*. This species may have associations with the teleomorph *Corynasca*, implying that the family name Corynascaceae could be applied if that is the case (Sivanesan 1996). Another well-supported but separate lineage contains the marine mangrove-associated species *Julella avicenniae* (Schoch et al. 2009a). The genus *Julella* has since been shown to be polyphyletic, with *Julella fallaciosa* showing a close relationship to species in Trypetheliales (Nelsen et al. 2011). *Julella avicenniae* has since been recombined as *Halojulella avicenniae* and the family Halojulellaceae proposed (Hyde et al. 2013).

Despite the high level of uncertainty remaining in family-level classification, recent

molecular data from multiple genes have steadily improved the phylogenetic context. Thus, the suborder Pleosporineae as circumscribed by Barr (1979) was emended to represent a well-resolved phylogenetic node showing common ancestry for five families (Zhang et al. 2009). This represents a majority of important Dothideomycetes plant pathogenic families and includes the type-bearing family, Pleosporaceae (Kodsueb et al. 2006a). This family represents the most intensively studied clade of Dothideomycetes with genome data available for several species, including *Cochliobolus* (with *Bipolaris* and *Curvularia* anamorphs), *Pyrenophora* (with *Drechslera* anamorphs), and several species in the anamorph genus *Alternaria* (Ohm et al. 2012), some tied to *Lewia* teleomorphs. Other families in the suborder are Cucurbitariaceae, Leptosphaeriaceae, and Phaeosphaeriaceae, which contain multiple plant pathogens in *Leptosphaeria* and *Phaeosphaeria* associated with *Phoma* and *Septoria* anamorphs (Aveskamp et al. 2010; de Gruyter et al. 2009, 2012; Zhang et al. 2009). Notably, the monophyly of Leptosphaeriaceae is only poorly to moderately supported in most recent molecular studies (e.g., de Gruyter et al. 2012; Zhang et al. 2009) (Fig. 6.2). A fifth family, Didymellaceae described with pseudoparaphyses deliquescing at maturity, contains the majority of *Phoma* anamorphs, although this large ubiquitous genus is interspersed throughout the suborder (de Gruyter et al. 2009, 2012). Finally, a dark-spored *Botryosphaeria*-like species, *Dothidotthia*, was recently removed from Botryosphaeriales based on multigene phylogenies and placed in a separate family, Dothidotthiaceae, within this suborder (Phillips et al. 2008).

Outside of Pleosporineae, the recently emended suborder Massarineae (Barr 1979; Zhang et al. 2012) contains families Montagnulaceae, Massarinaceae, Lentitheciaceae, Morosphaeriaceae, and Trematosphaeriaceae. These families contain a majority of saprobic species, and some may occur in aquatic environments. The type-bearing family, Massarinaceae, was traditionally treated as closely related to Lophiostomataceae (Barr 1987). Phylogenetic analysis shows that Lophiostomataceae is more distantly related, and like most other families in Pleosporales, more narrow concepts are now applied to these two families (Mugambi and Huhndorf 2009a ; Zhang et al. 2011).

Outside of Massarineae, the partially lichenized *Arthopyrenia* species are grouped with *Roussoella* and *Roussoellopsis*, species isolated from bamboo (Tanaka et al. 2009). These groups have clear morphological differences, so this relationship is surprising, prompting the need for more in-depth sampling and analysis. The variety of fungi isolated from bamboo is further seen in a new family, *Tetraplosphaericeae*, described for species predominantly isolated from this host (Tanaka et al. 2009). However, some studies could not corroborate unambiguous support for this taxon (e.g., Schoch et al. 2009a; Zhang et al. 2011). Additional pleosporalean families recently described, including Aigialaceae and Lindgomycetaceae, contain majorities of marine and freshwater species, respectively (Shearer et al. 2009; Suetrong et al. 2009).

The remaining ecological niche in Pleosporales comprises species growing in animal dung. This is the characteristic ecology in two families where coprophilic species are interspersed with saprobic lineages. In Pleosporales, these species are found in Sporormiaceae and Delitschiaceae (Kruys and Wedin 2009; Kruys et al. 2006), and although the relation is only a distant one, the related ascospores of both Sporormiaceae and Delitschiaceae have germ slits. In the first large-scale phylogenetic studies of Dothideomycetes, Delitschiaceae appeared as the earliest diverging lineage in Pleosporales (Liew et al. 2000; Kruys et al. 2006; Schoch et al. 2006). An isolate of *Zopfia rhizophila* has also been shown to be sister to this clade as representative of the large and poorly defined family Zopfiaceae, often isolated from soil and associated with roots (Kruys et al. 2006). However, a more recent paper placed a well-sampled clade of Massariaceae as the earliest diverging clade in Pleosporales (Voglmayr and Jaklitsch 2011). Members of this family were shown to be weakly parasitic or hemibiotrophic and were found to have high host specificity. As is the case for several families across Pleosporales (and Dothideomycetes), each of the

morphological characteristics used to define Massariaceae also occurs in other, unrelated groups. Therefore, characterization required the combination of these characters with a strong reliance on DNA sequence comparisons.

Hysteriales and Mytlinidiales. The hysteriaceous fungi are characterized by apothecioid ascomata that consist of persistent, carbonaceous structures that dehisce by a longitudinal cleft that allows for the exposure of the hymenium and can be closed in response to changes in humidity. This particular ascomatal structure, with its boat-shaped outline, has been referred to as a hysterothecium and has been described in several taxa now known to belong to Lecanoromycetes, Arthoniomycetes, and Dothideomycetes. The convergence of this morphology is illustrated in a photo plate that shows ascomata from various unrelated lineages in dothideomyceta (Fig. 6.3). Initially, taxa producing these structures were thought to reflect a single genealogical group and classified under the family Hysteriaceae, later raised to the order Hysteriales. Thus the original description of Hysteriales by Lindau (1897) included four families of which only one has bitunicate asci. Zogg (1962) monographed the remaining bitunicate species in the order and classified them in two families, Hysteriaceae and Mytilinidiaceae (as Lophiaceae). Because of similarities in the centrum, some authors have also placed Hysteriaceae under Pleosporales (Barr 1987) and Mytilinidiaceae in Melanommatales (Barr 1987, 1990), while others do not accept a second family (Luttrell 1973).

The role that DNA-based phylogenies can play in revealing the unreliability of morphological characters is displayed well in the hysteriaceous fungi. Earlier phylogenetic studies included a few of these taxa (Liew et al. 2000; Schoch et al. 2006), but only recently could large-scale comparisons be made (Boehm et al. 2009a, b; Mugambi and Huhndorf 2009b). The shared conclusion of these recent papers was that a high degree of convergence occurs in virtually all important morphological characters and that morphologically defined groups hide an unexpected genetic diversity. The results were that only a narrow concept of Hysteriales including the majority of Hysteriaceae can be accepted, and a new order, Mytilinidiales, was proposed for species in Mytilinidiaceae with thin-walled mussel-shaped ascomata. A species, *Rhytidhysteron rufulum*, included in an unrelated family, Patellariaceae, was placed in Hysteriales, and many other genera were shown to be polyphyletic, for example, *Hysterium*, *Hysterographium*, and *Gloniopsis*. In addition, some taxa previously included in Mytlinidiaceae based on these morphologies may also reside in Hysteriales, for example, *Ostreichnon*. Indicating additional poorly sampled diversity, a number of species could not be accommodated in the newly defined orders. *Farlowiella* and *Glonium* could be placed as Pleosporomycetidae incertae sedis (Boehm et al. 2009b), but members of the Patellariaceae (Patellariales) did not group with any defined subclass or order (Schoch et al. 2009a). An additional lineage of hysteriaceous species previously described in *Glonium* was also placed in Pleosporales under the genus *Anteaglonium* (Mugambi and Huhndorf 2009b). Members of this genus are now placed in the new family *Anteagloniaceae* (Hyde et al. 2013). In addition, *Cenococcum geophilum*, one of the most commonly isolated ectomycorrhizal fungi on a global scale, has a close relationship to *Glonium* species in the Gloniaceae (Schoch et al. 2009a; Spatafora et al. 2012). In conclusion, the latest phylogenetic results indicate at least two lineages corresponding to the orders Hysteriales and Mytilinidiales, with the Hysteriales as a sibling clade of Pleosporales and both sharing a common ancestry with Mytilinidiales (Schoch et al. 2009a) (Figs. 6.2 and 6.3).

Jahnulales. Based on moderate phylogenetic statistical support and the presence of pseudoparaphyses, Jahnulales was tentatively added to subclass Pleosporomycetidae (Schoch et al. 2009a), but more resolved phylogenies are required to verify this. Although the genus *Jahnula* was validated in 1936 (Kirschstein 1936), it was only by 1999 that additional species in the genus were described (Hyde and Wong 1999). In 2001, *Aliquandostipite khaoyaiensis* was described with extraordinarily broad hyphae, stalked ascomata, and the presence of pseudoparaphyses in its centrum, suggesting a link to Pleosporales. However, a DNA-sequence-based

phylogeny found no clear association with this order, and a new family was described, Aliquandostipitaceae. Subsequently, a new order was described, including *Aliquandostipite*, *Jahnula*, and *Patescospora* (Pang et al. 2002). The latter genus was later synonymized with *Aliquandostipite*, and the ordinal description emended to include hyphae wider than 10 μm and more variable ascospore morphology (Campbell et al. 2007). This small order now contains more than 40 species that are almost always found in freshwater habitats. The species exhibit a range of variation, with ascospores filled with lipid guttules, equipped with a variety of gelatinous appendages and sheaths, and broad vegetative hyphae (10–40 μm) that attach the fungi to submerged wood. In addition to four possible lineages in Jahnulales, a mangrove-associated species, *Manglicola guatemalensis,* which is able to grow in marine environments, was added and a new family, Manglicolaceae, described (Suetrong et al. 2010, 2011).

2. Dothideomycetidae

Dothideomycetidae was emended in Schoch et al. (2006) from an earlier designation that used the subclass extension as applied to the whole Dothideomycetes (Kirk et al. 2001). This means that Dothideomycetidae sensu Schoch et al. (2006) is confined to the generally aparaphysate orders Dothideales, Myriangiales, and Capnodiales.

Capnodiales. Capnodiales represents the second largest order of diverse species in Dothideomycetes after Pleosporales. The current concept of the order was expanded from Luttrell's original, based on a multigene phylogeny with the presence of ostiolar periphyses proposed as possible synapomorphy (Schoch et al. 2006). Like Dothideales, this order lacks pseudoparaphyses but does contain several species with periphysoids and periphyses.

The terms periphyses and ostiolar periphysoids are sometimes applied interchangeably to the same cells in species descriptions in Capnodiales. One reason for this is that periphysoids can be reoriented during ascus discharge from hanging downward in the ascoma to being oriented toward the ostiolar region (Reynolds 1998). This convergent character is also shared with unrelated loculoascomyetes in Chaetothyriomycetidae (Eurotiomycetes).

The diverse and expanded Capnodiales now includes the epiphytic sooty mold family Capnodiaceae, rock-inhabiting and lichenized species, as well as foliar epiphytes and species associated with human hair in the genus *Piedraia*. In Fig. 6.2, the earliest diverging lineage in Capnodiales represents a lichenized genus, *Racodium* (Muggia et al. 2008). Another lichenized species, *Cystocoleus ebeneus*, is represented as being unrelated to any of the known families and in Schoch et al. (2009a) formed a single clade (clade C) together with a number of rock-inhabiting species and other extremophiles. Whether this represents a novel family will require further analysis, but it appears likely. RIF occur throughout the order Capnodiales and are diverse in both classes. *Piedraia hortae*, a specialized parasite of human hair in the tropics, was shown by Schoch (2006) to belong to Capnodiales. Piedraiaceae appeared to cluster within Teratosphaeriaceae (Schoch et al. 2009a), but in some cases only with poor support (Crous et al. 2009a), and should likely still be regarded as Capnodiales incertae sedis. Several additional lineages appear unresolved in Fig. 6.2.

Davidiellaceae was introduced for the genus *Davidiella* with *Cladosporium* anamorphs (Braun et al. 2003; Schoch et al. 2006). *Cladosporium* is one of the most commonly isolated hyphomycete anamorphs in environmental samples and currently contains only approximately 100 species, many without any sexual state (Seifert et al. 2011). It is one of the most commonly isolated fungal forms from environmental samples and contains numerous species that are endophytic, fungicolous, and pathogenic on humans and plants. Based on molecular phylogenies, several important species, such as the tomato pathogen *Cladosporium fulvum* (now *Passalora fulva*), were removed from this genus only in the past decade and are now classified in different families (Thomma et al. 2005). The older family name *Cladosporiaceae* was recently

reintroduced in place of *Davidiellaceae* (Hyde et al. 2013).

The original concept of the Capnodiales was proposed based on the sooty molds, diverse fungi that occur together in a common sooty mass. It is now clear that this ecological guild consists of convergent but unrelated species. The order was proposed to consist of three families, Antennulariaceae, Capnodiaceae, and Coccodiniaceae (Woronichin 1925). The majority of sooty mold species with DNA sequence data currently comprise the single family Capnodiaceae, while a number of additional families previously named in this ecological guild await sequence sampling. Members of Coccodiniaceae were recently shown to belong to Chaetothyriales, Eurotiomycetes (Crous et al. 2009a). This in contrast with an older study that placed a species in a clade now defined as Teratosphaeriaceae (Winka et al. 1998). Species in Capnodiaceae tend to occur on the leaf surfaces of live plants where they absorb nutrients from honeydew produced by insects and other nutrients in the phyllosphere. The group is highly pleomorphic with several anamorphs with characteristically darkly pigmented hyphae usually found on leaf surfaces (Hughes 1976). Anamorphic states tend to be mainly pycnidial with small hyaline conidia (Hughes 1976). Most sooty molds do not grow in axenic culture, and only a small number of them have yielded DNA sequence data to date. An initial phylogeny based on 18S rDNA sequences indicated that those species in Capnodiaceae form well-defined groups with affinities to the other aparaphysate orders (Reynolds 1998), and a monophyletic Capnodiaceae was recovered in more recent analyses (Chomnunti et al. 2011; Crous et al. 2009a). The family also includes a lineage collected from ant nests. These undescribed species reinforce the nest walls and are nourished by the ants with honeydew, indicating an expansion of possible niches for these fungi (Schlick-Steiner et al. 2008). Another family containing sooty molds, Chaetothyriaceae, was initially described as possibly related to Capnodiales by Woronichin (1925). Members of this family are now classified in Chaetothyriales with the help of some recently generated DNA sequence comparisons (Chomnunti et al. 2012). They are found in the same niches as the Dothideomycetes sooty mold lineages and their main ecological distinctions from Capnodiaceae are a lack of insect associations. Morphologically, these two families can be differentiated by the tendency for Chaetothyriaceae to produce ascostromata containing multiple locules, as opposed to single locules in the ascomata of Capnodiaceae (Chomnunti et al. 2011, 2012).

The majority of plant pathogenic species in Capnodiales reside in Mycosphaerellaceae (Arzanlou et al. 2008), with a smaller number in the more ecologically diverse Teratosphaeriaceae (Crous et al. 2009b). DNA sequence comparisons have revealed that several generic taxa in both families are polyphyletic. For example, the large genus *Mycosphaerella* is proposed to consist of several distantly related species, and some lineages have been proposed to be renamed based on anamorphs, for example, *Ramularia*, *Zymoseptoria*, and *Pseudocercospora* (Crous et al. 2009b; Quaedvlieg et al. 2011). On a family level, the stability of both Mycosphaerellaceae and Teratosphaeriaceae also varies in different phylogenetic analyses with nonoverlapping sample sets. This is evident in Fig. 6.2, where Teratosphaeriaceae can be found with 98 % frequency in bootstrap resampling but Mycosphaerellaceae are only found in 49 %. The opposite trend was true in a previous study that sampled taxa broadly in Capnodiales but only used nuclear ribosomal sequences (Crous et al. 2009a). Using a combined set of ribosomal and protein coding sequences, Schoch et al. (2009) also indicated that families containing the most economically important plant pathogens (Mycosphaerellaceae, Schizothyriaceae) may share common ancestry. This phylogeny suggested that Capnodiales is analogous to Pleosporales in the sense that most plant pathogenic lineages are found in derived nodes with saprobes and RIF dispersed in early diverging lineages.

Several other diverse lineages await comprehensive sampling. The RIF (discussed earlier) contain numerous underdescribed lineages in Capnodiales (Ruibal et al. 2009). Recent studies on the sooty blotch and flyspeck (SBFS) complex that causes blemishes on apple and pear

fruit revealed a comparable level of poorly described complexity (Batzer et al. 2005, 2008; Yang et al. 2010). The rediscovery and analysis of a previously described species, *Torula compniacensis* (now *Baudoinia compniacensis*) (Scott et al. 2007), has also yielded unexpected additions to this group. This is another extremophile member of Teratosphaeriaceae with a high tolerance of ethanol. It is often found growing on surfaces near the vents of distilleries, where it metabolizes ethanol vapors.

Dothideales and Myriangiales. These two orders are well resolved as sister taxa in most modern phylogenetic analyses (Schoch et al. 2006, 2009a). Myriangiales has spherical asci in monascous locules in pseudoparenchyma and consists of two families. Myriangiaceae is saprophytic, often in association with scale insects, while Elsinoaceae is biotrophic, with several species involved in plant disease such as *Elsinoe fawcettii* and *Elsinoe australis*, the causal agents of citrus scab (Chung 2011). Dothideales contains two families, Dothioraceae and Dothideaceae, which remain poorly resolved in DNA-based phylogenies (e.g., Schoch et al. 2009a). Dothideales also contains several so-called black yeast anamorphs (de Hoog and Hermanides-Nijhof 1977) that are only distantly related. One of the most common morphological types in this group is the species in the *Aureobasidium pullulans* complex. It is a genetically diverse set of similar morphological species (Manitchotpisit et al. 2009) that contains producers of pullulan, a promising and commercially valuable polysaccharide currently used for the packaging of food and drugs, among other applications (Singh et al. 2008). *Aureobasidium pullulans* is a cosmopolitan species found on leaves and various surfaces such as concrete, wood, painted walls, and indoor environments. It has also been isolated from extreme environments, including hypersaline waters in salterns, as well as glacial and subglacial ice (Zalar et al. 2008). A comprehensive phylogenetic treatment of this complex remains to be completed, with several genome sequences in preparation (P. Zalar personal communication).

3. Incertae Sedis Lineages

A number of monotypic orders remain unplaced in any subclass. These include Acrospermales, a proposed order based on morphology only (Minter et al. 2007) (Fig. 6.2), with only one species included in Fig. 6.2. Patellariales was proposed in a similar fashion (Hawksworth and Eriksson 1986) and was briefly discussed here under the apothecioid orders Hysteriales and Mytilinidiales. The remaining incertae sedis lineages are covered subsequently in more detail.

Botryosphaeriales. The single-family Botryosphaeriaceae, now containing 2,000 species names, was described in 1918 (Theissen and Sydow 1918). The members of this group generally have pseudoparaphyses present, but they are not persistent and mature specimens may appear to be aparaphysate. Because of this and other intermediate characters, members were initially placed by different authors in either Pleosporales or Dothideales (Kirk et al. 2001; Luttrell 1955). Despite the fact that a new order, Botryosphaeriales, was proposed (Schoch et al. 2006), the latest classwide DNA sequence comparisons can still not support a clear relationship with either Pleosporomycetidae or Dothideomycetidae (Schoch et al. 2009a). The taxonomy of species in this group has been much changed by the use of DNA-based comparisons. This resulted in a much narrower definition of the type genus *Botryosphaeria*. Several new genera have been proposed, such as *Spencermartinsia* and *Barriopsis*, while other groups continue to be referred to by their anamorph names, such as *Dothiorella* and *Diplodia* (Crous et al. 2006; Phillips et al. 2008).

Species in Botryosphaeriales are often isolated as endophytes and can become causal agents of disease when plant hosts become stressed. This suggests that species in this group could be important indicators of climate change (Slippers and Wingfield 2007). Several anamorphs are prominent as disease agents in woody hosts. Anamorph features have traditionally been the most informative to taxonomists. They can be coelomycetes or

hyphomycetes, and conidial pigmentation and development, septation, and morphology have been used to define genera. Several cryptic species continue to be detected relying on molecular methods (e.g., Inderbitzin et al. 2010), and it is clear that, given the amount of undescribed species, an initial single family classification understates the genetic diversity. Several family-level lineages have recently been described, including *Guignardia* and its *Phyllosticta* anamorphs (Phyllostictaceae), species in Saccharataceae, Melanopsaceae (not shown in Fig. 6.2), and Aplosporellaceae (Slippers et al. 2013).

Microthyriaceae, Asterinaceae, and other thyriothecioid fungi. This group of species produces small, shield-shaped, flat structures that develop superficially (thyriothecia) on a broad range of substrates, including living and dead plant surfaces and other fungi. The ascomata can be removed from the substrate surface without any damage and are often visible as black specks resembling insect exudates. Thus they are broadly and indistinctly referred to as fly speck fungi (Hofmann and Piepenbring 2006). Many thyriothecioid species that are epiphytic and biotrophic can be found in the tropics and subtropics. A number of related species that occur as hyperparasites and saprobes also tend to occur in more temperate areas. Traditionally, thyriothecioid species are separated into several families based on ecology and variations in the ascomata, and the largest of these are Asterinaceae and Microthyriaceae. It is thought that Asterinaceae and Microthyriaceae obtain nutrients by penetrating plants with hyphopodia forming specialized structures. An important morphological feature is the upper layer of cells shielding the thyriothecium, called the scutellum. Traditional taxonomy treated the variable ways in which the scutellum opens to release the ascospore as an important character for distinguishing genera and families in these fungi (von Arx and Müller 1975). Asterinaceae opens with star-shaped or irregular fissures while Mycrothyriaceae has a central pore. Kirk et al. (2008) lists Microthyriaceae with Micropeltidaceae and Leptopeltidaceae as members of the order Microthyriales [first introduced by Arnaud (1918)]. It is clear that thyriothecioid species represent a polyphyletic convergent group, but with very few DNA sequences obtained from any species, much of the phylogenetic affinities remain uncertain. The small numbers of molecular characters obtained so far have shown that some members of the thyriothecioid Schizothyriaceae and Micropeltidaceae, for example, *Stomiopeltis versicolor* and *Aulographina pinorum*, should be classified in Capnodiales, likely close to Mycosphaerellaceae (Yang et al. 2010) (Fig. 6.2). A single representative was analyzed for Microthyriaceae, an unverified isolate of *Mycrothyrium microscopicum* (Schoch et al. 2009a) (Fig 6.2). This was placed outside of the main subclasses and sister to Venturiaceae with moderate support. More recently, DNA sequence data were expanded, underlining the **high divergence for thyriotheciate species** with morphologies resembling Microthyriaceae (Wu et al. 2011). Another species in *Stomiopeltis, S. beltulae*, was shown to be likely related to *Mycrothyrium microscopicum*, and species in *Micropeltis, Muyocopron, Neomicrothyrium, Paramicrothyrium*, and *Tothia* were all shown to be related to divergent lineages.

The Asterinaceae was recently placed in Capnodiales (Kirk et al. 2008) but according to recent results should also be placed in a position similar to Microthyriaceae (Hofmann et al. 2010; Wu et al. 2011). Unlike Hofmann et al. (2010), we do not find support for a sister relationship of Asterinaceae with Venturiaceae, although such support is found for Microthyriaceae (Fig. 6.2). In any event, character and taxon sampling requires expansion, and it appears likely that Asterinales (Barr and Huhndorf 2001) could be reinstated in a similar fashion as Microthyriales. Both these taxa contain large numbers of species, mainly segregated on their isolation from various plant hosts, and this will have to be tested in more comprehensive DNA sequence comparisons (Hofmann 2009). A number of remaining families of thyriothecioid taxa occurring on plant leaves still requires DNA sequence data: Brefeldiellaceae, Engerulaceae, Parmulariaceae, Polystomellaceae, and Vizellaceae (Hofmann 2009).

Tubeufiaceae. This family currently contains approximately 32 genera and more than

200 species (Kirk et al. 2008) and was first described by Barr (1979) for species within the Pleosporales that possess superficial, often pallid to bright, perithecioid ascomata, which may darken at maturity. Species in the family can be found in diverse ecological niches and range from being parasites on scale insects and other fungi to growing on rotting plant material, often in freshwater environments. Members of Tubeufiaceae frequently have anamorphs that produce distinctive coiled helicosporous conidia. These can be found in the genera *Helicoma*, *Helicomyces*, and *Helicosporium* (Tsui et al. 2006). Although members of the family have a pleosporalean centrum, initial DNA-sequence-based phylogenies could not place them with certainty within Pleosporales (Kodsueb et al. 2006b), and more comprehensive phylogenies now clearly place them outside of Pleosporomycetidae (Schoch et al. 2009a). They may constitute a new order, but several genera as currently circumscribed remain polyphyletic (Promputtha and Miller 2010; Sánchez et al. 2012; Tsui and Berbee 2006; Tsui et al. 2007), and more descriptive work will be required.

Venturiales and related species. Traditionally, members of Venturiaceae were placed under Pleosporales due to the presence of a hamathecium of pseudoparaphyses that often deliquesces at maturity. The order Venturiales was introduced for the core genera in Venturiaceae with the description of a new family, Sympoventuriaceae (Zhang et al. 2011) (not indicated in Fig. 6.2). Species in Venturiaceae produce ascospores from small ascomata, often with greenish or olivaceous ascospores but sometimes becoming dark brown (Barr 1987). Anamorphs include species in *Fusicladium*, *Spilocaea*, and *Stigmina*. The type species of the family, *Venturia inaequalis*, is the causal agent of apple scab and therefore well studied as a plant pathogen (Bowen et al. 2011). Sympoventuriaceae contains the genera *Sympoventuria* as well as the anamorph-only genera *Veronaeopsis* and *Fusicladium* (Zhang et al. 2011).

The use of DNA-sequence-based phylogenetics has now shown that **the core set of taxa traditionally placed in Venturiaceae resides outside of Venturiales** (Kruys et al. 2006; Winton et al. 2007). Multigene phylogenies confirmed the placement of several other species previously placed in Venturiaceae, for example, *Venturia*, *Protoventuria*, *Metacoleroa*, *Apiosporina*, and *Dibotryon* (Crous et al. 2007a, b; Winton et al. 2007; Zhang et al. 2011). Recent phylogenies have also shown that *Tyrannosorus pinicola*, not previously placed in Venturiaceae, belongs in this family (Zhang et al. 2011). This species produces ascomata with remarkably long, sharp spines and ascospores with multiple germ slits and was isolated from *Pinus* wood (Untereiner et al. 1995). Outside of the Venturiales clade a number of lineages grouped with good statistical support in recent large-scale phylogenies, for example, the coprophilic fungi in Phaeotrichaceae and a single isolate from Microthyriaceae (Schoch et al. 2009a). Shared ancestry with members of Asterinaceae is implied by some studies (Hofmann 2009; Hofmann et al. 2010) but not supported in Fig. 6.2 as well as some recent publications (Zhang et al. 2011). Still, it appears that a core set of lineages surrounding Venturiales may represent an additional subphylum taxon besides Pleosporomycetidae and Dothideomycetidae. More complete sequence sampling including protein coding genes would be required before proposing this taxon with a reasonable amount of confidence.

Trypetheliales, Strigulaceae, and Monoblastiaceae. These three lichenized lineages may represent separate lichenization events in Dothideomycetes, although their phylogenetic placement remains unresolved (Nelsen et al. 2011). The order Trypetheliales is a monotypic order containing approximately 200 species of tropical and subtropical crustose pyrenocarpous lichens. The first unambiguous support for a classification within Dothideomycetes was provided by Del Prado et al. (2006) after initial phylogenetic results by Lutzoni et al. (2004). Subsequently the order was erected using morphological criteria only (Aptroot et al. 2008) and was then corroborated to be distinct from other lineages in Dothideomycetes using DNA sequence comparisons (Nelsen et al. 2009, 2011; Schoch et al. 2009) (Fig. 6.2). Members of this order have variable perithecial morphologies but share a hamathecium of

thin, anastomosing pseudoparaphyses embedded in a stiff gelatinous matrix. Hyaline ascospores with diamond-shaped septa are distinctive for these groups but are often reduced or absent in species with muriform or multiseptate ascospores (Aptroot et al. 2008; Nelsen et al. 2009). In addition, a number of lichenized lineages remain outside of Trypetheliales and placed incertae sedis in Dothideomycetes. They are noted as long branches of uncertain affinity in recent phylogenies but in all likelihood represent clades separate from the nonlichenized dothideomycete orders (Nelsen et al. 2009). Strigulaceae (120 species) and Monoblastiaceae (130 species) are mainly tropical families with a similar ascus type and generally one- or three-septate ascospores (Aptroot et al. 2008; Lücking 2008). One of the most striking shared morphologies for either family is related to their asexual states (when present). Monoblastiaceae contain species with unique pycnidia, but conidia are embedded in a gelatinous matrix, while Strigulaceae has conidia with terminal gelatinous appendices (Nelsen et al. 2009). The most recent phylogenetic analysis showed that nonlichenized saprobic species, *Heleiosa barbatula* and *Funbolia dimorpha*, may also be members of Monoblastiaceae (Nelsen et al. 2011).

B. Arthoniomycetes

Earlier studies of ascomatal ontogenies suggested that Arthoniales was a distinct entity not closely related to the Dothideales or Myriangiales (Henssen and Thor 1994, 1998). A separate class, Arthoniomycetes, was thus described to account for the distinctiveness of this group (Eriksson and Winka 1997). Members of Arthoniomycetes were also included in the first phylogenetic analysis integrating lichenized and nonlichenized fungi (Gargas et al. 1995), and their monophyly was later confirmed (Lutzoni et al. 2001). The class is now generally accepted (Kirk et al. 2008; Lumbsch and Huhndorf 2010), and recent phylogenetic studies solidified the recognition of sister relationship with Dothideomycetes. Nelsen et al. (2009) applied the informal name (rankless taxon) dothideomyceta (introduced in Schoch et al. 2009b) to indicate the common ancestry of Arthoniomycetes and Dothideomycetes. Members of Arthoniomycetes were treated as a monotypic lineage with the sole order Arthoniales until Ertz et al. (2013) described the order Lichenostigmatales as a sister group of Arthoniales. Lichenostigmatales (not shown in Fig. 6.2) contain the lichenicolous fungus *Lichenostigma maureri* (with its *Phaesporobolus* anamorph) and RIF. Typically, the species in Lichenostimatales grow by budding of cells (either representing black yeasts, or species in which conidiomata and ascomata are made of agglomerated spherical cells). This is in sharp contrast to the hyphal growth found in Arthoniales. Ertz et al. (2013) also found that other species of *Lichenostigma* belong to Dothideomycetes and are there grouped with *Lichenothelia*, a genus also containing species representing so-called borderline lichens (Muggia et al. 2013).

The classification within Arthoniomycetes was for a long time based on few phenotypic cardinal characters, such as thallus, ascomata, and ascospore morphology. However, Ertz and Tehler (2011) noticed that **many phenotypic characters in Arthoniomycetes were of limited use in classification**. They interpreted this limitation as a possible result of a long phylogenetic history of adaptation and a slow evolutionary rate (according to the loci so far studied). The hypothesis of slow evolutionary rates was based on the idea that the precursors to *Roccella* were already widely distributed before the split of the Atlantic Ocean in the Mesozoic (Tehler et al. 2009). Some newly discovered relationships are indeed in contrast to previous classification. Growth form was earlier seen as a family-separating character, but now crustose and fruticose members are found in the same genus. As in other large groups of Fungi, the phenotypic flexibility has been severely underestimated, and the notion of slow evolutionary rate may be revised with the analysis of genomic data in the future.

Recent decades have witnessed a trend toward reducing the number of families in Arthoniomycetes, as it has been commonly accepted that the classification concepts of the

so-called Zahlbruckner era were artificial [e.g., Zahlbruckner (1907) accepted nine families]. Grube (1998) presented a review of the order, which comprised only three families: Arthoniaceae, Chrysotrichaceae, and Roccellaceae. This classification used ascus and secondary compound differences to keep the classification as clear and simple as possible. Recent molecular evidence again promotes the expansion of family numbers. The monophyletic Roccellaceae, with more or less cylindrical asci as accepted by Grube (1998), is now split up into several families (Ertz and Tehler 2011). Their phylogenetic study supports Opegraphaceae and Roccellographaceae as families. They also propose Lecanographaceae as an additional family candidate in Arthoniomycetes, although it is still poorly supported as a monophyletic group. The concept of Roccellaceae has become narrow again, and phenotypic recognition of families appears more complicated now (compare Table 1 of Ertz and Tehler 2011).

Arthoniaceae. Arthoniaceae are characterized by globose to clavate asci with strongly thickened apical and side walls. All species develop thalli with crustose shapes, unless they are growing in other lichens or mosses, for example, *Dawsophila*. Clavate asci are known also from Chrysotricacaceae (see below), whereas asci in other families are more cylindrical with thinner side walls. A few representatives with passive spore release have asci with thin to evanescent walls. The type genus *Arthonia* of Arthoniales is the most speciose. Approximately 500 species have been described, although the number may increase with ongoing taxonomic revisions. It is characterized by crustose thalli bearing rounded, maculiform, or lirellate ascomata with poorly developed exciples and transversely septate ascospores (e.g., Coppins and Aptroot 2009; Grube 2007). The transversely septate ascospores of *Arthonia* species were used as a diagnostic character to distinguish the genus *Arthothelium* with muriform ascospores (approximately 80 species). However, the validity of this concept has always been questioned. Apart from the septation of the ascospores, other characters are shared among *Arthonia* and *Arthothelium* species, and some species, which only differ in spore septation, are strikingly similar in all other respects (e.g., Coppins and James 1979; Grube and Giralt 1996). Therefore, Grube (2007) retained several *Arthothelium* species in *Arthonia*. However, recent results suggest that the phenotypically distinct genus *Cryptothecia*, usually kept in a separate family, Cryptotheciaceae, is nested in a group comprising *Arthonia* and *Arthothelium*. *Cryptothecia* is characterized by poorly delimited ascomatal aggregates and dispersed, globose asci. Recent data show that *Cryptothecia* is not monophyletic either (Frisch et al. 2014). In comparison with other families in Arthoniomycetes, sampling is still limited in the early diverging groups consisting of the unresolved genera *Arthonia* and *Arthothelium*, and major rearrangements are expected. Current data indicate two major clades in Arthoniaceae, one containing lichenized and lichenicolous species with generally blackish, adnate, moderately to strongly convex ascomata, and another clade, which is morphologically more diverse and includes the genera *Cryptothecia*, *Stirtonia*, and *Herpothallon* (Frisch et al. 2014).

The ascomatal structures of Arthoniaceae display a high degree of phenotypic heterogeneity, especially within the bulk genus *Arthonia*. For example, some tropical *Arthonia* and *Arthothelium* species with red ascomatal pigments and hydrophobic ascomata were placed in the genus *Coniarthonia* (Grube 2001b). The new genus *Crypthonia* (Frisch and Thor 2010) lacks such red pigments but also has hydrophobic fruit bodies and is related to *Herpothallon* (Frisch et al. 2014).

With a broader sampling, characters of ascomatal construction, for example hyphal textures and locule arrangement, may appear as phenotypic markers of phylogenetic relationships in Arthoniaceae. Arthoniaceae also includes *Tylophoron*, a peculiar genus with mazaediate ascomata and passive spore dispersal (Nelsen et al. 2009). Recent data show that the sterile, sporodochia-producing genus *Blarneya* is included in *Tylophoron* (Ertz et al. 2011). Additionally, a lichenicolous coelomycete, which could be grown in pure culture, was recently classified in Arthoniaceae. The new genus *Briancoppinsia* was introduced for

a species previously classified in *Phoma* (Diederich et al. 2011), a coelomycete genus usually associated with members of Dothideomycetes. Molecular data have also classified the hyphomycete *Reichlingia* as a member of the Arthoniaceae (Ertz and Tehler 2011). This species was earlier interpreted as a lichenicolous fungus on an unidentified host but apparently represents the interesting case of a lichenized hyphomycete related to *Arthonia*.

Chrysotrichaceae. Previously, Chrysotrichaceae were principally distinguished from other families in Arthoniomycetes by bright yellow pigments, both in the thallus and in the ascomata (due to production of pulvinic acid derivates). Moreover, all species in Chrysotrichaceae associate with coccal green algae and have crustose thalli. Therefore, it came as a surprise when Nelsen et al. (2009) demonstrated that *Arthonia caesia* with pruinose, but not yellow, ascomata grouped together with *Chrysothrix*. However, this placement also agreed with the presence of coccal green photobionts. Despite the presence of coccal green photobionts, the placement of *Arthonia medialla* in Chrysotricaceae is more surprising because this species has only dark, melaninlike pigments in the cell walls (Frisch et al. 2014). Nevertheless, present data strongly support Chrysotrichaceae as a separate family and as a sister group to all remaining Arthoniomycetes (Ertz and Tehler 2011; Nelsen et al. 2009). Future work will show whether further species currently placed in *Arthonia* might actually represent lineages in or near Chrysotrichaceae. This is at least the case with *Arthonia leucopellaea*, a species with distinct ascomata, atypical for Arthoniaceae (Frisch et al. 2014).

Lecanographaceae. Ertz and Tehler (2011) proposed Lecanographaceae as a family that is comprised of the genera *Zwackhia* and *Alyxoria* (with species previously assigned to *Opegrapha*) with the genus *Lecanographa*. *Zwackhia* and *Alyxoria* have asci with slightly different hemiamyloid (blue in alkaline Lugol's solution) internal staining, comprising the Vulgata and Varia types, respectively (whereas the Grumulosa type is found in *Lecanographa*). This family also includes the lichenicolous genera *Plectocarpon* and *Phacographa* (Frisch et al. 2014).

Opegraphaceae. Recent phylogenetic studies suggest a substantial reclassification of Opegraphaceae. *Opegrapha* was the second largest genus in Arthoniales, and traditionally recognized by distinctly developed, often elongate and dark colored exciples (outer layers). In their three-gene phylogeny study focusing on Opegraphaceae, Ertz et al. (2009) found that *Opegrapha atra* and *O. calcarea* are in fact closely related to the type species of *Arthonia* and were thus transferred to this genus. This placement is supported by similarities in asci, spores, and pigments. Other *Opegrapha* species are now placed in Lecanographaceae (e.g., in *Zwackhia* and *Alyxoria*). *Paralecanographa*, *Paraschismatomma*, *Paraingaderia*, and *Sparria* (for *Arthonia endlicheri* and *Sclerophyton cerebriforme*) are new genera in this family.

Roccellographaceae. This small family, described by Ertz and Tehler (2011), comprises—so far—crustose and subfruticose species. It includes the new genera *Dimidiographa* and *Fulvophyton*, in addition to the known genus *Roccellographa* (including *Sclerophyton muriforme* and *Peterjamesia circumscripta*).

Roccellaceae. The concept of Roccellaceae has significantly changed, and this family includes crustose and fruticose representatives of quite different phenotypic appearance. The monophyletic grouping of *Enterographa* and *Erythrodecton* represents a basal lineage in the family according to Ertz and Tehler (2011). *Enterographa* is now accepted in a narrower sense than before (Ertz et al. 2009). Interestingly, *Dendrographa*, originally a fruticose lichen, includes several newly transferred crustose species of other genera (of *Schismatomma* and *Roccellina*, as well as the sterile lichen *Lecanactis latebrarum*) (Ertz & Tehler 2011).

The family and genus concept in Arthoniomycetes has been changing significantly following the most recent phylogenetic studies, and accepted genera and families, which turned out to be paraphyletic, are currently being restructured. So far, the emerging

concept is hardly reflected by phenotypic cardinal characters. Moreover, the splitting of families raises the question of whether more families should be accepted for basal clades comprising phenotypically rather distinct members of currently accepted families. Since a considerable number of phenotypically enigmatic species have not yet been included in molecular phylogenies, further higher-level classification should be undertaken with caution. Some of these species represent new genera; for example, the monotypic genus *Phoebus* has opegraphoid ascomata and thalli with an orange quinoid pigment (Harris and Ladd 2007). A serious drawback of the current concept is the poor representation of lichenicolous fungi so far, although some of them are described as phenotypically distinct genera (e.g., *Phacothecium*, *Arthophacopsis*, *Paradoxomyces*, *Perigrapha*, *Phacographa*, *Plectocarpon*). Recent analyses suggest that the lichen-parasitic lifestyle has emerged multiple times in Arthoniales and are so far found in four lineages within Arthoniaceae (Frisch et al. 2014).

Two aspects of the evolutionary biology of Arthoniomycetes should finally be mentioned. The first concerns the species-pair concept, i.e., two closely related species with vegetative and sexual dispersal strategies. This concept was originally derived from observations on the geographic distribution of closely related (foliose) species in Lecanoromycetes, where the primary sexual species have usually restricted ranges and the vegetative secondary species is widespread (Poelt 1970). Such a concept has not been confirmed in Arthoniomycetes (Tehler et al. 2009). The dispersal mode is modulated by other processes but not sufficient to describe species. On the other hand, rapid radiation has been suggested for the *Roccella galapagoensis* group and a high fraction of endemic taxa in Roccellaceae (Tehler et al. 2009). Geographically limited ranges of species are also found in *Arthonia* (Ertz et al. 2010; Grube and Lendemer 2009; Grube et al. 2004). It remains to be tested whether widespread phenotypically recognized species in Arthoniales (and particularly polymorphic *Arthonia* species) actually represent swarms of multiple species the is expected to be characterized by molecular data.

V. Maintenance and Culture

The majority of saprobic and plant-pathogenic species in Dothideomycetes can be cultured on synthetic media using techniques commonly used in other fungi (Jong and Birmingham 2001). Marine species, such as members of Aigialaceae and Morosphaeriaceae, often require the extra addition of seawater or aquarium salt and trace elements to grow optimally (Kohlmeyer and Kohlmeyer 1979). Other species, growing in extreme environments, such as members of Teratosphaeriaceae, may require additions to growth media, reflecting their growth habit. For example, primary isolations of the *Baudoinia compniacensis* from habitats rich in alcohol vapors required the addition of 5 ppm ethanol (Scott et al. 2007). However, several large groups of species remain poorly represented in culture collections. One such group of species, commonly referred to as sooty molds, only rarely grows in axenic culture. As mentioned earlier, a large number of these species appear to reside in Capnodiaceae. Other diverse groups with poor representation in culture collections and DNA databases include the biotrophic Asterinaceae, Microthyriaceae, and Parmulariaceae (Hofmann 2009; Wu et al. 2011).

Lichenized species of Dothideomycetes and Arthoniomycetes are also poorly represented in public culture collections, partly due to the problems in initiating growth. Generally, axenic cultures of lichenized fungi are generated either from ejected (asco)spores or from tiny fragments of surface-sterilized thalli (after homogenizing the thalli or by microdissection from thalli), both of which are maintained on standard media. Ertz et al. (2009) made use of multispore cultures of Arthoniales to avoid the fairly common contaminations by other fungi, which co-occur with the species with inconspicuous, bark-inhabiting thalli. Growth of lichenized fungi can be improved when

their natural ecology is mimicked, for example, by supplying polyols to media. Some useful hints on culturing lichen fungi are found in Stocker-Wörgötter and Hager (2008) and references therein.

Often the **lack of robust cultures translates into poor representation of reliable sequences** in public DNA databases. However, techniques to isolate DNA from herbarium and fresh specimens are steadily improving. Success from old, but well-preserved, specimens are encouraging, and examples include sequences obtained from a more than 35-year-old *Didymella* species (Zuccaro et al. 2008) and a *Septoria* specimen of more than a century old (Quaedvlieg et al. 2011). Nevertheless, **the need for having well-represented DNA databases tied to well-vouchered specimens remains compelling** (Pleijel et al. 2008). Several recent efforts to extend this include epitypification (Hyde and Zhang 2008) and DNA barcoding (Begerow et al. 2010; Schoch et al. 2012).

VI. Conclusions

Dothideomyceta represents a well-resolved node on the fungal tree of life, still with some level of uncertainty as to which class is its nearest neighbor (Schoch et al. 2009b). Members of this group, consisting of sister classes Dothideomycetes and Arthoniomycetes, **share distinctive morphological characters regarding their asci and ascomata** but **clear differences in their ascoma, development, and major ecological roles**. The **classification for these organisms remains in a transitory state,** where several concepts proposed by earlier taxonomists relying on morphological, biochemical, and developmental comparisons are still being tested and adapted to correlate with phylogenetic hypotheses determined by comparisons of molecular characters.

As the phylogenetic resolution improves, researchers can also begin to investigate the evolutionary history of this group. Dothideomyceta have been recognized infrequently in the fossil record, and one of the oldest known fossils likely belongs to Pleosporales from the Eocene (55–35 MYA) (Mindell et al. 2007). Several sooty mold species with likely members in Capnodiales have also been described from amber older than 100 million years ago (Schmidt et al. 2014). Calibrated molecular clock analyses point to a much older origin for the shared ancestor of Dothideomycetes and Arthoniomycetes. Although fossil calibration points remain scarce and the interpretation of their placement can be contentious, a recent paper hypothesized a late Devonian origin (Gueidan et al. 2011). Much remains uncertain about the ancestral ecology that gave rise to these two classes, but **RIF** exhibit extremely high diversity in dothideomyceta, and, being highly tolerant to stress, they **may represent an ancient pool of diversity** giving rise to variable ecologies in extant lineages. This will have to be tested further with densely sampled phylogenies and well-documented biological data. Another important question to ponder will be how evolution shapes morphological convergence and plasticity. As mentioned earlier in this review, **several morphologies are shared with members of Eurotiomycetes,** and it remains challenging to place some species in either class using only morphology (Rossman et al. 2010).

Finally, it is hoped that massive environmental sampling, barcoding, and comparative genomics will rapidly fill in several gaps in our knowledge of these fungi. Recent comparative genomics studies are already uncovering a potentially novel system of chromosomal regulation unique to filamentous ascomycetes (Pezizomycotina). This is especially striking in Dothideomycetes. Mesosynteny (designated to distinguish it from micro- and macrosynteny) indicates a pattern where genes are conserved within homologous chromosomes, but with randomized orders and orientations (Hane et al. 2011; Ohm et al. 2012). Additionally, a recent 18-genome comparison of Dothideomycetes found differences in gene numbers of Pleosporales and Capnodiales, despite a constant set of core genes (Ohm et al. 2012). Several of these **genes involved in pathogenesis are positioned close to areas enriched in transposable elements** (Ohm et al. 2012). Other potential evolutionary mechanisms to be investigated

with expanded genome data include the **horizontal transfer** of genetic material (Richards 2011).

It should be obvious that continued study in dothideomyceta, with its huge genetic and ecological diversity, has tremendous potential. A focus on the addition of data from understudied saprobic and lichenized species will enrich comparative studies and continue to inform our concepts of fungal ecology, evolution, and biology.

Acknowledgements We appreciate the photos provided by Sabine Huhndorf (Field Museum) and Walter Obermayer (Karl-Franzens-University). We thank the Centraalbureau voor Schimmelcultures (CBS) and Studies in Mycology for allowing us to use illustrations from the 1975 publication by J. von Arx and E. Müller. Permission to use an adaptation from an illustration presented in the Ph.D. thesis of Tina Hoffman (Johann Wolfgang Goethe-University) is also appreciated. CLS acknowledges the Intramural Research Program of the National Institutes of Health, National Library of Medicine. We would like to thank Göran Thor (Swedish University of Agricultural Sciences) and Barbara Robbertse (NCBI) for critical readings of the manuscript.

References

Aptroot A, Lücking R, Sipman HJM, Umaña L, Chaves JL (2008) Pyrenocarpous lichens with bitunicate asci: a first assessment of the lichen biodiversity inventory of Costa Rica. Biblioth Lichenol 97:1–162

Arnaud G (1918) Lés Asterinées. Annals d'École National d'Agriculture de Montpellier série 2 16:1–288

Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauff F, Lutzoni F (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst Biol 58:283–297

Arzanlou M, Groenewald JZ, Fullerton RA, Abeln ECA, Carlier J, Zapater MF, Buddenhagen IW, Viljoen A, Crous PW (2008) Multiple gene genealogies and phenotypic characters differentiate several novel species of *Mycosphaerella* and related anamorphs on banana. Persoonia 20:19–37

Aveskamp MM, de Gruyter J, Woudenberg JH, Verkley GJ, Crous PW (2010) Highlights of the Didymellaceae: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60

Barr ME (1979) A classification of Loculoascomycetes. Mycologia 71:935–957

Barr ME (1987) Prodromus to class Loculoascomycetes. The Author, Amherst, MA

Barr ME (1990) Melanommatales (Loculoascomycetes). North American Flora Series II 13:1–129

Barr ME, Huhndorf SM (2001) Loculoascomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol VII, Part A: systematics and evolution. Springer, Berlin, pp 161–177

Batzer JC, Gleason ML, Harrington TC, Tiffany LH (2005) Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. Mycologia 97:1268–1286

Batzer JC, Arias MM, Harrington TC, Gleason ML, Groenewald JZ, Crous PW (2008) Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. Mycologia 100:246–258

Bellemère A (1994) Asci and ascospores in ascomycete systematics. In: Hawksworth DL (ed) Ascomycete systematics: Problems and Perspectives in the nineties. Plenum, New York, NY, pp 111–126

Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. Appl Microbiol Biotechnol 87:99–108

Berbee ML (1996) Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470

Berbee ML, Taylor JW (1992) Two Ascomycete classes based on fruiting-body characters and ribosomal DNA sequence. Mol Biol Evol 9:278–284

Boehm EW, Mugambi GK, Miller AN, Huhndorf SM, Marincowitz S, Spatafora JW, Schoch CL (2009a) A molecular phylogenetic reappraisal of the Hysteriaceae, Mytilinidiaceae and Gloniaceae (Pleosporomycetidae, Dothideomycetes) with keys to world species. Stud Mycol 64:49–83

Boehm EWA, Schoch CL, Spatafora JW (2009b) On the evolution of the Hysteriaceae and Mytilinidiaceae (Pleosporomycetidae, Dothideomycetes, Ascomycota) using four nuclear genes. Mycol Res 113:461–479

Boustie J, Grube M (2005) Lichens, a promising source of bioactive secondary metabolites. Plant Genet Resour 3:273–287

Bowen JK, Mesarich CH, Bus VGM, Beresford RM, Plummer KM, Templeton MD (2011) *Venturia inaequalis*: the causal agent of apple scab. Mol Plant Pathol 12:105–122

Braun U, Crous PW, Dugan F, Groenewald JZ, de Hoog GS (2003) Phylogeny and taxonomy of *Cladosporium*-like hyphomycetes, including *Davidiella* gen. nov., the teleomorph of *Cladosporium* s. str. Mycol Prog 2:3–18

Campbell J, Ferrer A, Raja HA, Sivichai S, Shearer C (2007) Phylogenetic relationships among taxa in the Jahnulales inferred from 18S and 28S nuclear ribosomal DNA sequences. Can J Bot 85:873–882

Chomnunti P, Schoch C, Aguirre-Hudson B, Ko-Ko T, Hongsanan S, Jones E, Kodsueb R, Phookamsak R,

Chukeatirote E, Bahkali A, Hyde K (2011) Capnodiaceae. Fung Divers 51:1–32
Chomnunti P, Ko TWK, Chukeatirote E, Hyde KD, Cai L, Jones EBG, Kodsueb R, Hassan BA, Chen H (2012) Phylogeny of Chaetothyriaceae in northern Thailand including three new species. Mycologia 104:382–395
Chung KR (2011) *Elsino fawcettii* and *Elsino australis*: the fungal pathogens causing citrus scab. Mol Plant Pathol 12:123–135
Cole GT, Sampson RA (1979) Patterns of development in conidial fungi, 2 vols. Academic, New York
Corlett M (1967) The developmental morphology of *Clathrospora heterospora* var. *simmonsii*. Can J Bot 45:221–227
Coppins B, Aptroot A (2009) *Arthonia*. In: Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW, Wolseley PA, Smith CW (eds) The lichens of Great Britain and Ireland. British Lichen Society, UK, pp 1–47
Coppins BJ, James PW (1979) New or interesting British lichens III. Lichenologist 11:27–45
Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006) Phylogenetic lineages in the Botryosphaeriaceae. Stud Mycol 55:235–253
Crous PW, Braun U, Groenewald JZ (2007a) *Mycosphaerella* is polyphyletic. Stud Mycol 58:1–32
Crous PW, Schubert K, Braun U, de Hoog GS, Hocking AD, Shin H-D, Groenewald JZ (2007b) Opportunistic, human-pathogenic species in the Herpotrichiellaceae are phenotypically similar to saprobic or phytopathogenic species in the Venturiaceae. Stud Mycol 58:185–217
Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, de Hoog GS, Groenewald JZ (2009a) Phylogenetic lineages in the Capnodiales. Stud Mycol 64:17–47
Crous PW, Summerbell RC, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andjic V, Barber PA, Groenewald JZ (2009b) Unravelling *Mycosphaerella*: do you believe in genera? Persoonia 23:99–118
Currey F (1856) Original communications: on the reproductive organs of certain fungi, with some remarks on germination. Q J Microsc Sci s1-4:192–200
De Bary A (1887) Comparative morphology and biology of the fungi, mycetozoa, and bacteria. Clarendon Press, Oxford
de Gruyter J, Aveskamp MM, Woudenberg JHC, Verkley GJM, Groenewald JZ, Crous PW (2009) Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. Mycol Res 113:508–519
de Gruyter J, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW (2012) Redisposition of *Phoma*-like anamorphs in Pleosporales. Stud Mycol 75:1–36
de Wit PJGM, Mehrabi R, van den Burg HA, Stergiopoulos I (2009) Fungal effector proteins: past, present and future. Mol Plant Pathol 10:735–747
Del Prado R, Schmitt I, Kautz S, Palice Z, Lücking R, Lumbsch HT (2006) Molecular data place Trypetheliaceae in Dothideomycetes. Mycol Res 110:511–520
De los Ríos A, Grube M (2000) Host-parasite interfaces of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). Mycol Res 104:1348–1353
Desmazières JBHJ (1843) Dixième notice sur quelques plantes cryptogames. Ann Sci Nat Bot Sér 2 19:335–373
Diederich P, Lawrey JD, Sikaroodi M, van den Boom PPG, Ertz D (2011) *Briancoppinsia*, a new coelomycetous genus of Arthoniaceae (Arthoniales) for the lichenicolous *Phoma cytospora*, with a key to this and similar taxa. Fung Divers 52:1–12
Eriksson OE (1981) The families of bitunicate ascomycetes. Opera Bot 60:1–220
Eriksson OE, Winka K (1997) Supraordinal taxa of Ascomycota. Myconet 1:1–16
Ertz D, Tehler A (2011) The phylogeny of Arthoniales (Pezizomycotina) inferred from nucLSU and RPB2 sequences. Fung Divers 49:47–71
Ertz D, Elix J, Grube M (2010) *Arthonia borbonica* (Ascomycota, Arthoniales), a new species from La Réunion. Plant Ecol Evol 143:222–224
Ertz D, Bungartz F, Diederich P, Tibell L (2011) Molecular and morphological data place *Blarneya* in *Tylophoron* (Arthoniaceae). Lichenologist 43:345–356
Ertz D, Lawrey JD, Common RS, Diederich P (2013) Arthoniomycetes sister to the primarily lichenized Arthoniales and composed of black yeasts, lichenicolous and rock-inhabiting species. Fung Divers 66:113–137
Ertz D, Miadlikowska J, Lutzoni F, Dessein S, Raspé O, Vigneron N, Hofstetter V, Diederich P (2009) Towards a new classification of the Arthoniales (Ascomycota) based on a three-gene phylogeny focusing on the genus *Opegrapha*. Mycol Res 113:141–152
Fries EM (1818) Observationes mycologicae. Gerhard Bonnier, Copenhagen
Frisch A, Thor G, Ertz D, Grube M (2014) The Arthonialean challenge: restructuring Arthoniaceae. Taxon 63:727–744
Frisch A, Thor G (2010) *Crypthonia*, a new genus of byssoid Arthoniaceae (lichenised Ascomycota). Mycol Prog 9:281–303
Fuckel L (1870) Symbolae Mycologicae. Beitrage zur Kenntniss der rheinischen Pilze, Wiesbaden 1870:329–330
Funk A, Shoemaker RA (1967) Layered structure in the bitunicate ascus. Can J Bot 45:1265–1266
Furtado JS, Olive LS (1971) Ascospore discharge and ultrastructure of the ascus in *Leptospharulina australis*. Nova Hedw 19:799–824
Gargas A, DePriest PT, Grube M, Tehler A (1995) Multiple origins of lichen symbioses in Fungi suggested by SSU rDNA phylogeny. Science 268:1492–1495

Greif MD, Gibas CFC, Tsuneda A, Currah RS (2007) Ascoma development and phylogeny of an apothecioid dothideomycete, *Catinella olivacea*. Am J Bot 94:1890–1899

Grube M (1998) Classification and phylogeny in the Arthoniales. Bryologist 101:377–391

Grube M (2001a) *Sporostigma* gen. nov., another calicioid genus in Arthoniales. Lichenologist 33:78–391

Grube M (2001b) *Coniarthonia* gen. a new genus of arthonioid lichens. Lichenologist 33:491–502

Grube M (2007) *Arthonia*. In: Nash TH III, Gries C, Bungartz F (eds) Lichen flora of the Greater Sonoran Desert region, vol 3, Lichens Unlimited. Arizona State University, Tempe, AZ, pp 39–61

Grube M, Giralt M (1996) Studies on some species of *Arthothelium* occurring in the western Mediterranean. Lichenologist 28:15–36

Grube M, Hafellner J (1990) Studien an flechtenbewohnenden Pilzen der Sammelgattung *Didymella* (Ascomycetes, Dothideales). Nova Hedw 51:283–360

Grube M, Lendemer JC (2009) *Arthonia rubrocincta*: belated validation of a name for a common species endemic to Sabal palmetto in the southeastern United States. Opusc Philolich 7:7–12

Grube M, Lücking R (2001) Ascogenous hyphae in foliicolous species of *Arthonia* and allied genera. Mycol Res 105:1007–1013

Grube M, Matzer M (1997) Taxonomic concepts of lichenicolous *Arthonia* species. Biblioth Lichenol 68:1–17

Grube M, Lücking R, Umana-Tenorio L (2004) A new, isidiate species of *Arthonia* (Ascomycota: Arthoniaceae) from Costa Rica. Mycologia 96:1159–1162

Gueidan C, Ruibal C, de Hoog S, Schneider H (2011) Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. Fung Biol 115:987–996

Hane J, Rouxel T, Howlett B, Kema G, Goodwin S, Oliver R (2011) A novel mode of chromosomal evolution peculiar to filamentous Ascomycete fungi. Genome Biol 12:R45

Harris RC, Ladd D (2007) New taxa of lichens and lichenicolous fungi from the Ozark ecoregion. Opusc Philolich 4:57–68

Hawksworth DL (1985) Problems and prospects in the systematics of the Ascomycotina. Proc Indian Acad Sci 94:319–339

Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. MycoKeys 1:7–20

Hawksworth DL (2012) Managing and coping with names of pleomorphic fungi in a period of transition. IMA Fungus 3:15–24

Hawksworth DL, Eriksson OE (1986) The names of accepted orders of Ascomycetes. Systema Ascomycetum 5:175–184

Henssen A, Jahns HM (1974) Lichenes. Eine Einführung in die Flechtenkunde. Georg Thieme, Stuttgart

Henssen A, Thor G (1994) Developmental morphology of the "Zwischengruppe" between Ascohymeniales and Ascoloculares. In: Hawksworth DL (ed) Ascomycete systematics. Problems and perspectives in the nineties. Plenum, New York, pp 43–61

Henssen A, Thor G (1998) Studies in taxonomy and developmental morphology in *Chiodecton*, *Dichosporidium*, *Erythrodecton* and the new genus *Pulvinodecton* (Arthoniales, lichenized Ascomycetes). Nordic J Bot 18:95–120

Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, Lutzoni F (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Mol Phylogenet Evol 42:543–555

Hofmann TA (2009) Plant parasitic Asterinaceae and Microthyriaceae from the Neotropics (Panama). PhD thesis, Faculty of Biological Sciences, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Hofmann TA, Piepenbring M (2006) New records and host plants of fly-speck fungi from Panama. Fung Divers 22:55–70

Hofmann TA, Kirschner R, Piepenbring M (2010) Phylogenetic relationships and new records of Asterinaceae (Dothideomycetes) from Panama. Fung Divers 43:39–53

Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F (2007) Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). Mol Phylogenet Evol 44:412–426

von Höhnel F (1909) Fragmente zur Mykologie IV No. 244. Revision der Myriangiaceen und der Gattung Saccardia. Sitz-berr Kaiserl Akad Wiss Wien 118:349–376

de Hoog GS, Hermanides-Nijhof EJ (1977) The black yeasts and allied Hyphomycetes. Stud Mycol 15:1–222

Hughes SJ (1976) Sooty molds. Mycologia 48:693–820

Hyde KD, Wong SW (1999) Tropical Australian freshwater fungi. XV The ascomycete genus *Jahnula*, with five new species and one new combination. Nova Hedw 68:489–509

Hyde KD, Zhang Y (2008) Epitypification: should we epitypify? J Zhejiang Univ Sci B 9:842–846

Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Bhat DJ,

Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, De Hoog S, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yacharoen S, Yan JY, Zhang M (2013) Families of Dothideomycetes. Fung Divers 63:1–313

Inderbitzin P, Bostock RM, Trouillas FP, Michailides TJ (2010) A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia 102:1350–1368

Ingold CT (1933) Spore discharge in the Ascomycetes. New Phytol 32:175–196

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fung Divers 35:1–187

Jong S-C, Birmingham JM (2001) Cultivation and preservation of fungi in culture. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol VII, Part B: Systematics and evolution. Springer, Berlin, pp 193–202

Kirk P, Cannon P, David J, Stalpers J (2001) Ainsworth and Bisby's dictionary of the fungi, 9th edn. CAB International, Wallingford, UK

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Ainsworth and Bisby's dictionary of the fungi, 10th edn. CAB International, Wallingford, UK

Kirschstein V (1936) Beiträge zur Kenntnis der Ascomyceten und ihrer Nebenformen besonders aus der Mark Brandenburg und dem Bayerischen Walde. Ann Mycol 34:180–210

Kodsueb R, Dhanasekaran V, Aptroot A, Lumyong P, McKenzie EHC, Hyde KD, Jeewon R (2006a) The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. Mycologia 98:571–583

Kodsueb R, Jeewon R, Vijaykrishna D, McKenzie EHC, Lumyong P, Lumyong S, Hyde KD (2006b) Systematic revision of Tubeufiaceae based on morphological and molecular data. Fung Divers 21:105–130

Kruys Å, Wedin M (2009) Phylogenetic relationships and an assessment of traditionally used taxonomic characters in the Sporormiaceae (Pleosporales, Dothideomycetes, Ascomycota), utilizing multigene phylogenies. Syst Biodiver 7:465–478

Kruys Å, Eriksson OE, Wedin M (2006) Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. Mycol Res 110:527–536

Kohlmeyer J, Kohlmeyer E (1979) Marine mycology: the higher fungi. Academic, New York

Lawrey J, Diederich P (2003) Lichenicolous fungi: interactions, evolution, and biodiversity. Bryologist 106:81–120

Lawrey JD, Diederich P, Nelsen MP, Sikaroodi M, Gillevet PM, Brand AM, Van den Boom P (2011) The obligately lichenicolous genus *Lichenoconium* represents a novel lineage in the Dothideomycetes. Fung Biol 115:176–187

Lawrey JD, Diederich P, Nelsen MP, Freeburg C, van den Broeck D, Sikaroodi M, Ertz D (2012) Phylogenetic placement of lichenicolous *Phoma* species in the Phaeosphaeriaceae (Pleosporales, Dothideomycetes). Fung Divers 55:195–213

Liew EC, Aptroot A, Hyde KD (2000) Phylogenetic significance of the pseudoparaphyses in Loculoascomycete taxonomy. Mol Phylogenet Evol 16:392–402

Lindau G (1897) Pyrenomycetineae. In: Engler A, Prantl K (eds) Die natürlichen Pflanzenfamilien. Wilhelm Engelmann, Leipzig, Germany

Liu K, Raghavan S, Nelesen S, Linder CR, Warnow T (2009) Rapid and accurate large-scale coestimation of sequence alignments and phylogenetic trees. Science 324:1561–1564

Liu YJ, Hall BD (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proc Natl Acad Sci U S A 101:4507–4512

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808

LoBuglio KF, Berbee ML, Taylor JW (1996) Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among the ascomycetes. Mol Phylogenet Evol 6:287–294

Lücking R (1995) Additions and corrections to the foliicolous lichen flora of Costa Rica (Central America). I. The family Arthoniaceae, with notes on the genus *Stirtonia*. Lichenologist 27:127–153

Lücking R (2008) Foliicolous lichenized fungi. Flora Neotropica Monograph 103:1–873

Lumbsch HT, Huhndorf S (2007) Whatever happened to the pyrenomycetes and loculoascomycetes? Mycol Res 111:1064–1074

Lumbsch HT, Huhndorf S (2010) Myconet volume 14. Part one. Outline of Ascomycota 2009. Part two.

notes on Ascomycete systematics 4751–5113. Fieldiana 1:1–64

Lumbsch HT, Lindemuth R (2001) Major lineages of Dothideomycetes (Ascomycota) inferred from SSU and LSU rDNA sequences. Mycol Res 105:901–908

Lumbsch HT, Lindemuth R, Schmitt I (2000) Evolution of filamentous ascomycetes inferred from LSU rDNA sequence data. Plant Biol 5:525–529

Lumbsch HT, Schmitt I, Lindemuth R, Miller A, Mangold A, Fernandez F, Huhndorf S (2005) Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (Leotiomyceta). Mol Phylogenet Evol 34:512–524

Lumbsch HT, Lücking R, Tibell L (2009) Molecular data place *Tylophoron* as an additional calicioid genus in the Arthoniales (Ascomycota). Biblioth Lichenol 99:285–296

Luttrell ES (1951) Taxonomy of Pyrenomycetes. Univ Missouri Stud 24:1–120

Luttrell ES (1955) The ascostromatic Ascomycetes. Mycologia 47:511–532

Luttrell ES (1965) Paraphysoids, pseudoparaphyses, and apical paraphyses. Trans Br Mycol Soc 48:135–144

Luttrell ES (1973) Loculoascomycetes. Academic, New York

Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lucking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Am J Bot 91:1446–1480

Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD (2012) A phylogenetic and taxonomic re-evaluation of the *Bipolaris–Cochliobolus–Curvularia* Complex. Fung Divers 56:131–144

Mangenot F, Reisinger O (1976) Form and function of conidia as related to their development. In: Weber DJ, Hess WM (eds) The fungal spore: form and function. Wiley, New York, pp 789–846

Manitchotpisit P, Leathers TD, Peterson SW, Kurtzman CP, Li XL, Eveleigh DE, Lotrakul P, Prasongsuk S, Dunlap CA, Vermillion KE, Punnapayak H (2009) Multilocus phylogenetic analyses, pullulan production and xylanase activity of tropical isolates of *Aureobasidium pullulans*. Mycol Res 113:1107–1120

Mayer KM, Ford J, Macpherson GR, Padgett D, Volkmann-Kohlmeyer B, Kohlmeyer J, Murphy C, Douglas SE, Wright JM, Wright JL (2007) Exploring the diversity of marine-derived fungal polyketide synthases. Can J Microbiol 53:291–302

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov 2010, New Orleans, pp 1–8

Mindell RA, Stockey RA, Beard G, Currah RS (2007) *Margaretbarromyces dictyosporus* gen. sp nov.: a permineralized corticolous ascomycete from the Eocene of Vancouver Island, British Columbia. Mycol Res 111:680–684

Minter D, Peredo H, Watson A (2007) *Acrospermum chilense* sp. nov. from Chile and Acrospermales ord. nov. Bol Soc Argent Bot 42:107–112

Mugambi GK, Huhndorf SM (2009a) Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae re-circumscribed (Pleosporomycetidae, Dothideomycetes, Ascomycota). Stud Mycol 64:103–121

Mugambi GK, Huhndorf SM (2009b) Parallel evolution of hysterothecial ascomata in ascolocularous fungi (Ascomycota, Fungi). Syst Biodiver 7:453–464

Muggia L, Hafellner J, Wirtz N, Hawksworth DL, Grube M (2008) The sterile microfilamentous lichenized fungi *Cystocoleus ebeneus* and *Racodium rupestre* are relatives of plant pathogens and clinically important dothidealean fungi. Mycol Res 112:50–56

Muggia L, Gueidan C, Knudsen K, Perlmutter G, Grube M (2013) The lichen connections of black fungi. Mycopathologia 175:523–535

Müller E (1981) The bitunicate ascus. In: Reynolds DR (ed) Ascomycete systematics: the Luttrellian concept. Springer, New York, pp 49–53

Nannfeldt JA (1932) Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. . Nova Acta Regiae Soc Sci Upsal/ Almqvist and Wiksells, Uppsala, Sweden

Nelsen MP, Lücking R, Grube M, Mbatchou JS, Muggia L, Rivas Plata E, Lumbsch HT (2009) Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta. Stud Mycol 64:135–144

Nelsen MP, Lücking R, Mbatchou JS, Andrew CJ, Spielmann AA, Lumbsch HT (2011) New insights into relationships of lichen-forming Dothideomycetes. Fung Divers 51:155–162

Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, Barry KW, Condon BJ, Copeland AC, Dhillon B, Glaser F, Hesse CN, Kosti I, LaButti K, Lindquist EA, Lucas S, Salamov AA, Bradshaw RE, Ciuffetti L, Hamelin RC, Kema GHJ, Lawrence C, Scott JA, Spatafora JW, Turgeon BG, de Wit PJGM, Zhong S, Goodwin SB, Grigoriev IV (2012) Diverse lifestyles

and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes Fungi. PLoS Pathog 8:e1003037

Onofri S, Selbmann L, Zucconi L, de Hoog GS, de los Rios A, Ruisi S, Grube M (2007) Fungal associations at the cold edge of life. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 735–757

Pang KL, Abdel-Wahab MA, Sivichai S, El-Sharouney HM, Jones EBG (2002) Jahnulales (Dothideomycetes, Ascomycota): a new order of lignicolous freshwater ascomycetes. Mycol Res 106:1031–1042

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21:29–55

Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, Sundberg P, Thollesson M (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol Phylogenet Evol 48:369–371

Poelt J (1970) Das Konzept der Artenpaare bei den Flechten. Vortrage aus dem Gesamtgebiet der Botanik. NF Dtsch Bot Ges 4:187–198

Pringsheim N (1858) Über das Austreten der Sporen von *Sphaeria scripi* aus ihren Schläuchen. Jahrb Wiss Bot 1:189–921

Promputtha I, Miller AN (2010) Three new species of *Acanthostigma* (Tubeufiaceae, Dothideomycetes) from Great Smoky Mountains National Park. Mycologia 102:574–587

Quaedvlieg W, Kema GHJ, Groenewald JZ, Verkley GJM, Seifbarghi S, Razavi M, Gohari AM, Mehrabi R, Crous PW (2011) *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. Persoonia 26:57–69

Rateb ME, Ebel R (2011) Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28:290–344

Reynolds DR (1971) Wall structure of a bitunicate ascus. Planta 98:244–257

Reynolds DR (1989) The bitunicate ascus paradigm. Bot Rev 55:1–52

Reynolds DR (1991) A phylogeny of fissitunicate ascostromatic fungi. Mycotaxon 42:99–123

Reynolds DR (1998) Capnodiaceous sooty mold phylogeny. Can J Bot 76:2125–2130

Richards TA (2011) Genome evolution: horizontal movements in the Fungi. Curr Biol 21:R166–R168

Rossman AY, Schoch CL, Farr DF, Nishijima K, Keith L, Goenaga R (2010) *Dolabra nepheliae* on rambutan and lychee represents a novel lineage of phytopathogenic Eurotiomycetes. Mycoscience 51:300–309

Rouxel T, Balesdent M-H (2005) The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters the genomic era. Mol Plant Pathol 6:225–241

Rouxel T, Grandaubert J, Hane JK, Hoede C, van de Wouw AP, Couloux A, Dominguez V, Anthouard V, Bally P, Bourras S, Cozijnsen AJ, Ciuffetti LM, Degrave A, Dilmaghani A, Duret L, Fudal I, Goodwin SB, Gout L, Glaser N, Linglin J, Kema GHJ, Lapalu N, Lawrence CB, May K, Meyer M, Ollivier B, Poulain J, Schoch CL, Simon A, Spatafora JW, Stachowiak A, Turgeon BG, Tyler BM, Vincent D, Weissenbach J, Amselem J, Quesneville H, Oliver RP, Wincker P, Balesdent MH, Howlett BJ (2011) Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by repeat-induced point mutations. Nat Commun 2:202

Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, Groenewald JZ, Muggia L, Grube M, Isola D, Schoch CL, Staley JT, Lutzoni F, de Hoog GS (2009) Phylogeny of rock-inhabiting fungi related to Dothideomycetes. Stud Mycol 64:123–133

Saccardo PA (1882–1931) Sylloge Fungorum, 25 vols. The Author, Padova

Sánchez RM, Miller AN, Bianchinotti MV (2012) A new species of *Acanthostigma* (Tubeufiaceae, Dothideomycetes) from the Southern Hemisphere. Mycologia 104:223–231

Santesson R (1952) Foliicolous lichens I. A revision of the taxonomy of the obligately foliicolous, lichenized fungi. Symb Bot Upsal 12:1–590

Schlick-Steiner BC, Steiner FM, Konrad H, Seifert B, Christian E, Moder K, Stauffer C, Crozier RH (2008) Specificity and transmission mosaic of ant nest-wall fungi. Proc Natl Acad Sci U S A 105:940–943

Schmidt AR, Beimforde C, Seyfullah LJ, Wege S-E, Dörfelt H, Girard V, Grabenhorst H, Gube M, Heinrichs J, Nel A, Nel P, Perrichot V, Reitner J, Rikkinen J (2014) Amber fossils of sooty moulds. Rev Palaeobot Palynol 200:53–64

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052

Schoch CL, Crous PW, Groenewald JZ, Boehm EW, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EB, Kohlmeyer J, Kruys A, Li YM, Lucking R, Lumbsch HT, Marvanova L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJ, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JH, Yonezawa H, Zhang Y, Spatafora JW (2009a) A class-wide

phylogenetic assessment of Dothideomycetes. Stud Mycol 64:1–15

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009b) The Ascomycota Tree of Life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Schoch CL, Wang Z, Townsend JP, Spatafora JW (2009c) Geoglossomycetes cl. nov., Geoglossales ord. nov. and taxa above class rank in the Ascomycota Tree of Life. Persoonia 22:129–138

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, Miller AN, Wingfield MJ, Aime MC, An KD, Bai FY, Barreto RW, Begerow D, Bergeron MJ, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz AR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJ, Crespo A, Cubas P, Cummings C, Damm U, de Beer ZW, de Hoog GS, Del-Prado R, Dentinger B, Dieguez-Uribeondo J, Divakar PK, Douglas B, Duenas M, Duong TA, Eberhardt U, Edwards JE, Elshahed MS, Fliegerova K, Furtado M, Garcia MA, Ge ZW, Griffith GW, Griffiths K, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Guo LD, Hagen F, Hambleton S, Hamelin RC, Hansen K, Harrold P, Heller G, Herrera G, Hirayama K, Hirooka Y, Ho HM, Hoffmann K, Hofstetter V, Hognabba F, Hollingsworth PM, Hong SB, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KD, James T, Johnson EM, Johnson JE, Johnston PR, Jones EB, Kelly LJ, Kirk PM, Knapp DG, Koljalg U, Kovacs GM, Kurtzman CP, Landvik S, Leavitt SD, Liggenstoffer AS, Liimatainen K, Lombard L, Luangsa-Ard JJ, Lumbsch HT, Maganti H, Maharachchikumbura SS, Martin MP, May TW, McTaggart AR, Methven AS, Meyer W, Moncalvo JM, Mongkolsamrit S, Nagy LG, Nilsson RH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaedvlieg W, Raja HA, Redecker D, Rintoul T, Ruibal C, Sarmiento-Ramirez JM, Schmitt I, Schussler A, Shearer C, Sotome K, Stefani FO, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh SO, Sung GH, Suzuki M, Tanaka K, Tedersoo L, Telleria MT, Tretter E, Untereiner WA, Urbina H, Vagvolgyi C, Vialle A, Vu TD, Walther G, Wang QM, Wang Y, Weir BS, Weiss M, White MM, Xu J, Yahr R, Yang ZL, Yurkov A, Zamora JC, Zhang N, Zhuang WY, Schindel D (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A 109:6241–6246

Scott JA, Untereiner WA, Ewaze JO, Wong B, Doyle D (2007) *Baudoinia*, a new genus to accommodate *Torula compniacensis*. Mycologia 99:592–601

Seifert K, Morgan-Jones G, Gams W, Kendrick B (2011) The genera of Hyphomycetes. CBS-KNAW, Utrecht

Shearer CA, Raja HA, Miller AN, Nelson P, Tanaka K, Hirayama K, Marvanov L, Hyde KD, Zhang Z (2009) The molecular phylogeny of freshwater Dothideomycetes. Stud Mycol 64:145–153

Shoemaker RA (2003) Proposal to conserve the name *Dothidea* with a conserved type (Fungi: Dothideomycetes). Taxon 52:623–625

Shoemaker RA, Hambleton S (2005) *Dothidea sambuci* and *Diaporthe spiculosa*. Can J Bot 83:484–490

Silva-Hanlin DMW, Hanlin RT (1999) Small subunit ribosomal RNA gene phylogeny of several loculoascomycetes and its taxonomic implications. Mycol Res 103:153–160

Simon UK, Bauer R, Rioux D, Simard M, Oberwinkler F (2005) The vegetative life-cycle of the clover pathogen *Cymadothea trifolii* as revealed by transmission electron microscopy. Mycol Res 109:764–778

Singh RS, Saini GK, Kennedy JF (2008) Pullulan: microbial sources, production and applications. Carbohydr Polym 73:515–531

Sivanesan A (1996) *Corynesporasca caryotae* gen. et sp. nov. with a *Corynespora* anamorph, and the family Corynesporascaceae. Mycol Res 100:783–788

Slippers B, Wingfield MJ (2007) Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fung Biol Rev 21:90–106

Slippers B, Boissin E, Phillips AJ, Groenewald JZ, Lombard L, Wingfield MJ, Postma A, Burgess T, Crous PW (2013) Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Stud Mycol 76:31–49

Spatafora JW, Mitchell TG, Vilgalys R (1995) Analysis of genes coding for small-subunit rRNA sequences in studying phylogenetics of dematiaceous fungal pathogens. J Clin Microbiol 33:1322–1326

Spatafora JW, Sung G-H, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser DM, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL (2006) A five-

gene phylogeny of Pezizomycotina. Mycologia 98:1018–1028

Spatafora JW, Owensby CA, Douhan G, Boehm EWA, Schoch CL (2012) Phylogenetic placement of the ectomycorrhizal genus *Cenococcum* in Gloniaceae (Dothideomycetes). Mycologia 104:758–765

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Syst Biol 57:758–771

Stocker-Wörgötter E, Hager A (2008) Appendix: culture methods for lichens and lichen symbionts. In: Nash TH (ed) III: Lichen Biology, 2nd edn. Cambridge University Press, Cambridge, UK, pp 353–363

Suetrong S, Schoch CL, Spatafora JW, Kohlmeyer J, Volkmann-Kohlmeyer B, Sakayaroj J, Phongpaichit S, Tanaka K, Hirayama K, Jones EB (2009) Molecular systematics of the marine Dothideomycetes. Stud Mycol 64:155–173

Suetrong S, Sakayaroj J, Phongpaichit S, Jones EBG (2010) Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis* (Jahnulales: Pezizomycotina; Dothideomycetes, Incertae sedis): new lineage of marine ascomycetes. Mycologia 102: 83–92

Suetrong S, Boonyuen N, Pang K-L, Ueapattanakit J, Klaysuban A, Sri-indrasutdhi V, Sivichai S, Jones E (2011) A taxonomic revision and phylogenetic reconstruction of the Jahnulales (Dothideomycetes), and the new family Manglicolaceae. Fung Divers 51:163–188

Sundin R, Tehler A (1998) Phylogenetic studies of the genus *Arthonia*. Lichenologist 30:381–413

Sutton BC (1980) The Coelomycetes. Fungi imperfecti with pycnidial acervuli and stromata. Commonwealth Mycological Institute, Kew

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya T (2009) Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with *Tetraploa*-like anamorphs, and notes on the phylogeny of selected species from bamboo. Stud Mycol 64:175–209

Tanaka K, Mel'nik VA, Kamiyama M, Hirayama K, Shirouzu T (2010) Molecular phylogeny of two coelomycetous fungal genera with stellate conidia, *Prosthemium* and *Asterosporium*, on Fagales trees. Botany 88:1057–1071

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Tehler A (1990) A new approach to the phylogeny of Euascomycetes with a cladistic outline of Arthoniales focusing on Roccellaceae. Can J Bot 68:2458–2492

Tehler A, Irestedt M (2007) Parallel evolution of lichen growth forms in the family Roccellaceae (Arthoniales, Ascomycota). Cladistics 23:432–454

Tehler A, Irestedt M, Bungartz F, Wedin M (2009) Evolution and reproduction modes in the *Roccella galapagoensis* aggregate (Roccellaceae, Arthoniales). Taxon 58:438–456

Theissen F, Sydow H (1918) Vorentwürfe zu den Pseudosphaeriales. Ann Mycol 16:1–34

Thomma BJ, van Esse HP, Crous PW, de Wit PJGM (2005) *Cladosporium fulvum* (syn. *Passalora fulva*), a highly specialized plant pathogen as a model for functional studies on plant pathogenic Mycosphaerellaceae. Mol Plant Pathol 6:379–393

Thor G (1990) The lichen genus *Chiodecton* and five allied genera. Opera Bot 103:1–92

Tsui CKM, Berbee ML (2006) Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. Mol Phylogenet Evol 39:587–597

Tsui CK, Sivichai S, Berbee ML (2006) Molecular systematics of *Helicoma*, *Helicomyces* and *Helicosporium* and their teleomorphs inferred from rDNA sequences. Mycologia 98:94–104

Tsui CKM, Sivichai S, Rossman AY, Berbee ML (2007) *Tubeufia asiana*, the teleomorph of *Aquaphila albicans* in the Tubeufiaceae, Pleosporales, based on cultural and molecular data. Mycologia 99:884–894

Untereiner WA, Straus NA, Malloch D (1995) A molecular morphotaxonomic approach to the systematics of the Herpotrichiellaceae and allied black yeasts. Mycol Res 99:897–913

U'Ren J, Lutzoni F, Miadlikowska J, Arnold A (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microb Ecol 60:340–353

van de Wouw AP, Howlett BJ (2011) Fungal pathogenicity genes in the age of 'omics'. Mol Plant Pathol 12:507–514

Voglmayr H, Jaklitsch W (2011) Molecular data reveal high host specificity in the phylogenetically isolated genus *Massaria* (Ascomycota, Massariaceae). Fung Divers 46:133–170

von Arx J, Müller E (1975) A re-evaluation of the bitunicate ascomycetes with keys to families and genera. Stud Mycol 9:1–159

Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW (2012) One fungus, one name promotes progressive plant pathology. Mol Plant Pathol 13:1–10

Winka K, Eriksson OE, Bang A (1998) Molecular evidence for recognizing the Chaetothyriales. Mycologia 90:822–830

Winton LM, Stone JK, Hansen EM (2007) The systematic position of *Phaeocryptopus gaeumannii*. Mycologia 99:240–252

Woronichin NN (1925) Über die Capnodiales. Ann Mycol 23:174–178

Wu H, Schoch C, Boonmee S, Bahkali A, Chomnunti P, Hyde K (2011) A reappraisal of Microthyriaceae. Fung Divers 51:189–248

Yang HL, Sun GY, Batzer JC, Crous PW, Groenewald JZ, Gleason ML (2010) Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the USA. Persoonia 24:29–37

Zahlbruckner A (1907) Lichenes. B. Specieller Teil. In: Engler A, Prantl K (eds) Die natürlichen pflanzenfamilien 1. Leipzig, pp 49–249

Zalar P, Gostincar C, de Hoog GS, Ursic V, Sudhadham M, Gunde-Cimerman N (2008) Redefinition of *Aureobasidium pullulans* and its varieties. Stud Mycol 61:21–38

Zhang Y, Schoch CL, Fournier J, Crous PW, de Gruyter J, Woudenberg JH, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, Hyde KD (2009) Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Stud Mycol 64:85–102

Zhang Y, Crous PW, Schoch CL, Hyde KD (2012) Pleosporales. Fung Divers 53:1–221

Zhdanova NN, Zakharchenko VA, Vember VV, Nakonechnaya LT (2000) Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. Mycol Res 104:1421–1426

Zogg H (1962) Die Hysteriaceae s. str. und Lophiaceae unter besonderer Berücksichtigung der mitteleuropäischen Formen. Beitr Kryptogamenfl Schweiz 11:1–190

Zuccaro A, Schoch CL, Spatafora JW, Kohlmeyer J, Draeger S, Mitchell JI (2008) Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. Appl Environ Microbiol 74:931–941

Nomenclature and Documentation

7 The Shifting Sands of Fungal Naming Under the ICN and the One Name Era for Fungi

Andrew M. Minnis[1]

CONTENTS

I. **Introduction** ... 179
II. **International Code of Nomenclature for Algae, Fungi, and Plants (ICN)** ... 180
A. Transition to One Name Per Fungus ... 182
1. History and Enactment of Rule Changes ... 182
2. How One Name Per Fungus Will Happen Under the New Rules ... 184
B. Other Major and Minor Changes in the ICN ... 189
1. Effective Publication via Electronic Publication ... 189
2. Latin or English for Valid Publication of Names of New Taxa ... 190
3. Registration of Fungal Names ... 190
4. Minor Changes ... 191
C. Practical Notes on Using the ICN ... 193
1. How Do I Describe New Taxa at the Rank of Species? ... 193
2. How Do I Describe New Taxa at the Rank of Genus? ... 195
3. What Is an Ex-type? ... 195
4. How and When Do I Designate a Lectotype for a Species? ... 195
5. How and When Do I Designate a Neotype for a Species? ... 196
6. How and When Do I Designate an Epitype for a Species? ... 196
7. How Do I Validly Publish New Combinations? ... 197
8. How Do I Validly Publish a Replacement Name Also Known as a Nomen Novum? ... 197
9. How Do I Correctly Give Author Citations for Taxa? ... 198
III. **Potential Changes to Nomenclatural Rules** ... 198
IV. **Conspectus and the Future of Fungal Naming** ... 199
References ... 200

I. Introduction

The accumulated knowledge about fungi and funguslike organisms is linked to names, and communication of this knowledge relies upon the use of these names. Naming of these organisms can be thought of simply as involving two principal components, taxonomy and nomenclature. Taxonomy is how a fungal entity is defined and grouped with other fungal entities into a system of classification. Although there is no universally agreed upon right or wrong way to do this, mycologists today generally base taxonomic concepts and classifications of fungi on evolutionary relationships inferred from phylogenetic analyses. Nomenclature includes the rules for formally establishing a name, determining which is correct, and establishing a standard to determine the application of a name, i.e., the type. The formal rules of nomenclature that govern the naming of fungi and funguslike organisms, excluding Microsporidia, are found in the current version of the International Code of Nomenclature for algae, fungi and plants, or ICN (Melbourne Code) (McNeill et al. 2012). New taxonomic concepts often lead to name changes, and newly recognized taxa are added as mycologists document the enormous quantity of fungi and funguslike organisms, estimated to include as many as 5.1 million fungal species (Blackwell 2011). Name changes also result from the application of and changes to the rules of the ICN.

[1]USDA-US Forest Service, Center for Forest Mycology Research, One Gifford Pinchot Dr., Madison, WI 53726, USA; e-mail: minnisncf@gmail.com

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

Though the name of a fungus might change, the fungus itself remains the same. Thus, tracking the different names used for a specific fungus allows all of the accumulated knowledge about it to be pooled, and in some cases misidentifications and changing concepts can be evaluated and information that is no longer clearly applicable to a specific fungus can be identified and reconsidered. The value of fungal naming and following the ICN thus lies in having one correct name for each fungus and funguslike organism that can be used for precise communication and an associated standard for determining what organism that name represents.

In the first edition of *The Mycota*, Hawksworth (2001) provided a scholarly and detailed summary of fungal naming that provided guidance for using the version of the International Code of Botanical Nomenclature (Tokyo Code) (Greuter et al. 1994) that was in effect at that time and integrated information about potential proposals for changes to fungal naming, some of which were never adopted, e.g., the BioCode. Subsequent to the completion of the original text of Hawksworth's (2001) publication, three different Codes have been used for fungal naming, and major changes to the procedures for naming fungi have been adopted, especially changes found in the current ICN (McNeill et al. 2012). Rather than restate the historical aspects and basics of fungal naming summarized by Hawksworth (2001), this chapter will provide an update and supplement to the previous version. Much has been written on the topic of fungal naming in recent years, and all of this will not be covered in detail here. The present chapter will focus on the transition to a one-name-per-fungus system of classification, other major changes to how fungi are named found in the ICN, and how to perform frequently used nomenclatural procedures correctly. Some attention will also be given to a number of current proposals and ideas on changes to fungal naming that may be adopted in the future.

II. International Code of Nomenclature for Algae, Fungi, and Plants (ICN)

The ICN is a set of rules for naming the organisms traditionally treated as algae, fungi, or plants (McNeill et al. 2012). Modifications of and additions to the rules are considered, and new Codes are adopted via a democratic process every 6 years at an International Botanical Congress (IBC), the next of which will occur in 2017 in Shenzhen, China. The Code may be thought of as a sort of gentlemen's agreement since it has no legal status; hence, it depends on users' willingness to voluntarily follow its rules, which has largely been done over the years. The Code itself is divided into a number of parts, and the organization and numbering of these in the current ICN has changed substantially from earlier Codes. Its language is complicated, and the finer details of the rules take a while to learn and appreciate; a single read is insufficient for most mycologists to gain anything close to a basic degree of mastery. Typically, nomenclatural experts must read and reread and reinterpret the Code depending on what nomenclatural procedures, some little used, and areas of interest have emerged during a taxonomic project. Unfortunately, many mycologists do not have time to learn the Code because the rigors of keeping up with other scientific matters in a quickly changing world take up a lot of time. It is hoped that the present chapter will serve mycologists as an elementary guide to the basic essentials of nomenclature.

Following a preface summarizing the changes found in the current Code, a key to the renumbering of parts, and a list of important dates in the Code, the ICN begins with a preamble that defines its purpose and the organisms it covers and summarizes its parts, among other items. Next comes the first of three divisions of the ICN, Principles. This single page contains the six major principles that form the basis of nomenclature, not the least of which are determining the application of names by nomenclatural types, priority of publication, and that each taxon may only bear one correct name, with a few exceptions.

The second division, Rules and Recommendations, contains nine chapters, some of which have additional sections. These chapters contain rules, which are set out in articles and sometimes clarified with notes, and recommendations, and examples are added occasionally for illustrative purposes. Rather than detail

all of these, a brief summary of important concepts and parts that should be useful for most mycologists follows. Chapter I covers taxa and their ranks. The principal ranks include kingdom, phylum, class, order, family, genus, and species, which we have all learned and loved. A bevy of secondary ranks are also presented for those inclined to produce complicated classifications. None of these ranks is a level, so studies that use the term "level," such as species level or level at other ranks, reflect the authors' nomenclatural ignorance. Such usage is best avoided by employing the term rank rather than level because there is no such thing as the latter (at least under the Code).

Chapter II covers status, typification, and priority of names. Effective publication (getting the word out) is defined as per the articles of Chapter IV, and this simply means that the name of a taxon has been presented to the world by appropriate means, for example, in a printed book or journal that is widely distributed rather than on a note left in a single university herbarium specimen. Valid publication, which produces a valid name, means that the articles of Chapter V, hybrids ignored herein, were followed during the naming process. To be a name, a name must be valid, and valid simply means meeting the basic requirements to exist or have status under the Code and be recognized by the scientific community. An invalid name does not have status (exist) under the Code and should not be recognized by the scientific community because it does not meet the basic requirements of the ICN. The terms valid and invalid are misused by a number of authors, often as a way of indicating disagreement with a particular taxonomic concept of someone else. A legitimate name is a valid name that is in accordance with the rules, while an illegitimate name is a valid one that is defined as such by the articles listed in ICN Art. 6.4. There are two common ways in which a name may be illegitimate. The first is by being a later homonym, or the same name validly published at a later date, like the imaginary cases of "*Amanita hibbettii* Vilgalys 2011" and the illegitimate "*Amanita hibbettii* J.W. Taylor 2013." The second is by being superfluous, which can be thought of as simply renaming an already named fungus, for example, I have "*Amanita hibbettii* Vilgalys 2011," but I call it "*Amanita justoi* Minnis 2014." The terms legitimate and illegitimate are also often misused by authors despite being clearly defined in the various Codes.

Typification is one of the most important principles of the Code because a type is a standard used to determine the application of a name, i.e., answer the question: what is this fungus? An analogous standard is the International Prototype Kilogram (IPK), which is used to ascertain the base unit of mass if and when questions about it arise (BIPM 2006). Needless to say, the IPK is rarely handled and protected from damage for good reason. A nomenclatural type functions similarly for the name of a fungus. As a single standard, a type may not encompass all of the variation of a biological entity, and it may not be the most representative element. A type is typically a specimen, but illustrations may serve as types in some cases. There are a large number of different kinds of types and terminology, and some of them are reviewed later in this chapter. The name of a genus is typified by the type of name of a species, and this may be indicated by citation of the species name. The name of a family is typified by the same type as the generic name on which it is based, and this may be indicated by citing the name of the genus. Above the rank of family, typification does not apply unless the name is based on a generic name, in which case the type is automatically the same as that of the genus. Thus, the Agaricomycetes has the same type as that of the genus *Agaricus*, the type of *Agaricus campestris* L.

Priority applies to names at the rank of family and below where each name may bear only one correct name, with a few exceptions. Names do not have priority outside of the rank at which they were published, and the correct name is the legitimate name that was effectively published first. Priority does not operate above the rank of family. Generally speaking, there are three major ways that the principle of priority may be limited for fungal names. For fungi, the valid publication of names is treated as beginning on 1 May 1753 with *Species Plantarum*, a work of Linnaeus, and fungal names that may

have existed before this date have no status under the Code. However, this has not always been the case, and the starting point for rusts, smuts, and gasteromycetes used to be Persoon's *Synopsis Methodica Fungorum*, while that of other fungi (excluding slime molds) was Fries' *Systema Mycologicum*. Because of the change in starting point for these groups and the resulting and unfortunate need to change many names, sanctioning was adopted to give priority against earlier names to names adopted in the works that used to be the former starting point for fungi; additional information relating to sanctioning and its history was summarized by Hawksworth (2001). Conservation via ICN Article 14 is another procedure that limits priority and is employed when strict application of the rules results in disadvantageous changes. Names may be conserved to have priority over competing names or to have a different type that preserves usage in order to serve stability. Rejection via ICN Article 56 is another procedure that limits priority. It is similar to conservation in some ways, but a rejected name may not be used and can be thought of as being taken out of play permanently, unless later conserved. Additional limitations to priority involve the transition to a one-name-per-fungus system of classification, which will be covered in more detail subsequently in this chapter.

Chapters of Division II not previously mentioned in detail include Chapter III, which shows how to construct names at the various ranks, Chapter VI, which shows how to give author citations that credit the appropriate authors of names, Chapter VII, which covers all forms of name rejection, Chapter VIII, which deals with the names of anamorphic fungi or those with a pleomorphic life cycle (discussed under the transition to one name per fungus below), and Chapter IX, on the orthography and gender of names, which simply means spelling and grammar issues. Division III, the last one of the Code, includes matters relating to governance of the Code. The last items of the Code appear as Appendix I on the names of hybrids, a glossary, and a couple of indexes. Seven additional appendices are to be published separately and will be available in print and electronically. For additional information on the ICN and matters not summarized herein, refer to the complete ICN (McNeill et al. 2012) and the earlier chapter by Hawksworth (2001).

A. Transition to One Name Per Fungus

Perhaps the most significant change to the naming of fungi found in the current ICN (McNeill et al. 2012) is the elimination of provisions that allowed multiple names for the same taxa of non-lichen-forming ascomycetous and basidiomycetous fungi, sometimes referred to as dual nomenclature. The following sections consider historical aspects and how the transition to a one-name-per-fungus system of classification was implemented as well as the specific rules of the ICN and how they will be used to make the transition happen. Terms such as anamorph, teleomorph, and holomorph, though still present in the ICN, are abandoned as much as possible in accordance with the preferences of mycologists including Hawksworth (2013) and Amy Y. Rossman, my recent mentor. Seifert (2014), however, has made a good case for the continued use of these terms. It would be hard for new students of mycology to understand the historical mycological literature without these terms and knowledge of earlier Codes of nomenclature.

1. History and Enactment of Rule Changes

Many fungi possess complicated life cycles that include a number of different states, both asexual and sexual. These states may look completely different, some may be found rarely or seemingly not at all, and sometimes they occur at different times and/or places. Thus, mycologists have not always known whether or not the variously observed states of fungi represent the same organism, and multiple names for the same fungus inevitably accumulated in the literature. During the formalization of the earliest Codes of nomenclature, mycologists were already linking the different states of a number of fungi. Nevertheless, and with less than universal agreement, multiple names were

allowed for certain ascomycetous and basidiomycetous fungi with so-called pleomorphic life cycles in early Codes for the sake of convenience of communication. Weresub and Pirozynski (1979) presented a historical account of pleomorphic fungi and fungal naming. Over the years, a number of modifications and clarifications to the rules of naming pleomorphic fungi and fungi known only from asexual states were adopted. These changes included rules regarding, for example, the invalidation of species names if the state of a genus differed from the state of a newly described species that was included in that genus, which changed to the mere illegitimacy of species names under the same circumstances, and went to legitimacy of species names but incorrect classification in the wrong genus. Terms like imperfect and perfect that describe asexual and sexual states, respectively, were replaced by terms like anamorph (asexual state), teleomorph (sexual state), and holomorph (fungus in all of its morphs) in formalized Codes. Because of the frequency of rule changes and their increasing complexity, many problems involving the names of fungi with pleomorphic life cycles were associated with the rules that had been in place when a significant amount of work was being performed on certain groups of fungi and how rules changes affected this work. Oftentimes, rules changed again before mycologists were able to address issues associated with the names of groups of fungi that were not receiving substantial attention. A study of rust nomenclature is particularly enlightening when it comes to understanding the impacts of variously changing Codes, for example, Judith and Rossman (2014).

As DNA sequence data became more readily obtainable and used in fungal taxonomy, a small but vocal group of mycologists came to the conclusion that multiple names for the same fungus were no longer convenient (Hawksworth 2011; Hawksworth et al. 2011; Taylor 2011). The debate about whether or not to transition to a one-name-per-fungus system of classification and how to do it went on for a number of years with the small but vocal group slowly increasing in numbers but never quite reaching a substantial majority of mycologists, as evidenced by the results of the nomenclature questionnaire from the International Mycological Congress (IMC 9) in Edinburgh (Norvell et al. 2010). In 2010, as the next IBC in Melbourne was approaching, Redhead (2010b) reported on the efforts of the latest group to formally consider the matter in a representative way, the Special Committee on Nomenclature of Fungi with a Pleomorphic Life Cycle. Redhead (2010b) summarized an ideological impasse in which no agreement could be reached on a course of action and noted the anarchy that was occurring in mycological publications regarding the naming of pleomorphic fungi. Following the report by Redhead (2010b), proponents of a one-name-per-fungus system of classification felt an immediacy to act on nomenclatural reform. This act came in the form of the "Amsterdam Declaration on Fungal Nomenclature" (Hawksworth et al. 2011). Early versions of the declaration were circulated to mycologists to garner their support, and despite significant support, a number of proponents for one name per fungus chose not to support the Amsterdam Declaration due to its general lack of details and the inclusion of unrelated issues, such as governance of the Code and the naming of environmental sequences. The published version of the Amsterdam Declaration (Hawksworth et al. 2011) made a strong statement on the principle of moving to a one-name-per-fungus system of classification, but the rule changes by which this was to occur were not detailed, and the aforementioned unrelated rider issues were excluded from the formal declaration but put up for future consideration. With knowledge that this issue was to be brought up on the floor of the IBC in Melbourne, "A Critical Response to the Amsterdam Declaration" was circulated to gain support by advocates of a continuation of multiple names per fungus who strongly opposed a transition to a one-name-per-fungus system of classification, but because of a lack of time before the IBC, it was not published formally by Gams and Jaklitsch (2011) until after the IBC, where the response was presented as a handout. The IBC itself was attended by a small number (13) of representative mycologists and a large number of botanists

(nearly all of the remaining 201 attendees). Following an inconclusive discussion and presentation of the competing principles on naming fungi with a pleomorphic life cycle, Redhead prepared to circulate the first of his three proposals that were hastily developed and not seen or considered by more than a handful of mycologists, some of whom were consulted in making them (Milius 2014; Norvell 2011b). The three Redhead proposals were arranged from the most extreme to a moderate proposal that was in line with the majority of mycologists on the Special Committee on Nomenclature of Fungi with a Pleomorphic Life Cycle (Redhead 2010b). The mostly botanists and other attendees at the IBC passed the first and most extreme proposal without even considering the latter two (Milius 2014; Norvell 2011b). A number of additional proposals for and against dual nomenclature with varying degrees and often involving a term called teleotypification had been made and considered prior to the IBC in Melbourne (McNeill and Turland 2011a; McNeill et al. 2011; Norvell 2011a), but these were not seriously considered at the IBC because most were withdrawn (McNeill et al. 2011). In this way, well over 100 years of fungal naming involving multiple names for fungi with pleomorphic life cycles passed into history, and the transition was made to a one-name-per-fungus system of classification.

2. How One Name Per Fungus Will Happen Under the New Rules

In the International Code of Botanical Nomenclature (Vienna Code), or ICBN (McNeill et al. 2006), immediately preceding the ICN (Melbourne Code) (McNeill et al. 2012), separate names for asexual and sexual states of non-lichen-forming ascomycetes and basidiomycetes were allowed by a number of rules that governed the naming of fungi, and Art. 59 of that ICBN included most of these rules. Redhead's floor proposal at the IBC in Melbourne and subsequent editorial modifications have more or less replaced Art. 59 and related provisions in their entirety, and separate names for the asexual and sexual states of non-lichen-forming ascomycetes and basidiomycetes are no longer allowed. Understanding how the transition to a one-name-per-fungus system of classification will occur, perhaps best thought of as the continuing taxonomic process, requires examination of basic nomenclatural mechanisms, special provisions for stability, and considerations on how to decide which names will be correct. Links are provided to articles of the Code for the sake of convenience, and the associated explanatory text is best considered concurrently with the text of the Code. Hawksworth (2012) and Rossman (2014) offer additional guidance on dealing with and understanding the rule changes. Braun (2012) does so as well using powdery mildews as an example.

The previous Art. 59 has been replaced in the ICN by the new Art. 59 (McNeill et al. 2012) in Chapter VIII, "Names of anamorphic fungi or fungi with a pleomorphic life cycle": http://www.iapt-taxon.org/nomen/main.php?page=art59.

The first thing to note about the new Art. 59 is that the changes are retroactive via ICN Principle VI. This means that all previous mycological literature must be interpreted as if these rules had been in place at the time they were published. Of course, they had not been, and the mycologists of other times were doing their best to follow whichever rules were in place at their respective times. This also means that all previous literature must be reconsidered to see how this will affect the fungal names. Indexes of names and other databases will take some time to update because the task is enormous, so scientists will have to check names on which they would like to publish themselves, and little existing literature involving pleomorphic fungi can be taken for granted as being nomenclaturally accurate.

For names of newly described taxa, authors should not publish more than one name for a fungus regardless of the number of states in its life cycle. This is because simultaneously published names that are alternative names for asexual and sexual states are invalid if published on or after 1 January 2013. Additionally, multiple state names published for named species, regardless of whether the named taxon at the same rank was typified by a sexual state or

asexual state, will be illegitimate as superfluous in most cases if published on or after 1 January 2013. Such a fungal name would be superfluous as defined by the ICN since an already named fungus would be renamed.

For names published prior to 1 January 2013 when multiple names were allowed for certain fungi and there was an intent or implied intent of their applying to or being typified by a particular morph or state, these may be valid and legitimate, and if so, they will compete for priority. If proposed at the same time, the names for the different states are not alternative names and are heterotypic. Priority (typically being validly published first), except when this is limited, will determine the correct name of taxa at the ranks of family and below, and this can be thought of as the basic default mechanism that requires no action. Regardless of the type of the state of the fungus, all names are treated equally, and this is a departure from earlier Codes that gave precedence to names associated with the sexual state when the whole fungus in all of its forms was considered. The following examples will illustrate how this works.

The Amsterdam Declaration (Hawksworth et al. 2011) and most mycologists currently working on the transition to one name per fungus primarily focus on the rank of genus. If a taxonomic study concludes that the following genera represent the same genus, and even if these were used for separate asexual and sexual states historically, priority determines the correct generic name, except when this principle is limited:

Competing names		Correct name
Chloridium Link 1809 (asexual) *Melanopsamella* Höhn. 1929 (sexual)	→	***Chloridium* Link 1809**

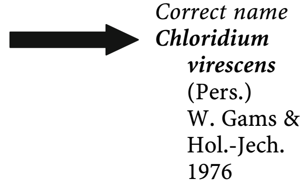
Though little considered prior to enactment of the new rules, a number of familial names apply separately to asexual and sexual states. In recent history, most authors gave priority to those typified by sexual states, and they were used widely in accordance with previous Codes. If a taxonomic study concludes that the following families represent the same family, and even if these were used for separate asexual and sexual states historically, priority determines the correct familial name, except when this principle is limited:

Competing names		Correct name
Chloridiaceae Nann. 1932 (asexual) Chaetosphaeriaceae Réblová, M.E. Barr & Samuels 1999 (sexual)	→	**Chloridiaceae Nann. 1932**

The situation at the rank of species is perhaps the most complicated because a large number of possibilities might occur, especially owing to the existence or potential creation of homonyms. In general, a taxonomic study looking at a fungus with existing names for multiple states should look to determine the correct name for each state and then use this to determine the correct name for the species. If a taxonomic study concludes that the following species represent the same species, and even if these were used for separate asexual and sexual states historically, priority determines the correct species name, except when this principle is limited:

Competing names	→	Correct name?
Chloridium virescens (Pers.) W. Gams & Hol.-Jech. 1976 (asexual) *Melanopsamella vermicularioides* (Sacc. & Roum.) Réblová, M.E. Barr & Samuels 1999 (sexual)		
Four possibilities:		
Chloridium virescens, based on the basionym, *Dematium virescens* Pers. 1797		

(continued)

Competing names Correct name?
Chloridium vermicularioides
Melanopsamella virescens
Melanopsamella vermicularioides, based on the basionym, *Eriosphaeria vermicularioides* Sacc. & Roum. 1883
In this case, we know that *Chloridium* has priority at the rank of genus, and the species epithet comes from *Dematium virescens*, which has priority at the rank of species:
 *Correct name* ***Chloridium virescens*** (Pers.) W. Gams & Hol.-Jech. 1976

In this simple case, no new combination was required. But this may not always be the case.

The principle of priority does not operate above the rank of family. Suppose only the ordinal name Chaetoshaeriales Huhndorf, A.N. Mill. & F.A. Fernández 2004 is available. An author could publish an additional name, for example, Chloridiales Minnis 2014, and use this as the correct name at that rank if it was desirable.

Author citations and types of names associated with pleomorphic fungi may be changed as a result of the deletion of various provisions of Art. 59.6 found in the previous ICBN (McNeill et al. 2006) found here: http://www.iapt-taxon.org/icbn/main.htm.

In some instances, like Example 6 of the previous Art. 59.6 (McNeill et al. 2006), the Code would automatically create a new name based on a type that matches the appropriate morph or state. This would effectively change the supposed new combination *Mycosphaerella aleuritidis* (Miyake) S.H. Ou based on *Cercospora aleuritidis* Miyake into a new species name, for example, *Mycosphaerella aleuritidis* S.H. Ou, with a different author citation, and the type would change from an old specimen with conidia to a newly created type that is a different specimen with asci and ascospores. *Cercospora aleuritidis* would remain typified by the specimen bearing conidia and only apply to the asexual state, and *M. aleuritidis* would apply to the sexual state and have precedence for the name of the whole fungus in all of its states. Under Art. 59 of the ICN (McNeill et al. 2012), Example 2 shows how in this situation the new species name reverts to a new combination, *Mycosphaerella aleuritidis* (Miyake) S.H. Ou, and the type changes from a specimen with asci and ascospores to the older specimen with conidia that typifies the basionym, a process I refer to as **detypification and retypification**. The specimen with asci and ascospores is no longer a type. It has been suggested (Hawksworth 2012) that this situation has not occurred very often. In my work updating the fungal databases at the U.S. National Fungus Collections (Farr and Rossman 2014, and ongoing), however, I found this situation to be more common than easily overlooked because of its rarity, especially for rust fungi. Authors addressing taxonomy and nomenclature should be aware of this phenomenon because it would be especially significant if the different types did not represent the same fungus, which seems to happen from time to time in the era of molecular splitting.

Universal recognition of one aspect of transitioning to a one-name-per-fungus system of classification was given to the need to provide some means for having stability of fungal names when the basic nomenclatural mechanisms did not allow for a smooth transition to a one-name-per-fungus system of classification. Along with typical conservation against competing synonyms via ICN Article 14.1 and rejection of names via ICN Article 56.1, additional provisions for the stability of fungal names were included in the ICN. The three major provisions follow.

The first of these is ICN Art. 14.13 (McNeill et al. 2012): http://www.iapt-taxon.org/nomen/main.php?page=art14.

Under this rule, fungal names, excluding lichen-forming fungi, may be added to lists of accepted names and treated as if conserved following submission of these lists to the General Committee (GC) of the International Association of Plant Taxonomists (IAPT) and subsequent review and approval by both the Nomenclature Committee for Fungi (NCF) and the GC. Accepted names on approved lists

are then added to Appendices of the Code along with competing names against which they are conserved. Subcommittees and international groups established by and in support of the NCF are to aid in the assembly of lists and make recommendations regarding their approval. Fortunately, proposals by Redhead (2010a) on having an option to publish some of the Appendices of the ICN separately and/or in electronic format only were approved at the IBC in Melbourne (McNeill and Turland 2011b; McNeill et al. 2011) since the Appendices are likely destined to become rather large during the transition to one name per fungus!

The second of the provisions is ICN Art. 56.3 (McNeill et al. 2012): http://www.iapt-taxon.org/nomen/main.php?page=art56.

Under this rule, fungal names, excluding those of lichen-forming fungi, may be added to lists and treated as if rejected under ICN Art. 56.1. The approval process is the same as the one that applies to Art. 14.13. Names on approved lists are also added to the Appendices of the Code. Names to be treated as rejected may become eligible for use only by subsequent conservation via ICN Art. 14.

The third provision is ICN Art. 57.2 (McNeill et al. 2012): http://www.iapt-taxon.org/nomen/main.php?page=art57.

This rule addresses pleomorphic fungi, excluding lichen-forming fungi, that have both widely used anamorph-typified and widely used teleomorph-typified names prior to 1 January 2013. In such cases, anamorph-typified names with priority are not to take precedence over teleomorph-typified names unless and until either a proposal to reject the anamorph-typified names via Art. 56.1 or to put them on a list to be treated as rejected via Art. 56.3 **or** a proposal to conserve the teleomorph-typified names via Art. 14.1 or to put them on a list to be treated as conserved via Art. 14.13 has been submitted and rejected. This rule is perhaps one of the most difficult to interpret because no guidance is given as to what it means to be widely used. In any case, the benefit of this is that any major revision of groups, where either both asexual and sexual state names were frequent and where sexual state names were given priority because of their state over older asexual state names, will have to proceed by a more democratic process that involves the mycological community. Historical precedence of teleomorph-typified names is also continued to some degree by default. It is worthwhile noting that the existing Example 3 involves two genera, *Magnaporthe* and *Pyricularia*, that are no longer considered to be congeneric (Luo and Zhang 2013), and ICN Art. 57.2 does not apply. The downside is that a liberal interpretation of the phrase “widely used” will create a lot of work for mycologists and the appropriate committees.

Though several options are available to protect against undesirable changes, ICN Arts. 56.1 and 56.3 should be employed judiciously and only after careful consideration since rejected names may not be used later if taxonomic revisions create a reason to do so without the names first being conserved under ICN Art. 14.

The NCF of the International Association for Plant Taxonomy (IAPT) plays a major role in reviewing and approving lists of accepted and rejected names, and the International Commission on the Taxonomy of Fungi (ICTF; http://www.fungaltaxonomy.org/) is assisting with the coordination of working groups on specific fungal groups that are charged with making these lists. Since it is desirable to make the transition to one name per fungus a community-wide and inclusive effort, authors are encouraged to contact the NCF and/or the ICTF before publishing major revisions of important taxonomic groups of pleomorphic fungi affected by the changes to a one-name-per-fungus system of classification.

In determining whether to accept the basic nomenclatural mechanisms or employ one of the tools allowing for the stability of fungal names, it is difficult but necessary to remain without prejudice because it is often easy to prefer certain names for arbitrary or personal reasons. A number of considerations on name choice are listed in what follows.

Should genus choice be correlated with higher ranks?

Trichoderma Pers. 1794 versus *Hypocrea* Fr. 1825

Trichodermataceae Fr. 1825 versus Hypocreaceae De Not. 1844
Hypocreales, Hypocreomycetidae

In this example, *Trichoderma* and Trichodermataceae have priority at their respective ranks, but higher rank classification is based on the name *Hypocrea*. Rossman et al. (2013a) recently proposed the use of *Trichoderma*, an asexual genus with priority, instead of *Hypocrea*, a sexual genus used as the correct name for the whole fungus under the previous Code, when both were widely used. Rossman et al. (2013a) also favored the name Hypocreaceae, though it lacks priority. In this case, the accepted name of the genus does not correlate with higher ranks, and *Hypocrea* will not be used at the rank of genus or below.

What if I don't like the familial name for these fungi?

Planistromellaceae M.E. Barr 1996
Kellermania Ellis & Everh. 1885
= *Piptarthron* Mont. ex Höhn. 1918
= *Alpakesa* Subram. & K. Ramakr. 1954
= *Planistroma* A.W. Ramaley 1991
= *Planistromella* A.W. Ramaley 1993

In this example, if all of these genera are treated as synonyms, as was done by Minnis et al. (2012), the only available familial name is Planistromellaceae, which is not based on an accepted generic name. Alternatives include living with this or describing the new family Kellermaniaceae Minnis 2014 and using one of the tools for fungal name stability such as ICN Arts. 14.1, 14.13, 56.1, and 56.3 to make the later name correct.

What results in the fewest changes or maximum stability?

Which genus has more species names in it?

Which genus appears most in the literature and in the most significant literature?

What morph or state is found most commonly in nature?

All four of these are worthy considerations. The case of *Cochliobolus* Drechsler 1934 versus *Bipolaris* Shoemaker 1959, as noted by Rossman et al. (2013b), is one in which going with the later name based on an asexual state results in the fewest changes, *Bipolaris* has more names, and the asexual state is found more commonly in nature. Significant literature regarding genetics and genomics, unfortunately, uses the name *Cochliobolus* (Rossman et al. 2013b).

Should we prioritize priority?

Scientific discovery is typically credited to the first person to find something new and publish on it. One of the six major principles of the ICN is the principle of priority, which means that names that were validly published first shall be correct in most instances and the author citations of these names are those of the authors that should receive credit. One of the issues with lists of accepted and rejected names of fungi is that such lists totally disregard the principle of priority and the authors who originally discovered and described fungi are not credited for this work. For this reason, some might not prefer to liberally or arbitrarily use the lists of accepted and rejected names at all or for a large number of cases. Others may feel it is not necessary to give credit where credit is due since other factors are believed to be more important.

What do users of fungal names such as plant pathologists and medical mycologists prefer and how do we get the word out?

Users of fungal names in more applied fields are often frustrated by frequent name changes associated with scientific progress. Perhaps it would be wise to consider how to make the transition to one name per fungus that resulted from philosophical differences in naming to be as painless as possible for users in these fields. Wingfield et al. (2012) and Zhang et al. (2013) have provided valuable explanations of the transition to one name per fungus and the benefits of doing this to the community of plant pathologists. Hoog et al. (2014) have provided a similar resource for the medical community.

Some general observations and thoughts on the transition to one name per fungus:

- Taxonomic revisions and new species descriptions are hardly justifiable without looking at the whole fungus in all of its morphs or states, for example, all of those existing names, and taxonomic integration is essential.
- Mycologists must value the discovery and description of the other morphs or states for named species as key taxonomic and mycological contributions because the recognition for doing so may not be the same as in the past.
- We will have what I refer to as "The rise of the anamorphs" since, in general, asexual states for many groups are more common and conspicuous than sexual states and generic concepts for them were elaborated more quickly [except when later discovered to be polyphyletic hodgepodges, e.g., *Acremonium* (Summerbell et al. 2011)] and asexual state names will tend to be favored by the basic nomenclatural mechanisms.
- Mycologists need to work together to make the transition to one name per fungus. Individuals should be discouraged from taking matters into their own hands and enforcing their preferences on others from positions of power, for example, a journal editor.
- Some groups will require significant study before a wise choice can be made. In rusts, for example, we have yet to phylogenetically place the type of the genus *Uredo*, *Uredo betae*. Crous et al. (2014) have provided suggestions for addressing types of genera and a call to address them.
- Obscure groups may not be dealt with as quickly.
- Groups such as the NCF and the International Commission on the Taxonomy of Fungi will make the process more democratic.

B. Other Major and Minor Changes in the ICN

A number of other significant changes to the naming of fungi are found in the current ICN (McNeill et al. 2012), and these have been reported by McNeill et al. (2011) and summarized variously by Hawksworth (2011), McNeill and Turland (2011b), and Norvell (2011b). The following sections consider the most significant changes.

1. Effective Publication via Electronic Publication

Earlier versions of the Code did not allow for effective publication via electronic formats since only printed matter was available for most of the time that a formal Code has been in existence. An urgent need to update the Code resulted from high costs associated with producing printed formats, the proliferation of electronic-only publications, and the issuance of material in both printed and electronic matter, not always at the same time, for the same publication. The Special Committee on Electronic Publication carefully considered the issue and presented a series of proposals to address effective publication via electronic publication (Chapman et al. 2010), and these were accepted at the IBC in Melbourne (McNeill et al. 2011) and implemented in the ICN. The relevant changes regarding effective publication by electronic means are found in Chapter IV of the ICN and are summarized in what follows. Knapp et al. (2011) provide additional guidance.

Electronic material distributed on or after 1 January 2012 in Portable Document Format (PDF) in an online publication with an International Serial Number (ISSN) or an International Standard Book Number (ISBN) is effectively published, a key component of valid publication. Electronic material distributed before this date is not effectively published. Online is defined as being accessible via the World Wide Web. Only the final version of an electronic publication is effectively published. In press or preliminary versions that may be subsequently altered and are not considered as being the final versions by the publisher are not yet effectively published. The content of electronic publications must not be altered after effective publication, and any such changes are not effectively published. Content in external sources, such as something accessed via a hyperlink, is not part of the publication. Content is that which stands alone as what the

publisher considers to be final, and preliminary page numbers or a lack of them does not prevent a publication from being effectively published, even if page numbers are added or altered later. This can be confusing in some cases because it must be determined which version is considered final by a publisher and what must be cited as the correct page numbers and date of effective publication. The date of effective publication is when the printed matter or electronic matter became available, and this dictates the date used to determine priority of names. When both electronic and printed materials are issued for the same publication, the date of effective publication is treated as being the **same** and is that of **whichever form comes first**.

2. Latin or English for Valid Publication of Names of New Taxa

ICN Art. 38.1 states, with a few exceptions and among other things, that valid publication of the name of a new taxon requires a description and/or diagnosis of the taxon or a reference to an effectively published description and/or diagnosis. In earlier versions of the Code, one of the other requirements was that the description or diagnosis of new fungal taxa must be in Latin, and the Preface of the ICN provides a brief history of this requirement and how its effective date was set to 1 January 1935. This requirement has not always been unanimously supported, and several attempts to modify or remove this requirement, some with success, were made in subsequent years (Hawksworth 2011; Smith et al. 2011). Like others who flouted the rules of the Code they did not like, Dearness (1941) refused to describe new species of fungi in Latin because it was a dead language and would take up too much valuable space in Mycologia. This particular instance of open disregard for the rules was noted during routine updating of the SMML Fungal Databases (cited currently as Farr and Rossman 2014), and Braun et al. (2009) validly published the previously invalid species of interest to them in accordance with the rules. Many mycologists continued to detest the Latin requirement, and there was substantial support for modifying this requirement at the International Mycological Congress (IMC 9) in Edinburgh (Norvell et al. 2010). Demoulin (2010) subsequently acted on the support at the IMC 9 and proposed that Latin or English be acceptable for naming of new fungal taxa. A number of additional and related proposals were made, including that any language be allowed (McNeill and Turland 2011a; Smith et al. 2011), which was not supported by the NCF (Norvell 2011a) and was rejected in the preliminary mail vote for the IBC in Melbourne (McNeill et al. 2011; Smith et al. 2011). At the IBC in Melbourne, the Demoulin (2010) proposal was adopted and modified so that the Latin or English requirement was broadened to include all organisms covered by the Code and set to take effect on 1 January 2012 (Hawksworth 2011; McNeill et al. 2011; Norvell 2011b; Smith et al. 2011). New names described from 1935 up until this date in 2012 must still have descriptions and/or diagnoses in Latin in order to be valid.

3. Registration of Fungal Names

The mycological literature has always been scattered, and taxonomists must be collectors if they are to be scholars. In the earliest days, relatively few copies of mycological publications were made, and their distribution relied upon relatively primitive means of transportation. Due to the slowness in getting the word out, species were often described multiple times. Early indexes of fungal names like Saccardo's *Sylloge Fungorum* gathered lists of described names, their place of publication, and relevant information about the fungi in one place. Hawksworth (2001) provided a list of many of the historical fungal indexes and noted in particular the value of the Index of Fungi. Index Fungorum (http://www.indexfungorum.org/), closely allied with the Index of Fungi, has and continues to be a valuable source as an online global fungal nomenclator. As with the issue of effective publication via electronic publication, the proliferation of large numbers of journals and in numerous formats provided challenges to mycologists, in

this case in the assembly and awareness of published fungal names. I remember having trouble with a conservation proposal due to not knowing about a recent article in a regional faculty journal, the *Revista Facultad Nacional de Agronomía Medellín*. As related challenges grew, financial support of existing indexes began to wane, and the necessary personnel for upkeep became increasingly challenged by the volume of work. The idea of a formal registration of names as a component of valid publication has been around for a while, but many attempts to implement it were not successful (Hawksworth et al. 2010; Hawksworth 2011). In recognizing the problem, the large number of undescribed fungi, and the need to have help in assembling the kind of index that mycologists need, MycoBank was launched as an experimental and voluntary repository for newly published fungal names and associated data on these fungi (Crous et al. 2004). A key component for success was requiring that the authors themselves be responsible for entering names and data. Use of MycoBank increased rapidly, and several mycological journals required that authors of fungal names employ it (Hawksworth et al. 2010; Hawksworth 2011). Significant support for formally requiring the registration of fungal names received support at IMC 9 in Edinburgh (Norvell et al. 2010). As a result, Hawksworth et al. (2010) formally proposed changing the Code so that key information would be entered into an approved repository and that the identifier provided during this process must be included in the publication of fungal names as a requirement for valid publication. The NCF recommended approval (Norvell 2011a), and registration of fungal names was formally approved at the IBC in Melbourne (Hawksworth 2011; McNeill and Turland 2011b; McNeill et al. 2011; Norvell 2011b). Article 42 of the ICN (McNeill et al. 2012) is the relevant article on the registration of fungal names (http://www.iapt-taxon.org/nomen/main.php?page=art42). Thus, all fungal names published on or after 1 January 2013 must cite an identifier issued by a recognized repository; otherwise, it is not valid. Identifiers are issued when minimum elements, for example, those necessary for valid publication of a fungal name, are given to repositories by the authors. The NCF officially approved three repositories, Fungal Names, Index Fungorum, and MycoBank, that had agreed to coordinate their efforts, and the NCF noted that a number of details would need further consideration (Redhead and Norvell 2013).

4. Minor Changes

The Code gets a name change. Botany has historically included the study of more than just plants and was inclusive of, for example, algae and fungi. As the phylogenetic differences between these groups became more and more emphasized and understood, botany tended to become more restricted to just plants, and some major departments even went so far as to change their name from Department of Botany to Department of Plant Biology. Mycologists, wondering where they might be included and feeling slighted, began to think that the name International Code of Botanical Nomenclature might be misleading. Rambold et al. (2013) have also discussed the importance of fungi and the need for more recognition of the field of mycology. To address this matter, Hawksworth et al. (2009) made a series of proposals to amend the ICBN that would include mycology in the title, add fungus-related terms throughout, and transfer some aspects of governance of fungal naming from the International Botanical Congress to the International Mycological Congress. Hawksworth et al. (2009) also noted that some mycologists preferred to break off and establish an independent code for mycology. At the nomenclature session of IMC 9 in Edinburgh, mycologists generally did not support an independent code, provided the name of the current ICBN was changed, but did support transfer of governance to an IMC (Norvell et al. 2010). The NCF recommended approval of a name change, the addition of fungal terminology, and the election of members of the NCF at an IMC, but the NCF did not support transfer of governance (Norvell 2011a). Proposals on a name change and adding fungal terminology were so well supported at the IBC in Melbourne that algae were also included in the title, and the

Code was renamed the International Code of Nomenclature for algae, fungi, and plants via a floor proposal (Hawksworth 2011; McNeill and Turland 2011b; McNeill et al. 2011; Norvell 2011b). Proposals on governance for fungal names were withdrawn on the understanding that a subcommittee would examine the issue at a future date (Hawksworth 2011; McNeill et al. 2011; Norvell 2011b). Mycologists did seem to get everything they really wanted or needed at the IBC and did benefit from having assistance from numerous experts in nomenclature who happen to study plants. As for the new title of the Code, apologies are extended to those who study slime molds and other funguslike organisms; lowercase fungi is the best that could be done to cover these organisms (Hawksworth 2011)!

Microsporidia excluded from ICN. Microsporidia are intracellular parasites that invade host cells via injection by polar filaments (see Didier et al., Chap. 5, Vol. VII, Part A). These organisms are medically important and have been studied historically by zoologists. It turns out that they are fungi, and Redhead et al. (2009) discussed the nomenclatural issues resulting from this realization. Basically, Microsporidia had been described under the rules of the International Code of Zoological Nomenclature (ICZN). This was problematic because Latin diagnoses were not provided, as was required for fungal descriptions, which meant that names of numerous Microsporidia would be invalid. Under the ICBN (Vienna Code) (McNeill et al. 2006), rules were put into place to address this situation, and these stated that if an author of a fungal name thought the organism was something covered by the rules of another code, then the name was validly published as long as that other code was followed, even if the organism was a fungus. This seemed to solve the problem, and a large number of names of Microsporidia were saved from being declared invalid, except that those working with Microsporidia began to say that they were fungi and still kept using the ICZN. Those researching Microsporidia subsequently asked for them to be excluded from the ICBN and covered by the ICZN, which could already do so under one of its articles. Redhead et al. (2009) discussed this and made formal proposals to modify the ICBN to do just that, and Demoulin (2010) provided another proposal to extend this idea to other organisms historically treated under other codes. These proposals passed at the IBC in Melbourne (Hawksworth 2011; McNeill and Turland 2011b; Norvell 2011b). Practically speaking for mycologists, if you want to examine nomenclatural issues of Microsporidia and/or describe new taxa, learn to use the ICZN (International Commission on Zoological Nomenclature 1999).

Typification of sanctioned names. Under the ICBN (Vienna Code) (McNeill et al. 2006), typification of sanctioned names was confusing, and sanctioned names could be typified "in the light of anything associated with the name in that work," meaning the sanctioning work. The protologue or original description of a sanctioned name often predated the sanctioning work, and other rules of the Code stated that a lectotype is to be chosen and designated from the original material (material associated with the protologue) used originally to describe a taxon if no holotype exists. However, material associated with the sanctioning work may not have been part of the original material, and it was unclear as to what such a type, if designated with later material, should be called and which of the potentially conflicting rules should be followed when typifying a sanctioned name. Other complications related to this matter are not discussed here, but Redhead et al. (2010) proposed a set of rule changes to address this matter that would either delete the rule about typifying in light of anything associated with the name in that sanctioning work (ICBN Art. 7.8) (McNeill et al. 2006) or modify a number of rules to create a new kind of type called the sanctiotype for sanctioned names. Perry (2011) provided competing proposals that would not employ the term sanctiotype. The NCF recommended Redhead's proposals relating to the introduction of the sanctiotype (Norvell 2011a). At the IBC in Melbourne, the proposers of these competing options and a few others met to resolve differences, and when it became apparent that the term sanctiotype was hated enough by most at the IBC to be rejected, a compromise was reached (Norvell

2011b). Under the ICN, **a sanctioned name may be typified by an element associated with the protologue and/or the sanctioning work, and this is a lectotype**. Several works (Hawksworth 2011; McNeill et al. 2011; Norvell 2011b) provide more details on the compromise and the changes to the typification of sanctioned names.

Problems with fungal cultures as types. Living fungal cultures may not serve as types of fungal names, and if they are proposed as the type of a new fungal name, that name is invalid. Cultures of fungi permanently preserved in a metabolically inactive state, however, are acceptable as types. Having read a number of publications, I have found it depressing to note how many fungal names are invalid because authors overlooked this requirement of valid publication. Additionally, many authors fail to note the preservation status of cultures, which requires subsequent investigation. Nakada (2010) noted this problem and several other issues such as culture collections periodically reviving cultures and cultures not being deposited in an inactive state. To help clarify matters related to valid publication, Nakada (2010) proposed that a recommendation be added to the Code that the phrase "permanently preserved in a metabolically inactive state" be given when a culture is designated as a type. This proposal was adopted at the IBC in Melbourne (Hawksworth 2011; McNeill et al. 2011; Norvell 2011b). It is often easiest to **dry a culture and deposit it in a herbarium as the type of a taxon and submit a subculture obtained prior to drying to culture collections as the ex-type**.

C. Practical Notes on Using the ICN

The following notes on how to perform frequently used nomenclatural procedures are taken and modified from a document prepared by A.M. Minnis, K.A. Seifert, S.A. Redhead, and R.E. Halling for Mycologia (http://www.mycologia.org/site/misc/FAQvers2.xhtml).

Nomenclatural Procedures FAQ (ICN/Melbourne Code): Help and Checklist for Authors.

This document is designed to help authors avoid common mistakes in frequently used nomenclatural procedures. For questions not covered here, please consult an expert and/or the current version of the International Code of Nomenclature for algae, fungi, and plants (ICN) at http://www.iapt-taxon.org/nomen/main.php?page=title. Following several changes that were adopted at the IBC in Melbourne in July 2011, this FAQ has been modified from earlier versions.

Note: In several examples that follow, the symbol ≡ is used to indicate a homotypic (sometimes called a nomenclatural or obligate) synonym, which is a synonym based on the same type. The symbol = should be used to indicate a heterotypic (sometimes called a taxonomic or facultative) synonym, which is a synonym based on a different type.

1. How Do I Describe New Taxa at the Rank of Species?

a) Validation

Ensure that the new name is in Latin (or is acceptably Latinized). Construct the name according to ICN Arts. 23 and 60. Make sure that the epithet conforms to recommendations and conventions if dedicating the name to a person or place or referring to growth on a substrate or host. Pay special attention to the gender of generic names and corresponding adjectival species epithets. (However, this rule does not apply to epithets that are nouns; they retain their own gender and never change their endings.)

Provide an English or Latin description or diagnosis for your taxon (or provide a full and direct reference to a previously published English or Latin description or diagnosis uniquely applicable to your fungus). Have the diagnosis/description checked by an expert in the language that is used.

- You may use a single English or Latin description or diagnosis (i.e., *a descriptio generico-specifica*) for both a new genus **and** a new species if there is a single species in the new genus and **both are new**.

Designate a holotype (authors must use the word "holotypus" or "holotype," alternatively

"typus" or "type") and cite the **single herbarium** or place the specimen is housed. See Index Herbariorum or other biorepository for proper institutional codes. The phrase "hic designatus" (designated here) is not required.

- Designate a single collection made at one place and time represented as a single specimen in a single institute (list the collector, date, and collection number);
- or a permanently metabolically inactive culture or tissue (e.g., frozen, dried, or pickled) designated by a unique reference and in a single institute (write "permanently preserved in a metabolically inactive state" and indicate the method of preservation to ensure that readers know the type is not a living culture or one that is in a temporarily inactive state);
- or an effectively published illustration (concurrently or previously published) **if and only if** there are technical difficulties preserving a collection of a microfungus. If previously published, a full and direct reference to the place of prior publication is required.

Do not indicate that the "holotype" is in several institutes. Duplicates of the holotype collection are isotypes when deposited elsewhere or are otherwise separate from the holotype. Cultures derived from the holotype, or used to generate the holotype, are themselves not types. Because preserved cultures can serve as "type," do not indiscriminately cite both a specimen and a culture as type. Ensure one is specifically designated as holotype and specifically state where that single type is located. (Otherwise the name will be invalid.)

Do not provide **alternative** Latin names for the same taxon. (Otherwise all will be invalid unless allowed by ICN Art. 59 for pleomorphic fungi until the end of 2012.)

Do not suggest that your new scientific name is tentative, provisional, a temporary fix or express any other doubt about accepting a name for a new taxon. (Otherwise it is invalid.)

You must register your name with MycoBank, obtain a MycoBank registration number, and present it in the protologue. Registration with another of two repositories, Fungal Names or Index Fungorum, that have been officially approved for this purpose (Redhead and Norvell 2013) and the listing of the identifier provided by that repository in the protologue is also acceptable for valid publication.

b) Legitimization

Ensure that you do not publish a later homonym, a name spelled exactly like an earlier valid name (regardless of whether this is legitimate or illegitimate), or one confusingly closely spelled. Later homonyms are illegitimate.

You can and should use MycoBank and Index of Fungi or Index Fungorum to check for earlier potential fungal homonyms and Index Kewensis and other sources via the International Plant Names Index or Tropicos or AlgaeBase for many "botanical" (covered by the ICN) names. Check Index Nominum Genericorum to ensure that the generic name in which you are publishing your species is uniquely fungal. If there are other valid "botanical" homonyms at the generic rank, consider that there is the potential for you to create a later homonym at the species rank to a species in that other genus. You should check GenBank and the World Wide Web for any such uses regardless of whether they are valid or legitimate.

Homonyms for Bacteria and Archaea and for animals (including protozoa) are **covered by other codes**. You may create homonyms, but **this should be avoided.**

Example (fictional; not intended for valid publication):

Lodgea pini E.E. Sm., sp. nov.

MycoBank MB9876543

English diagnosis: this species is distinguished from others in the genus by its brown pileus and yellow pore surface.

Typus: USA: Idaho, Valley Co., near McCall, on soil associated with stands of *Pinus* sp., 07/17/1910, coll. *E. E. Smith*, *E.E. Smith 22* (F).

Seifert and Rossman (2010) have provided some additional guidance on describing new fungal species.

2. How Do I Describe New Taxa at the Rank of Genus?

Construct the name according to ICN Arts. 20 and 60. Follow recommendations 20A.1(h) and 60B when dedicating the name of the genus to a person. Check databases (see earlier discussion) to make sure the generic name has not been used already. A MycoBank, Fungal Names, or Index Fungorum registration number must be obtained and listed for all new names.

An English or Latin diagnosis or description must be supplied when describing a new genus or any other taxon. Have the diagnosis/description checked by an expert in the language that is used.

Designate the type of the genus by citing the name of one previously or concurrently validly published species. Use the word "typus" or "type."

Example (fictional; not intended for valid publication):
Lodgea E.E. Sm., gen. nov.
MycoBank MB457896
Latin diagnosis: *Similis* Hygrocybe *sed hymenio poroso differt.*
Typus: *Lodgea pini* E.E. Sm.

3. What Is an Ex-type?

A living culture obtained from a type may be referred to as an ex-type (see ICN Article 8 for more information). It is linked to the type, but it is not the same as the type. Depending on the nature of the type, it may be called, for example, an ex-holotype, an ex-neotype, or an ex-epitype. Such cultures, as well as the place where the living culture is preserved, should be indicated in publications, especially for new taxa. This information is often listed next to the type designation.

Example (fictional; not intended for valid publication):
Lodgea pini E.E. Sm., sp. nov.
MycoBank MB457896
Latin diagnosis: *Pileo brunneo. Poris luteis.*
Typus: USA: Idaho, Valley Co., near McCall, on soil associated with stands of *Pinus* sp., 07/17/1910, coll. *E. E. Smith*, *E.E. Smith 22* (F); ex-type CBS 4567493.

4. How and When Do I Designate a Lectotype for a Species?

A lectotype is designated when there was no holotype in the original description or if it has been lost or destroyed. Rarely, a lectotype may be designated when the holotype belongs to more than one taxon (see ICN Art. 9 for more information).

A lectotype is a designated specimen or illustration that is part of the original material. Simply speaking, original material consists of specimens and published or unpublished illustrations that were definitely used in the original description of a name. For sanctioned fungal names, the former material and/or any element associated with the name in the sanctioning treatment (equivalent to original material) may be used for lectotypification (see ICN Articles 9.2, 9.3, and 9.10).

When designating a lectotype for a name that is not sanctioned, priority must be given to the following types of materials in the order given:

1. Isotype (see ICN Art. 9.4);
2. Syntype (also possibly an isosyntype) (see ICN Art. 9.5);
3. Paratype (see ICN Art. 9.6);
4. Uncited specimen, uncited illustration, cited illustration.

On or after 1 January 1990, the herbarium housing the specimen or unpublished illustration must be cited, and on or after 1 January 2001, the term "lectotypus" or "lectotype" must be given along with the phrase "hic designatus" or "designated here." A full and direct reference to the place of publication of previously published illustrations should be given, and it is ideal if the illustration can be reproduced in the current work. Lectotypification is only achieved through effective publication. In the case of accepted names based on a basionym (legitimate, previously published name on which a new combination or name at a new rank is based and that provides the final epithet or stem of such a name) or replaced synonym

(valid name on which a replacement name is based and that does not provide the final epithet, etc.), the basionym or replaced synonym should be the name that is lectotypified.

Example (fictional):

Pseudocercospora nyssicola (Peck) Peck, Mycologia 3: 377. 1911.

≡ *Cercospora nyssicola* Peck, Mycologia 1: 100. 1909.

Lectotypus of *Cercospora nyssicola* (hic designatus): USA: Louisiana, near LSU, on leaves of *Nyssa*, 07/12/1907, coll. *Peck, Peck 1239* (BPI).

5. How and When Do I Designate a Neotype for a Species?

A neotype is designated when no original material (specimens and published or unpublished illustrations that were definitely used in the original description of a name) exists. See earlier notes on sanctioned fungal names. With rare exception, a lectotype designated from original material supersedes a neotype. Thus, it is important not to overlook any original material when considering a neotype designation.

A neotype is a specimen or illustration, preferably the former. Special consideration should be given so that the designated neotype matches the material described in the protologue in nearly every regard. For example, a *Puccinia* on *Rosa* from China should not be chosen as a neotype specimen for a *Puccinia* species described on *Potentilla* from Ireland since there is a significant risk that they may not represent the same taxon.

On or after 1 January 1990, the herbarium housing the specimen or unpublished illustration must be cited, and on or after 1 January 2001, the term "neotypus" or "neotype" must be given along with the phrase "hic designatus" or "designated here." A full and direct reference to the place of publication of previously published illustrations should be given, and it is ideal if the illustration can be reproduced in the current work. Neotypification is only achieved through effective publication. In the case of accepted names based on a basionym or replaced synonym, the basionym or replaced synonym should be the name that is neotypified.

Example (fictional):

Amanita nyssae (Peck) Peck, Mycologia 5: 9. 1913.

≡ *Agaricus nyssae* Peck, Mycologia 2: 39. 1910.

Neotypus of *Agaricus nyssae* (hic designatus): USA: Louisiana, near Baton Rouge, scattered, associated with *Nyssa sylvatica*, 10/25/2001, coll. *Tulloss, Tulloss 2211* (NYS).

6. How and When Do I Designate an Epitype for a Species?

An epitype is designated when the existing nomenclatural type (holotype, lectotype, or neotype) or all the original material is not sufficient to allow for precise application of a name. An example of this would be an agaric species where the stipe of the holotype is missing but the stipe is critical for species recognition. In this case, an epitype with a stipe displaying the critical features may be designated to support the existing holotype. Many mycologists working with culturable fungi designate epitype specimens associated with a separate living culture so that DNA data and cultural characters needed to recognize a species are associated with the type of a name. Others use the epitype to link asexual and sexual states of the same fungus.

An epitype is a specimen or illustration, but a specimen should nearly always be employed. Only one epitype is allowed per name. Thus, it must be carefully chosen, and authors should ensure that the epitype represents the same taxon as the type it supports.

For an epitypification to be effected, the herbarium housing the specimen or unpublished illustration must be cited or, in the case of a published illustration, a full and direct bibliographic reference must be given, and on or after 1 January 2001, the term "epitypus" or "epitype" must be given along with the phrase "hic designatus" or "designated here." Additionally, the nomenclatural type (holotype, lectotype, or neotype) that the epitype supports must be explicitly cited. Epitypification is only achieved through effective publication. In the case of accepted names based on a basionym or replaced synonym,

the basionym or replaced synonym should be the name that is epitypified.

Example (fictional):

Amanita nyssae (Peck) Peck, Mycologia 5: 9. 1913.

≡ *Agaricus nyssae* Peck, Mycologia 2: 39. 1910.

Neotypus of *Agaricus nyssae* (designated by R.E. Tulloss, Mycotaxon 82: 54. 2002): USA: Louisiana, near Baton Rouge, scattered, associated with *Nyssa sylvatica*, 10/25/2001, coll. *Tulloss*, *Tulloss 2211* (NYS).

Epitypus of *Agaricus nyssae* (hic designatus): USA: Louisiana, near Baton Rouge, solitary, associated with *Nyssa sylvatica*, 10/31/2007, coll. *Methven*, *ASM 55891* (EIU).

Notes: The stipe of the neotype is missing and its preservation in chemicals prevents PCR amplification. Here, we designate a supporting epitype with stipe that is associated with DNA sequence data.

7. How Do I Validly Publish New Combinations?

The rules for publishing new combinations are covered in large part and in more detail in ICN Arts. 35, 37, and, especially, 41. The basionym must be cited with a clear and direct reference to its place of valid publication. For this, authors making new combinations must include the journal and volume or book title, the page where the protologue begins (be sure not to cite the entire pagination of the whole publication that includes the protologue), and the date. Authors should make sure that adjectival species epithets agree grammatically with the genus in making new combinations (e.g., *Agaricus americanus* becomes *Lepiota americana* instead of *Lepiota americanus*). A MycoBank, Fungal Names, or Index Fungorum registration number must be obtained and listed for new combinations.

Examples (both fictional):

Alternaria nyssicola (Peck) E.G. Simmons, comb. nov.

MycoBank MB124578

≡ *Stemphylium nyssicola* Peck, Mycologia 3: 375. 1911 (basionym).

≡ *Ulocladium nyssicola* (Peck) Minnis, Mycologia 100: 22. 2008.

Pseudocercospora nyssicola (Peck) A.H. Sm., comb. & stat. nov.

MycoBank MB654826

≡ *Cercospora apii* var. *nyssicola* Peck, Mycologia 3: 376. 1911 (basionym).

In the second example, the new combination also changes the rank from variety to species.

8. How Do I Validly Publish a Replacement Name Also Known as a Nomen Novum?

Replacement names are similar to new combinations, but they are made in cases where there is an illegitimate later homonym or when the epithet of the basionym is already occupied in the genus where a new combination is required. The replaced synonym (not a basionym since the epithet is not being used in the new name) must be cited with a clear and direct reference to its place of valid publication. For this, authors making replacement names must include the journal and volume or book title, the page where the protologue begins (be sure not to cite the entire pagination of the whole publication that includes the protologue), and the date. Authors should make sure that species epithets agree grammatically with the genus of their new name. It is also suggested that authors include a citation including a full and direct reference for the earlier homonym or species name already occupying a genus that necessitates the replacement name. A MycoBank, Fungal Names, or Index Fungorum registration number must be obtained and listed for replacement names.

Examples (both fictional):

Nectria peckii Rossman, nom. nov.

MycoBank MB124669

≡ *Nectria cinnabarina* Peck, Mycologia 3: 377. 1911 (replaced synonym), non *Nectria cinnabarina* (Tode : Fr.) Fr., Summa vegetabilium Scandinaviae 2: 388. 1849.

In the preceding example, there is an illegitimate later homonym (Peck's "*cinnabarina*") and a cited earlier legitimate homonym (Fries' "*cinnabarina*").

Phoma braunii Rossman, nom. nov.

MycoBank MB222223

≡ *Phyllosticta cinnabarina* Peck, Mycologia 5: 123. 1913 (replaced synonym), non *Phoma cinnabarina* Fr., Summa vegetabilium Scandinaviae 2: 390. 1849.

In the preceding example, the epithet "*cinnabarina*" is already occupied in *Phoma*. The new combination "*Phoma cinnabarina*" based on Peck's species would create a later homonym.

9. How Do I Correctly Give Author Citations for Taxa?

Complete details about author citations for taxa are found in ICN Arts. 46–50. For existing fungal names, correct author citations may be found (usually) in Index Fungorum and MycoBank. In detailed taxonomic studies, authors should attempt to carefully verify that these databases are correct since they are not perfect. For new names, including new combinations, authors should include author citations for such taxa. These author citations are not necessarily the same as the authorship for the whole publication. Abbreviations for authors of fungi and plants should follow the standards established by the International Plant Names Index (IPNI), and in cases where a standardized abbreviation does not yet exist, authors should still attempt to conform to IPNI practices. Authors should be linked by the use of an "&" and the serial comma is not employed.

Example (fictional):
Chaetomium oregonense T.C. Harr., H.Y. Su & Spatafora, sp. nov.

III. Potential Changes to Nomenclatural Rules

A number of possible modifications to the ICN are currently being considered, and some of these may be implemented in the future. In the following text, some of these ideas and background information are given, but in general, details from cited works are not because the proposals are preliminary and have not yet been adopted in the Code.

More changes relating to Art. 59 and the transition to one name per fungus: During the debate about whether or not to transition to a one-name-per-fungus system of classification, strong feelings about philosophical principles of maintaining dual nomenclature or abandoning it were voiced. Unfortunately and in general, relatively little attention was given to the actual details of potential rules to make a transition to one name per fungus happen, and numerous experts of nomenclature that really know the Code well, but opposed the transition, did not assist the voices calling loudly for change. Gams et al. (2012a, b) brought up a number of considerations, challenges, and needed clarifications about the adopted rules for the transition shortly after it was passed, and many of the finer details may have to be addressed through additional modification of the ICN (Hawksworth 2014).

A number of possible proposals that would tinker with the current transition to a one-name-per-fungus system of classification have already been presented. Hawksworth et al. (2013) have suggested that if a new species name were erected for a morph or state of an already existing species name having the corresponding morph or state under dual nomenclature and the two species names shared the same epithet, the later name should be treated as a new combination rather than a new species name and share the same type as the earlier species name. The later type would then have no standing under the ICN. This approach may be overly complicated since it requires an understanding of old rules that have been deleted from the Code. There is a danger that the two species names do not represent the same fungus, and the application of the name based on the later type might be more prevalent in the literature. In noting existing confusion about terminology associated with lists of fungal names to be treated as conserved via ICN Art. 14.13 or treated as rejected via ICN Art. 56.3, Hawksworth (2014) has proposed that the term "**protected**" be introduced for the former situation and "**suppressed**" for the latter. Hawksworth (2014) has also proposed that fungal names listed via ICN Art. 14.13 be protected

against all unlisted names. Informal discussion of the NCF also suggests that the term "widely used" and Art. 57.2 in general are likely to be modified in the next Code.

Registration of typifications of existing names: The same problems of scattered mycological literature and the proliferation of numerous journals that make it valuable to construct a fungal index through required registrations of fungal names also plague later typifications, such as lectotypification, neotypification, and epitypification, of existing names. Shortly after Hawksworth et al. (2010) proposed registration of fungal names as a requirement for valid publication, Gams (2010) proposed that a similar type of registration be required for typification of existing names. The NCF recommended that this proposal be adopted (Norvell 2011a), but it was rejected at the IBC in Melbourne (McNeill et al. 2011). Hawksworth (2014) has since then suggested draft proposals for registration of typifications of existing names, and MycoBank now has a utility for just such an action.

Naming of environmental sequences: With the emergence of new DNA sequencing technologies, the accumulation of large amounts of DNA sequence data from environmental samples in a short time has outpaced the speed at which taxonomists address the multitude of undescribed fungi, some of which are seemingly known only from environmental data. As a result, a few mycologists have proposed that these sequences be formally named and have proposed some possible mechanisms by which this might happen (Hawksworth et al. 2011; Hibbett and Taylor 2013; Hibbett et al. 2011; Taylor 2011). Challenges include sequencing errors, incomplete sampling of named taxa, and intragenomic variation of markers like the ITS, among others (Hibbett and Taylor 2013; Hibbett et al. 2011; Lindner and Banik 2011; Lindner et al. 2013; Taylor 2011). It has also been proposed that fungal naming be completely automated by computer programs (Hibbett and Taylor 2013; Taylor 2011). Typification of taxa known only from environmental samples remains a problem because it is not clear whether such material or data obtained from it would qualify as a type under the ICN, and many mycologists object to the idea that a gram of soil or piece of cow manure filled with large numbers of organisms, some as a spore or two, can serve as a suitable standard for the application of a name or names. Nevertheless, some authors have begun to explore the following questions: What is the absolute minimum that is required to publish a new fungal name? And can this be done with only DNA sequence data? The NCF is currently debating many complicated aspects brought up by these questions in deliberations on whether or not some recently and minimally described fungi are valid names (Tripp and Lendemer 2012). The online serial publication provided by Index Fungorum (http://www.indexfungorum.org/Names/IndexFungorumRegister.htm) has other similar examples. Large-scale naming of fungi based on environmental sequences will likely require modification of the ICN. Given that next-generation sequencing technology is advancing rapidly, the field of molecular ecology is still in its relative infancy, and many taxonomists feel that ITS data alone are entirely inadequate, it may be hasty to rush into the formal description and naming of a large number of environmental sequences based on one marker at this time.

Other changes being considered: Hawksworth (2014) has offered a grab bag of possible proposals for consideration that include issues such as extension of sanctioning to additional works, extending conservation to additional ranks, and more. These will not be considered in detail herein. Kirk et al. (2013) have shown an interest in expanding the coverage of the lists for dealing with the transition to a one-name-per-fungus system of classification to a protected list for all fungal genera via existing rules of the ICN, even though this was not the intent of Redhead's proposal.

IV. Conspectus and the Future of Fungal Naming

Precise communication about fungi and funguslike organisms relies upon the use of names, and the application of a name is determined by means of a nomenclatural type, i.e., a standard. This chapter provides a basic treat-

ment of changes to fungal nomenclature as implemented by the current International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), or ICN. The transition to a one-name-per-fungus system of classification has begun. Several working groups or subcommissions have already organized in loose association with the International Commission on the Taxonomy of Fungi, and a number of them have made progress on lists of names, such as that offered by the Leotiomycetes Working Group (Johnston et al. 2014). The NCF is currently in the process of beginning to look at the lists of names recommended by these groups. It remains to be seen how much progress will be made before the next International Botanical Congress in Shenzhen in 2017. Additionally, essential and basic nomenclatural rules and terminology and notes on how to perform frequently used nomenclatural procedures correctly are covered in this chapter. Most nomenclature must be self-taught because of the lack of courses and experts, and this chapter attempts to provide guidance in its use.

What is the future of fungal naming? Although some mycologists (Money 2013) feel that we should give up on the naming of fungi, fungal names will continue to provide an essential tool for communicating everything that is known about these important organisms. With the incredibly large number of undescribed fungi, the prospects of cataloging this diversity are certainly exciting and daunting for those who are willing and able to do so. Names will be needed to discuss critical ecological questions on how environmental change affects major ecosystems and human welfare. Names will be needed to put together the Fungal Tree of Life and to fill in missing clades as well as help understand fungal evolution and the origins of structural and biochemical diversity (McLaughlin et al. 2009). We mycologists have learned over the years that modifications to the Code create more work and instability of names, but it is hoped that completing the transition to a one-name-per-fungus system of classification will make it easier to communicate about fungi for generations to come. Samuels (2011) provided valuable insights as to his own intellectual struggles regarding a possible transition to a one-name-per-fungus system of classification, and perhaps his most valuable lesson for fungal systematists is to make sure that the most productive times in a career are spent doing exciting and worthwhile mycology. In the words quoted by Walter J. Sundberg, my sorely missed doctoral advisor, are we having fun yet? As for the future of fungal naming, I guess we will find out what we make of it.

References

Blackwell M (2011) The fungi: 1,2,3…5.1 million species? Am J Bot 98:426–438

Braun U (2012) The impacts of the discontinuation of dual nomenclature of pleomorphic fungi: the trivial facts, problems, and strategies. IMA Fungus 3: 81–86

Braun U, Farr DF, Minnis AM (2009) Nomenclatural notes on some cercosporoid hyphomycetes. Schlechtendalia 19:81–84

Bureau International des Poids et Mesures (BIPM) (2006) Le Système international d'unités (SI), 8th edn. STEDI Media, Paris

Chapman AD, Turland NJ, Watson MF (2010) Report of the special committee on electronic publication. Taxon 59:1853–1862

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. Stud Mycol 50: 19–22

Crous PW, Giraldo A, Hawksworth DL, Robert V, Kirk PM, Guarro J, Robbertse B, Schoch CL, Damm U, Trakunyingcharoen T, Groenewald JZ (2014) The genera of Fungi: fixing the application of type species of generic names. IMA Fungus 5:141–160

Dearness J (1941) New species of Tennessee fungi. Mycologia 33:360–366

Demoulin V (2010) (185–190) Proposals to amend Articles 15, 36 and 45. Taxon 59:1611–1612

Farr DF, Rossman AY (2014) Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases. Cited 19 Jun 2014

Gams W (2010) (183–184) Proposals to require deposition of information concerning typification of names of fungal taxa, with an associated recommendation. Taxon 59:1610–1611

Gams W, Jaklitsch W (2011) Fungal nomenclature 3. A critical response to the 'Amsterdam Declaration'. Mycotaxon 116:501–512

Gams W, Baral HO, Jaklitsch WM, Kirschner R, Stadler M (2012a) Clarifications needed concerning the new Article 59 dealing with pleomorphic fungi. IMA Fungus 2:175–177

Gams W, Humber RA, Jaklitsch W, Kirschner R, Stadler M (2012b) Minimizing the chaos following the loss

of Article 59: suggestions for a discussion. Mycotaxon 119:495–507

Greuter W, Barrie FR, Burdet HM, Chaloner WG, Demoulin V, Hawksworth DL, Jørgensen PM, Nicolson DH, Silva PC, Trehane P, McNeill J (eds) (1994) International Code of Botanical Nomenclature (Tokyo Code) adopted by the Fifteenth International Botanical Congress Yokohama, August–September 1993. Regnum vegetabile no. 131. Koeltz Scientific Books, Königstein

Hawksworth DL (2001) The naming of fungi. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol VII, Systematics and evolution, Part B. Springer, Berlin, pp 171–192

Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. IMA Fungus 2:155–162

Hawksworth DL (2012) Managing and coping with names of pleomorphic fungi in a period of transition. IMA Fungus 3:15–24

Hawksworth DL (2013) Mycospeak and biobabble. IMA Fungus 4:1

Hawksworth DL (2014) Possible house-keeping and other draft proposals to clarify or enhance the naming of fungi within the International Code of Nomenclature for algae, fungi, and plants (ICN). IMA Fungus 5:31–37

Hawksworth DL, Crous PW, Dianese JC, Gryzenhout M, Norvell LL, Seifert KA (2009) (016–020) Proposals to amend the Code to make clear that it covers the nomenclature of fungi, and to modify its governance with respect to names of organisms treated as fungi. Taxon 58:658–659

Hawksworth DL, Cooper JA, Crous PW, Hyde KD, Iturriaga T, Kirk PM, Lumbsch HT, May TW, Minter DW, Misra JK, Norvell LL, Redhead SA, Rossman AY, Seifert KA, Stalpers JA, Taylor JW, Wingfield MJ (2010) (117–119) Proposals to make the pre-publication deposit of key nomenclatural information in a recognized repository a requirement for valid publication of organisms treated as fungi under the Code. Taxon 59:660–662

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, Abaci O, Aime C, Asan A, Bai FY, de Beer ZW, Begerow D, Berikten D, Boekhout T, Buchanan PK, Burgess T, Buzina W, Cai L, Cannon PF, Crane JL, Damm U, Daniel HM, van Diepeningen AD, Druzhinina I, Dyer PS, Eberhardt U, Fell JW, Frisvad JC, Geiser DM, Geml J, Glienke C, Gräfenhan T, Groenewald JZ, Groenewald M, de Gruyter J, Guého-Kellermann E, Guo LD, Hibbett DS, Hong SB, de Hoog GS, Houbraken J, Huhndorf SM, Hyde KD, Ismail A, Johnston PR, Kadaifciler DG, Kirk PM, Kõljalg U, Kurtzman CP, Lagneau PE, Lévesque CA, Liu X, Lombard L, Meyer W, Miller A, Minter DW, Najafzadeh MJ, Norvell L, Ozerskaya SM, Öziç R, Pennycook SR, Peterson SW, Pettersson OV, Quaedvlieg W, Robert VA, Ruibal C, Schnürer J, Schroers HJ, Shivas R, Slippers B, Spierenburg H, Takashima M, Taşkn E, Thines M, Thrane U, Uztan AH, van Raak M, Varga J, Vasco A, Verkley G, Videira SIR, de Vries RP, Weir BS, Yilmaz N, Yurkov A, Zhang N (2011) The Amsterdam declaration on fungal nomenclature. IMA Fungus 2:105–112

Hawksworth DL, McNeill J, de Beer ZW, Wingfield MJ (2013) Names of fungal species with the same epithet applied to different morphs: how to treat them. IMA Fungus 4:53–56

Hibbett DS, Taylor JW (2013) Fungal systematics: is a new age of enlightenment at hand? Nat Rev Microbiol 11:129–133

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH (2011) Progress in molecular and morphological taxon discovery in fungi and options for formal classification of environmental sequences. Fungal Biol Rev 25:38–47

Hoog GS de, Chaturvedi V, Denning DW, Dyer PS, Frisvad JC, Geiser D, Gräser Y, Guarro J, Haase G, Kwon-Chung K-J, Meiss JF, Meyer W, Pitt JI, Samson RA, Taylor JW, Tintelnot K, Vitale RG, Walsh TJ, Lackner M (2014) Name changes in medically important fungi and their implications for clinical practice. J Clin Microbiol. doi:10.1128/JCM.02016-14

International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature, 4th edn. International Trust for Zoological Nomenclature, London

Johnston PR, Seifert KA, Stone JK, Rossman AY, Marvanová L (2014) Recommendations on generic names competing for use in Leotiomycetes (Ascomycota). IMA Fungus 5:91–120

Judith C, Rossman A (2014) Revised scientific names of the genus *Hemileia* (Pucciniales) based on the new ICN. MycoKeys 8:1–10

Kirk PM, Stalpers JA, Braun U, Crous PW, Hansen K, Hawksworth DL, Hyde KD, Lücking R, Lumbsch TH, Rossman AY, Seifert KA, Stadler M (2013) A without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi, and plants. IMA Fungus 4:381–443

Knapp S, McNeill J, Turland NJ (2011) Changes to publication requirements made at the XVIII International Botanical Congress in Melbourne: what does e-publication mean for you? Taxon 60: 1498–1501

Lindner DL, Banik MT (2011) Intragenomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus*. Mycologia 103:731–740

Lindner DL, Carlsen T, Nilsson RH, Davey M, Schumacher T, Kauserud H (2013) Employing 454 amplicon pyrosequencing to reveal intragenomic

divergence in the internal transcribed spacer rNDA region in fungi. Ecol Evol 3:1751–1764
Luo J, Zhang N (2013) *Magnaporthiopsis*, a new genus in Magnaporthaceae (Ascomycota). Mycologia 105:1019–1029
McLaughlin DJ, Hibbett DS, Lutzoni F, Spatafora JW, Vilgalys R (2009) The search for the fungal tree of life. Trends Microbiol 17:488–497
McNeill J, Turland NJ (2011a) Synopsis of proposals on botanical nomenclature—Melbourne 2011: a review of the proposals concerning the International Code of Botanical Nomenclature submitted to the XVIII International Botanical Congress. Taxon 60:243–286
McNeill J, Turland NJ (2011b) Major changes to the Code of Nomenclature—Melbourne, July 2011. Taxon 60:1495–1497
McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (eds) (2006) International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress, Vienna, Austria, July 2005. Regnum vegetabile no. 146. Gantner, Ruggell
McNeill J, Turland NJ, Monro AM, Lepschi BJ (2011) XVIII International Botanical Congress: preliminary mail vote and report of Congress action on nomenclature proposals. Taxon 60:1507–1520
McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ (eds) (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum vegetabile no. 154. Koeltz Scientific Books, Königstein
Milius S (2014) The name of the fungus: genetic advances spur mycologists to put their kingdom in order. Sci News 185:22–26
Minnis AM, Kennedy AH, Grenier DB, Palm ME, Rossman AY (2012) Phylogeny and taxonomic revision of the Planistromellaceae including its coelomycetous anamorphs: contributions towards a monograph of the genus *Kellermania*. Persoonia 29:11–28
Money NP (2013) Against the naming of fungi. Fungal Biol 117:463–465
Nakada T (2010) (138) A proposal on the designation of cultures of fungi and algae as types. Taxon 59: 983
Norvell LL (2011a) Report of the nomenclature committee for fungi: 17. Taxon 60:610–613
Norvell LL (2011b) Fungal nomenclature. 1. Melbourne approves a new Code. Mycotaxon 116:481–490
Norvell LL, Hawksworth DL, Petersen RH, Redhead SA (2010) Fungal nomenclature. 1. The IMC9 Edinburgh Nomenclature Sessions. Mycotaxon 113: 503–511
Perry G (2011) Comments on proposals for the Melbourne Congress dealing with original material and the typification of sanctioned names. Taxon 60:916–919
Rambold G, Stadler M, Begerow D (2013) Mycology should be recognized as a field in biology at eye level with other major disciplines—a memorandum. Mycol Prog 12:455–463
Redhead SA (2010a) (098–100) Proposals to limit hardcopy publication of Appendices II–V. Taxon 59: 311
Redhead SA (2010b) Report on the special committee on the nomenclature of fungi with a pleomorphic life cycle. Taxon 59:1863–1866
Redhead SA, Norvell LL (2013) Report of the nomenclature committee for fungi 19: official repositories for fungal names. Taxon 62:173–174
Redhead SA, Kirk PM, Keeling PJ, Weiss LM (2009) (048–051) Proposals to exclude the phylum Microsporidia from the Code. Taxon 58:669
Redhead SA, Norvell LL, Pennycook SR (2010) (223–232) Proposals to amend articles regulating the typification of names in sanctioning works. Taxon 59: 1910–1913
Rossman AY (2014) Lessons learned from moving to one scientific name for fungi. IMA Fungus 5:81–89
Rossman AY, Seifert KA, Samuels GJ, Minnis AM, Schroers HJ, Lombard L, Crous PW, Põldmaa K, Cannon PF, Summberbell RC, Geiser DM, Zhuang W-Y, Hirooka Y, Herrera C, Salgado-Salazar C, Chaverri P (2013a) Genera in Bionectriaceae, Hypocreaceae, and Nectriaceae (Hypocreales) proposed for acceptance or rejection. IMA Fungus 4:41–51
Rossman AY, Manamgoda DS, Hyde KD (2013b) Proposal to conserve the name *Bipolaris* against *Cochliobolus* (Ascomycota: Pleosporales: Pleosporaceae). Taxon 62:1331–1332
Samuels G (2011) Some thoughts about naming anamorphs and teleomorphs. MycoAfrica 4(2):11–14
Seifert KA (2014) In defence of the terms holomorph, teleomorph, and anamorph. IMA Fungus 4:55–56
Seifert KA, Rossman AY (2010) How to describe a new fungal species. IMA Fungus 1:109–116
Smith GF, Figueiredo E, Moore G (2011) English and Latin as alternative languages for validating the names of organisms covered by the International Code of Nomenclature for algae, fungi, and plants: the final chapter? Taxon 60:1502–1503
Summerbell RC, Gueidan C, Schroers H-J, de Hoog GS, Starink M, Rosete YA, Guarro J, Scott JA (2011) *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. Stud Mycol 68:139–162
Taylor JW (2011) One fungus = one name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2:113–120

Tripp EA, Lendemer JC (2012) (4–5) Request for binding decisions on the descriptive statements associated with *Mortierella sigyensis* (fungi: Mortierellaceae) and *Piromyces cryptodigmaticus* (fungi: Neocallimastigaceae). Taxon 61:886–888

Weresub LK, Pirozynski KA (1979) Pleomorphism of fungi as treated in the history of mycology and nomenclature. In: Kendrick B (ed) The whole fungus; the sexual-asexual synthesis. National Museums of Canada, Ottawa, pp 17–30

Wingfield MJ, de Beer ZW, Slippers B, Wingfield BD, Gronewald JZ, Lombard L, Crous PW (2012) One fungus, one name promotes progressive plant pathology. Mol Plant Pathol 13:604–613

Zhang N, Rossman AY, Seifert K, Bennett JW, Cai G, Cai L, Hillman B, Hyde KD, Luo J, Manamgoda D, Meyer W, Molnar T, Schoch C, Tadych M, White JF Jr (2013) Impacts of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) on the scientific names of plant pathogenic fungi. Online. APSnet Feature. American Phytopathological Society, St. Paul, MN. http://www.apsnet.org/publications/apsnetfeatures/Pages/Melbourne.aspx. Cited 19 Jun 2014

8 The Role of Herbaria and Culture Collections

Gerard J.M. Verkley[1], Amy Rossman[2], Jo Anne Crouch[2]

CONTENTS

I. Introduction 205
II. Best Practices for Fungal Herbaria..... 207
A. Preparing Fungal Reference Specimens 207
B. Preparing Dried Culture Specimens . 209
C. Numbering and Labeling Fungal Specimens............................ 209
D. Storage and Organization of Dried Specimens............................ 209
E. Requesting, Annotating, and Returning Specimens 210
F. Use of Fungal Herbarium Specimens in Molecular Studies 211
1. Introduction........................ 211
2. Contamination of Herbarium DNA Samples............................ 212
3. Extraction of DNA from Herbarium Specimens 212
4. DNA Quality from Herbarium Specimens 214
III. Maintenance of Living Cultures........ 214
A. Introduction 214
B. Metabolically Active Preservation ... 215
1. Storage on Agar with Periodic Transfer 215
2. Storage on Agar Under Mineral Oil and Distilled Water 216
C. Metabolically Inactive Preservation . 217
1. Cryopreservation (Cryogenic Storage)............................ 217
2. Freeze Drying 220
IV. Conclusions............................ 222
References............................... 222

[1]CBS Biodiversity Center, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; e-mail: g.verkleij@cbs.knaw.nl

[2]Systematic Mycology & Microbiology Laboratory, USDA-ARS, Rm. 246, B010A 10300 Baltimore Ave., Beltsville, MD 20705, USA; e-mail: amy.rossman@ars.usda.gov; joanne.crouch@ars.usda.gov

I. Introduction

Living cultures and dried reference specimens of fungi preserved in herbaria document the occurrence of fungi over time and space; we will never be able to return to the past to determine exactly which fungi were present (Rossman 1996). Cultures and specimens from the past are the only true representation of what existed then and, thus, are very important. In addition to fungal cultures and specimens deposited in the past to reveal the historical mycota, plant specimens, including those of fossils preserved with their fungal associates, have proven to be a useful source for discovering rarely collected fungi on living plants, such as *Uredophila* (Rossman 1987), determining the worldwide spread of plant pathogens (Ristaino 2006; Crouch and Tomaso-Peterson 2012), or discovering the past fungal diversity associated with plant fossils (Bidartondo et al. 2011). One practical reason to preserve fungal specimens when describing a new taxon is to fulfill the requirement of the ***International Code for the Nomenclature of algae, fungi, and plants.*** The code requires the deposition of a holotype specimen in a recognized herbarium (McNeill et al. 2006; Seifert and Rossman 2010). This will allow future scientists to know the precise characteristics of a specific taxon; thus, these **type specimens serve as the "standard" for defining a fungal species.** Having them available for study is essential for increasing scientific knowledge, especially concerning fungal systematics. What a joy to encounter a plentiful, well-preserved type specimen with appropriate morphological features! While it may not

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

always be possible to deposit such a specimen, a well-preserved slide as a type specimen serves the same purpose. Although a living culture in a metabolically inactive state is allowed to serve as a holotype specimen, a dried culture specimen is also useful because after a period of time cultures in some groups of fungi will no longer produce their diagnostic reproductive structures.

At present, it is generally agreed that only 5–10 % of the fungi that exist have been described and that the total number of fungal species may range from 1.5 to 5 million species (Blackwell 2011; Hawksworth 2004). **With the growing interest in biological diversity, many new species and habitats for fungi are being discovered that should be documented with herbarium specimens and living cultures**. For fungi as for flowering plants, herbaria themselves are a source of new knowledge of diversity (Bebber et al. 2010; Brock et al. 2009), but the data need to be digitized and disclosed for usage. Recent support from funding bodies, such as the National Science Foundation to digitize 35 fungal collections in the USA, underpins the importance of such collections. In addition, environmental sampling by extracting, cloning, and sequencing DNA suggests that the biological diversity of fungi is even greater than has been hypothesized. Even new phylogenetic lineages are being discovered using these techniques (Schadt et al. 2003; Jones et al. 2011). Although documented with sequences, the environmental samples themselves from which the DNA has been extracted may be deposited in herbaria, where they will be made available for future analyses using as yet unknown methods for describing biological diversity. Thus, as for collections of all organisms, the **role of fungal collections** may be even more important than currently recognized in ecological and environmental research of the future (Pyke and Ehrlich 2010).

Beyond systematics and biological diversity, fungal cultures and specimens should be deposited to document fungal research of all kinds, including ecology, genetics, and plant pathology. **Reference or voucher specimens** are essential for the repeatability or later verification of that research. For example, new diseases caused by fungi are reported regularly, with the causal agent identified by a scientific name. Over time, the concept of that species may change with increased knowledge. As an example, the concept of *Armillaria mellea* has changed significantly over the past 50 years as this broadly conceived species has been divided into many more narrowly circumscribed taxa that have a more specific biology (Coetzee et al. 2003). Often the species concept is initially revised based on molecular sequence data, but upon reexamination of specimens morphological clues to these newly recognized species are found. Once found, previously deposited specimens can be reidentified using the accurate species concepts and the biology of these more narrowly defined species (Rossman and Palm-Hernandez 2008).

Most sequence data available for fungi today are generated from DNA extracts from fresh fruiting bodies of fungi collected in the field or from isolates preserved in culture collections. Many different extraction protocols have been developed for these materials, and the reader may consult other publications for more details (Eberhardt 2012). With the ability to extract DNA, fungal herbarium specimens are becoming increasingly useful in, for example, precisely identifying type specimens or determining the historical distribution of specific populations of plant pathogens. Ristaino (1998, 2006) successfully tracked the movement of populations of *Phytophthora infestans* throughout the 1800–1900s using historical specimens from various herbaria (May and Ristaino 2002, 2004; Ristaino et al. 2001). Later in this chapter is a section with suggested protocols for DNA extraction from herbarium specimens along with the problems that may be encountered. This should only be done with permission from the herbarium that issued the loan and in cases where the specimen is abundant.

The protocols and techniques presented subsequently represent an overview of these subjects. More detailed accounts of these subjects have been published for herbaria in general (Forman and Bridson 1989; Savile 1962) and for fungal herbaria specifically (SAFRINET 1999; Wu et al. 2004). A useful overview of practical aspects in dealing with fungi is

provided by Mueller et al. (2004) in which measuring and monitoring the biological diversity of all kinds of fungi are considered. Hawksworth (1974) provides a general account of the systematics of fungi.

II. Best Practices for Fungal Herbaria

Fungal herbaria have historically been included in botanical institutions. Each herbarium has an official **acronym**: for example, K stands for Kew Botanical Gardens and BPI is the official acronym for the U.S. National Fungus Collections based on its previous affiliation with the USDA Bureau of Plant Industry. Information about fungal herbaria can be found at the Index Herbariorum Web site, http://sciweb.nybg.org/science2/IndexHerbariorum.asp, including listings of directors and associated staff, herbarium Web sites, lists of major scientists' specimens deposited at those institutions, and rules governing the loan of specimens. These data are continuously updated. This Web site should be consulted before requesting specimens on loan. Spooner and Cannon (2010) recently coined the term "fungarium" for collections of dried fungal specimens, underlining the fact that the fungi are now generally accepted as a kingdom in their own right and not to be seen as part of the kingdom of plants.

Increasingly the information associated with fungal herbarium specimens, especially types, is available online (Farr and Farr 2004), and the herbarium Web sites with these data should be consulted prior to requesting a specimen. In addition, the specimens themselves along with the data are increasingly being digitized and made available on the Internet, as for example in the USA (Baker 2011). This allows users to select the specimen most useful to them; in some cases, just seeing the specimen may be all that is required to answer the user's question. In addition to preventing damage to the invaluable specimen itself, this also saves time and money for both the herbarium management and user.

A. Preparing Fungal Reference Specimens

When **collecting a fungal specimen** to be deposited in an herbarium, as much material as possible should be gathered so that multiple specimens can be sent to various institutions for establishing the fungus in pure culture and for DNA extraction. Fungal specimens including lichens should be **timely and thoroughly dried** to prevent mold and bacterial growth prior to beginning the preservation process. **Characteristics** such as color, shape, size, and surface texture of fleshy fungi that will disappear upon drying should be recorded and included with the specimen. It is often useful to photograph specimens before they are dried. If material is to be used later for **DNA extraction**, a portion of the specimen should be placed in a cryovial with or without a buffer solution.

Air-drying is usually sufficient to adequately dry small fungal specimens, but large fleshy or woody fungi may require the use of a dehydrator or drying cabinet to prevent the specimen from becoming moldy. Large polypores and mushrooms can be cut in slices; mushroom caps and stipes may be separated to fit more easily into specimen packets or boxes and save storage space. Care should be taken to preserve diagnostic characters such as the reproductive structures. In the case of delicate specimens such as myxomycetes or hyphomycetes, material should be glued into boxes in the field to prevent crushing.

Fungi on living or dead leaves or other thin substrates and folious lichens should be pressed in paper towels and newspaper in a plant press or under a heavy weight, such as a pile of books, similar to the procedure used for pressing and drying plants. The material should be spread flat in one layer. Once dried, material can be cut with scissors or folded into packet-sized specimens, always taking care to protect the diagnostic structures. After drying, specimens are ideally stored in a labeled glassine envelope, especially if the specimen is fragile. It is useful to include a pressed voucher specimen of the substrate when collecting fungal

specimens in order to obtain an accurate host identification; thus, a plant press is extremely useful in the field.

Fungi on woody substrates or twigs should be trimmed to a size that will fit in a packet or box, then dried. Unnecessary substrate should be removed, taking care to preserve the diagnostic fungal structures. A knife or good clippers are useful for this purpose. For large woody specimens, a bandsaw can be used to trim material to a reasonable size.

For **fleshy fungi** or specimens preserved for exhibition, freeze drying or lyophilizing reduces shrinkage and color loss. However, freeze-dried specimens readily absorb moisture from the environment and must be maintained at a low relative humidity. They are also fragile and vulnerable to breakage (Moore 1999). For this reason, this practice is not recommended as standard procedure for fungal herbarium specimens.

All newly accessioned specimens should be placed in a −20 °C freezer for 3–5 days to kill potential pests before adding them to the collection. Polypores and mushrooms are particularly susceptible to insect infestation. This is also good practice for specimens coming to or leaving other herbaria and even specimens that have been removed from the herbarium environment for a period of time.

Unlike plant specimens, fungi are not directly mounted on herbarium sheets. **Paper packets** can be used to contain small specimens. Machine-cut or prefolded packets, sometimes called bryophyte packets or fragment folders, can be purchased from an herbarium supply company, or packets can be folded by hand from 100 % cotton, acid-free, A4 (21.0 × 29.7 cm) paper. Whatever folding method is used, the packets should stay closed without the use of clips, to prevent the loss of small pieces of the specimen, and open flat. Paper may be made into a packet by folding about one-third of the paper from the bottom and a short-third from the top, then folding back about 2.5 cm on both sides.

The following items may be added to specimen packets if necessary:

- A thin piece of cardboard in the packet for added rigidity; crustose lichens should be attached to a card to keep the substrate from disintegrating and prevent the specimen from sliding around in the packet.
- Cotton wadding to preserve delicate structures or prevent the specimen from sliding to the bottom of the packet.
- Acid-free glassine envelopes to contain dusty or fragile specimens, such as thin, dried leaves, within the paper packets.
- Zip-top polyethylene bags to store specimens inside paper packets.

Variously sized specimen packets are glued to standard herbarium **mounting sheets** using archival-quality adhesive. Mounting sheets can be obtained from an herbarium supply store. In gluing a packet to a sheet, a small drop of adhesive should be applied to the center of the back of the packet so that the side flaps can be unfolded to open the packet. A **label with the specimen data** generated from a database should be attached to the front of each packet. Multiple packets of the same fungal species on the same host may be attached to one mounting sheet to conserve space. A sheet with multiple packets is then labeled with the fungus and host names in the bottom right corner.

Large or heavy specimens, such as agarics and polypores, and delicate specimens that need protection from crushing, such as puffballs, should be placed in appropriately sized **cardboard boxes**. Myxomycetes are sometimes glued to box interiors, especially shallow boxes such as matchboxes or pill boxes; these are then placed inside specimen boxes or packets. Fungi growing on insect hosts may also need to be stored in boxes. Specimens may be stored in zip-top polyethylene bags inside boxes.

Permanent slide mounts or dried cultures can be stored in cardboard slide mailers within specimen packets or boxes. If a separate slide collection is maintained, slides should be cross-referenced with their corresponding specimen packets. Photographs, illustrations, descrip-

tions, spore prints, dried cultures, and any associated material should also be stored with specimens or cross-referenced.

B. Preparing Dried Culture Specimens

Dried specimens of living cultures can be included in a fungal herbarium. One technique for making dried cultures requires a **cardboard slide mailer**, water-soluble glue such as Elmer's, and silica gel in a tight-fitting box. This technique was described by Rossman and Simmons (1999) and is described and illustrated at http://www.ars.usda.gov/Services/docs.htm?docid=9405.

The steps are as follows:

1. Label a cardboard slide mailer with the name and number of the fungus to be preserved as well as the kind of agar on which it is growing. If the fungus is on only one kind of agar, use a single slide mailer, but if it is on two kinds of agar, a double slide mailer can be used placing a strip of fungus from each kind of agar into each place of a slide.
2. Squeeze a line of glue directly onto the slide holder and smear it around. Cut a slice of agar with the diagnostic features of the fungus from a Petri plate and place on the thin layer of glue in the slide mailer.
3. Then place the slide mailer, lid open, in a container with dried (blue) silica gel about 1 cm deep. Place a tight-fitting lid on the container.
4. The glue and agar will dry at about the same rate, becoming hard within 2–3 days. The slide mailer with the lid closed to protect delicate structures may then be considered as a separate specimen or placed in a packet with the specimen from which the culture was derived.

Other techniques exist for making dried cultures (Wu et al. 2004). A culture in a plastic Petri plate may simply be allowed to dry up if the lid is left unsealed. The resulting flat circle of media with the fungus can be removed from the Petri plate and placed in a glassine envelope. However, such cultures may stick to the plate and be difficult to remove without breaking. In addition, fragile and delicate aerial structures may be crushed in the packet.

Although a metabolically inactive culture may serve as a holotype specimen, usually a dried reference specimen from a culture is **designated as the holotype, epitype, or other kind of type specimen,** with the living culture serving as an ex-holotype, and deposited in a publicly available culture collection.

C. Numbering and Labeling Fungal Specimens

When starting a fungal herbarium, it is important to decide on a **numbering system** and stick with that system. The best approach is to decide on or obtain a **unique acronym** from *Index Herbariorum* and start with number 1, giving each specimen a unique number. In addition, entering specimen data into a database will greatly facilitate making labels, handling specimens, and keeping track of loaned material. Some programs exist that do this, such as SPECIFY, which is available from the University of Kansas: http://specifysoftware.org/.

At a minimum, packets and boxes should be labeled with the **scientific name of the fungus and host, complete collection locality data, collector name, determiner name, and collection date.** Labels should be printed on acid-free paper and attached to a packet or box lid with archival-quality adhesive.

D. Storage and Organization of Dried Specimens

Like plant collections, fungus collections should be **protected from excessive heat, moisture, and sunlight**. Cabinets should be well sealed and collection areas kept clean to help prevent **insect infestations.**

Specimen packets attached to herbarium sheets can be stored in standard metal **herbarium cabinets.** As mentioned earlier, specimens of the same genus or species or species on a single host should be grouped in labeled folders depending on the number of specimens present. Alternatively, each species can be placed in a separate folder, but this requires more space than grouping specimens of several species into generic folders. Specimen boxes may be placed in larger boxes or trays that fit on herbarium cabinet shelves, with the larger boxes labeled with the range of specimens they contain.

Another approach to storing fungal specimens involves storing individual specimen

packets and boxes vertically in cabinets with drawers, such as 10.2 × 15.2 cm card file drawers. While this storage method is space efficient and might provide easier access to specimens, it can cause damage to fragile specimens that fall to the bottom of the packet. In addition, it does not allow for the placement of variously sized packets and boxes in the same place in the herbarium.

No standard **arrangement scheme** exists for fungal herbaria. The best arrangement for an herbarium depends on the size of the herbarium and the needs and knowledge level of the users. For example, alphabetical arrangement by genus and species might work well for a small collection or a collection used by nonspecialists, but a large collection might benefit from having the specimens placed in taxonomic order so that users do not have to move to different parts of the herbarium to find specimens in related groups. Reference tables and labels should be posted on cabinets to aid in filing and finding specimens.

Taxonomic and alphabetical arrangements can be combined, as in the following examples:

- Arrange fungal genera in taxonomic order by major groups.
- Arrange fungal species in alphabetical order within genera.
- Arrange multiple specimens of a single fungal species in alphabetical order by host genera and species.

This system can be altered so that fungal genera are sorted alphabetically under classes or families, which are arranged taxonomically following a recent classification scheme such as that of Lumbsch and Huhndorf (2010) for ascomycetes. For groups of parasitic fungi with narrow host ranges such as rusts, arranging specimens of a single species by host family is useful. Within genera or species, specimens might also be arranged geographically, as is commonly done for plant specimens. For example, separate sheets or folders can be used for specimens from the main region of interest, with regions less well represented grouped together.

E. Requesting, Annotating, and Returning Specimens

Herbarium specimens may be requested on loan from herbaria throughout the world for use in studies of fungi. Usually specimens are loaned from one herbarium to another with the request agreement communicated between the directors of the respective institutions. When **requesting a specimen**, the requestor should include as much information as possible about the specimen desired. If the specimen data are available on the Internet, specimens should be requested by number as well as fungal name. If type specimens are being requested, the scientific name of the species, any synonyms, its place of publication, and the data associated with the type specimen should be included in the request. This will assist herbarium personnel in locating the specimen. The institutions where specimens of important mycologists are housed are listed in Kirk et al. (2008) under "Author Names" using the abbreviations in *Index Herbariorum*.

In requesting specimens for loan, a letter or e-mail from the director of the requesting herbarium should be addressed to the director of the loaning herbarium, with a brief statement about who will be using the specimens and for what purpose. Each specimen requested should be listed with details so that the specimens can be easily located. Most herbaria loan specimens for a period of 6 months to a year, although the time period for the loan of type specimens may be shorter. It is critical that specimens be returned in a timely matter. Return of overdue loans has been the source of considerable consternation in the herbarium community. At one point in time, personnel at the invaluable Saccardo herbarium (PAD) in Padova, Italy, refused to loan specimens until the fungal community as a whole returned their overdue specimens. Often the only way to obtain overdue loans is to decline requests for loans of specimens to others at the same institution.

When **examining specimens on loan** from other institutions, the **regulations provided by that institution must be followed.** Usually

these regulations require that specimens be stored in a safe place such as a metal herbarium cabinet. When examining the specimen, the smallest amount of material possible should be used in order to preserve the specimen for the future. This is especially true for type specimens that can be degraded to nothing after being examined by a few mycologists. In the case of type specimens, most institutions require that all portions of the specimen, including the microslides, be returned with the specimen. The problem of destroyed specimens has become so dire that some type specimens, such as those of Fries, are no longer available for examination.

Examined herbarium specimens should always be annotated with a small slip of paper that includes an evaluation of its identity, as much as can be determined. Annotations should be typed or made in legible handwriting on small pieces of paper that include the herbarium and specimen number, an evaluation of the identity of that specimen, and the examiner's name and date. **Annotations provide a history for future users and may serve to prevent further destruction of that specimen.**

F. Use of Fungal Herbarium Specimens in Molecular Studies

1. Introduction

In recent years, molecular systematists have been increasingly using DNA extracted from fungal herbarium specimens as part of their research. The widespread use of so-called **ancient DNA (aDNA)** for molecular studies arose shortly after the development of the polymerase chain reaction (PCR), which allowed scientists to perform selective enrichment of targeted DNA sequences using very small quantities of heterogeneous DNA (Saiki et al. 1985, 1988), even from poorly preserved sources, such as fossils, museum specimens, and archaeological remains (Pääbo et al. 2004). The importance of DNA analysis in modern systematics is placing additional demands on herbarium resources and has introduced a new set of challenges for institutions. Decisions regarding the use of specimens for molecular analysis need to be carefully considered to maintain a balance between preserving valuable resources and enabling robust scientific inquiry. A working knowledge of the many factors that influence the successful outcome of research using aDNA, as detailed in the following sections, will assist in the development of effective policies in this emerging area.

Specific policies for destructive sampling of fungal reference specimens for the purpose of aDNA analysis vary depending on the individual herbaria. Most generally, when permitted, sampling for DNA extraction must be preapproved in writing by curatorial staff. The majority of herbaria have strict policies prohibiting destructive sampling of type specimens and important historical samples but are generally more permissive with other samples.

At the U.S. National Fungus Collections and other herbaria, decisions regarding destructive sampling for aDNA extractions are made on a case-by-case basis and are influenced by many factors, including the quantity and quality of material available for analysis, the availability of other suitable material, the experience of those requesting permission, and the likelihood of success. Some institutions, such as the Wageningen Herbarium (WAG), explicitly limit sampling to a maximum of 5 % of available tissue up to a maximum of 50 mg (Telle and Thines 2008). Others, such as the Harvard University Herbaria, require researchers to submit a written protocol for proposed molecular analysis in advance of loan approval (Wood et al. 1999).

Frequently, material to be used for aDNA extractions will be excised by herbarium personnel before loans are provided to scientists. Alternatively, scientists may perform the excision of tissue on site at the herbarium under the supervision of curatorial staff. Digital imaging of specimens prior to the loaning of material is a labor-intensive practice but may be reluctantly adopted by some institutions in an attempt to safeguard collections from unauthorized sampling.

At the completion of the research, from 50 to 100 % of the extracted aDNA is typically returned to the herbarium because the loaning institution retains ownership of all material derived from the loaned specimen. The mycological specimen associated with the aDNA

extraction is annotated with details of the data generated from the research, including the success or failure of DNA extraction attempts and GenBank accession numbers of sequence data. Although uncommon, some institutions, such as WAG, are beginning to provide access to stores of aDNA extracted from their collections to the scientific community.

2. Contamination of Herbarium DNA Samples

The potential for contamination of aDNA from external sources is a serious concern that must be taken into consideration before embarking on any study that uses herbarium-derived DNA. Researchers should be prepared to incorporate rigorous **controls to minimize sources of error derived from contaminant DNA** (Cooper and Poinar 2000; Pääbo et al. 2004). Contamination of ancient samples with contemporary DNA as a complicating factor was notorious in early studies of macroorganisms (e.g., Zischler et al. 1995) and remains pervasive in studies involving microbial pathogens (e.g., Gilbert et al. 2004). There are several points at which herbarium specimens targeted for molecular analysis may become contaminated by external sources: (1) during initial collection, (2) during storage in the herbarium, (3) during examination while on loan, and (4) during the DNA manipulation process. Regardless of when the contaminant is introduced, the high level of sensitivity of the PCR process used in molecular research ensures that the presence of even small amounts of foreign DNA can be detected, compromising research conclusions. For example, some PCR assays are sensitive enough to reproducibly detect the presence of just two fungal spores, as demonstrated from studies of the rust fungus *Phakopsora pachyrhizi* (Barnes et al. 2008).

How pervasive is contamination of mycological herbarium specimens? A recent survey of specimens at the fungal herbarium at the Royal Botanic Gardens in Kew, UK, collected between the 1950s and the present day showed that contamination can be a relatively common occurrence, with 11 % of 263 ITS sequences derived from contaminant fungi such as *Penicillium* rather than the targeted specimen (Brock et al. 2009). At present, there is no way to know whether this level of contamination is representative; comprehensive studies on this topic are needed. Given the capacity of fungal spores and other structures to travel unseen through air currents or to move as passengers on, for example, hands, clothing, or tweezers, many fungal herbarium specimens may contain some level of cross contamination from the environment. The typical fungal herbarium environment seems especially vulnerable to **cross contamination** since samples are routinely stored in close proximity to one another and multiple specimens are often stored in adjacent packets on a single herbarium sheet. The occurrence of mites or insect pests, and their movement from one specimen to the next, may add to the problem of contaminant transfer. For obscure specimens that have remained unopened since their initial collection, the potential for the introduction of intermediate contaminants may be nominal. In contrast, specimens that have been subjected to repeated examination over the course of decades or centuries are more likely to be contaminated. Likewise, specimens on loan with specialists may be exposed unintentionally to any number of fungal contaminants from the environment and run the risk of exposure to similar species that might go undetected if appropriate experimental control measures are not implemented.

3. Extraction of DNA from Herbarium Specimens

A strict set of best practice criteria for the generation of authentic, uncontaminated aDNA data has been proposed and refined over the past three decades. Though it is impossible to eliminate past contaminants introduced during routine storage and handling, careful adherence to aDNA authentication criteria can help researchers identify the presence of contaminants and minimize the introduction of new contaminants during handling. A discussion of the experimental controls required to produce robust, authenticated aDNA data is

beyond the scope of the present review, and readers are referred to detailed summaries of these approaches presented elsewhere (Cooper and Poinar 2000; Pääbo et al. 2004). The following section is limited to the **technical considerations involved in extracting aDNA from mycological specimens.**

New contaminants can take the form of living environmental sources such as spores or previously amplified PCR products that may be present in the lab. During the process of extracting DNA from herbarium specimens, it is essential that work be performed in a specially prepared clean area using specially prepared tools. Ideally, this will mean working in a laboratory space where the experimental organism has never been present, distant from places where contemporary sources of the organism might be encountered. Ventilation systems for heating and air conditioning may provide unanticipated connections between seemingly isolated laboratories in a single building or introduce contaminants from adjacent environments such as greenhouses and experimental plots.

Throughout all experiments, work should be performed on a surface thoroughly cleaned with oxidants (bleach solution, UV light) within an environment such as a laminar flow hood or PCR hood where air flow is restricted and continuously filtered to eliminate spores, dust, and other contaminants. The **work area** should contain only the tools needed to perform the extraction. Exterior surfaces of specimen packets are wiped with a dry, lint-free towel to dislodge any spores or other contaminants before introducing the sample into the clean work area. Only one sample is handled at a time within the prepared work space using fresh, sterile tools, generally a pair of tweezers, and a sharp knife or scalpel with a fresh blade. Loose or long-sleeved clothing that could harbor contaminants should not be worn, and hands and lower arms are to be thoroughly washed with soap and water before manipulating specimens. Fresh disposable latex or nitrile laboratory gloves should be worn and changed between specimens. Specimens should only be manipulated using fresh, sterile tools and excision of tissue performed on a sterile surface, such as a disposable plastic Petri plate.

The quantity of **tissue excised from the specimen** for DNA extraction may be dictated by the herbarium but in all cases should cause as little destruction to the sample as possible. Tissue is always excised from the least obvious area of the specimen and must never destroy unique morphological structures or features. For plant-associated fungi preserved in a host plant matrix, avoid the "best" lesions, pustules, or other structures that serve as visual exemplars. Instead, sample from less striking areas or from broken pieces of lesser value. For plant-associated fungal specimens, both fungus and host tissue are included in the extraction process, with the plant tissue serving as a DNA carrier, although inclusion of excess, uncolonized plant tissue should be avoided when possible. For specimens of both fleshy and plant-associated fungi, a 2–4 mm^3 section may be more than adequate for many specimens and will allow the extraction process to be performed in a single microcentrifuge tube. If the amount of tissue for a specimen is minimal, less tissue must be used, and never more than 5 % of the total specimen must be removed. Indeed, it has recently been demonstrated that only 2 mg of grass tissue infected with either *Sclerospora graminicola* or *Albugo* sp. was needed for successful aDNA extractions (Telle and Thines 2008). Excised tissue is cut into small sections, then placed in a sterile 1.5 mL microcentrifuge tube until needed.

As with any DNA extraction from fungi, the first step is **cell lysis** to release the DNA from the confines of the cell wall, a process that is accomplished through mechanical, chemical, or enzymatic disruption of the cells. Countless different protocols are available, with the choice of methodology dictated by the organism being studied and the DNA extraction protocol used. **Chemical or enzymatic lysis** may be difficult to achieve for some fungi, although many successful examples are found in the literature (e.g., Fredericks et al. 2005; Ristaino et al. 2001). Some laboratories grind the prepared tissue into a fine powder inside the microcentrifuge tube using liquid nitrogen and

a glass rod, although this can result in the loss of valuable tissue if the liquid nitrogen is allowed to accidentally bubble out of the tube. Increasingly, **bead beating** using silica or iron beads and specialized vortexing equipment are used for fungal cell lysis (e.g., Crouch and Szabo 2011; Fredericks et al. 2005; Telle and Thines 2008). Regardless of the method employed, clean work areas as described in the preceding paragraphs must be maintained throughout the entire extraction process. It is advisable to dedicate pipetters and other equipment in the laboratory as DNA-free and utilize them only for transferring reagents. Barrier tips should always be used for transfers of DNA to minimize the risk of cross contamination.

Researchers currently make use of several different approaches for **extracting DNA from fungal herbarium specimens**, including conventional or optimized CTAB-based methods (Brock et al. 2009; Cubero et al. 1997; Ristaino et al. 2001; Telle and Thines 2008) or, increasingly, solution- or column-based commercial kits (Crouch and Szabo 2011; O'Gorman et al. 2008; Sokolski et al. 2004; Telle and Thines 2008). Unfortunately, systematic comparisons of different methodologies for extracting DNA from fungal herbarium specimens are rare. In the single study where a range of common extraction methods were tested using samples of the oomycetes *Sclerospora graminicola* and *Albugo* sp., it was shown that the extraction method plays a significant role in the quantity and quality of yield and affects the success of downstream PCR amplification (Telle and Thines 2008). Similar research for the true fungi is needed before recommendations can be made for the ideal approach to maximize yield, minimize failures, and conserve valuable specimens.

4. DNA Quality from Herbarium Specimens

The degradation of DNA that occurs through **enzymatic and microbial processes** after an organism dies is well documented and presents problems for researchers working with herbarium specimens. This topic is reviewed in detail by Pääbo et al. (2004). Compared to DNA extracted from fresh samples, herbarium DNA is shorter in length due to postmortem strand breakage caused by enzymatic degradation, single-stranded nicks caused by nonenzymatic hydrolytic cleavage of phosphodiester bonds in the phosphate–sugar backbone, and strand breakage due to chemical changes at abasic sites (Friedberg et al. 1995; Lindahl 1993). On average, the **length of ancient DNA** is between 100 and 500 bp (Hofreiter et al. 2001). Some publications report fragments as long as 1 kb (e.g., Lambert et al. 2002; Telle and Thines 2008); however, many researchers interpret the presence of lengthy fragments as an indicator of contamination (Gilbert et al. 2004), and others provide evidence that long fragments may be artifacts (Lister et al. 2008).

In addition to the universal postmortem degradation processes experienced by all organisms, **the quality of herbarium specimen DNA may be further degraded as a result of unique storage and maintenance practices.** Specimens that have been air dried, stored at temperatures below 42 °C, and not exposed to chemical treatment will yield higher-quality aDNA than tissues that have been treated otherwise (Taylor and Swann 1994). Tissues preserved in silica gel or anhydrous $CaSO_4$ may contain highly degraded aDNA, as will specimens exposed to high temperatures. Fumigation and other disinfecting treatments, such as the application of mercuric chloride and microwaving, may also negatively impact aDNA quality (Hall 1981; Hill 1983; Metsger and Byers 1999; Taylor and Swann 1994).

III. Maintenance of Living Cultures

A. Introduction

Fungi that can be successfully isolated in **axenic culture** open a plethora of possibilities to study life cycles, metabolism, antimicrobial activity, and many other aspects of their biology, including taxonomy. When the original source specimen for such cultures is lodged in an herbarium, the culture is even more valuable to taxonomic studies and can, for example, also

serve as material to be selected for diagnostic tools such as DNA barcoding.

This section is primarily intended for scientists who want to establish or improve their own working collections of living strains and focuses on **preservation methods**. Handling live cultures of human and animal pathogens is subject to **national legislation for biosafety**, and dispatch of these organisms is strictly bound to **transport regulations**. Plant-pathogenic fungi can be subject to national phytosanitary regulations and international transfers to quarantine regimes. Culture collections that offer or plan to establish public services for distribution or accession of cultures should consider applying for a membership in a national or international organization for culture collections, such as the World Federation of Culture Collections (WFCC, http://www.wfcc.info/). The World Data Center for Microorganisms (WDCM), maintained by WFCC, contains information on 585 culture collections established in 68 countries related to their organization, management, services, and scientific interests. Regional networks, such as the Asian Biological Resources Center Network (http://www.abrcn.net/) and the European Culture Collections Organisation (ECCO, http://www.eccosite.org/) regularly organize scientific meetings to exchange information and discuss common problems and strategies. **Most networks also provide practical training courses for collection staff on topics like collection management, quality standards, and preservation.** The OECD Best Practice Guidelines for Biological Resource Centres (OECD 2007) provides a baseline for the operation of service collections and deals with important issues such as **quality control, data management, traceability, biosafety, and biosecurity**.

Many service collections furnish their strains under a **material transfer agreement (MTA)**, which clarifies the qualifications and responsibilities of the recipient and settles with the recipient the terms of use, matters of intellectual property, and warranties. Most MTAs also cover the obligations of the **Convention on Biological Diversity** (CBD, http://www.cbd.int/convention/default.shtml/) on matters of **access and benefit sharing (ABS)** in relation to the country of origin of the material. The consequences for culture collections of the recently published *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity* are currently being discussed in the community of microbial resource centers with the aim of proposing workable solutions that will contribute to the conservation and sustainable use of microbial diversity in the spirit of the CBD, without seriously hampering international access to cultures for taxonomic and noncommercial research.

Living fungi can be preserved as cultures in either a metabolically active or metabolically inactive state, as described in what follows. Fungal cultures that are intended as the actual holotype specimens must be stored in a metabolically inactive state and indicated as such when designated (McNeill et al. 2006).

B. Metabolically Active Preservation

Metabolically active preservation methods are used widely to preserve fungi, and although some strains may survive for decades, most fungi cannot be expected to be kept viable for more than 5–10 years using these methods. For some methods that use refrigeration (5 °C), metabolic activity is strongly reduced yet never completely arrested. A number of other methods suitable for particular fungal groups have been described in detail by Jong and Birmingham (2001) and Nakasone et al. (2004), including a method for the production and storage of sclerotia-forming fungi or other resting structures and methods based on the colonization of organic (wood chips) and inorganic (sand, soil, silica gel) substrata, which can be refrigerated or frozen to −20 °C. Below the most frequently used methods are briefly described and discussed.

1. Storage on Agar with Periodic Transfer

Pure cultures can be maintained actively growing on agar slants by **periodic subculturing**. No expensive equipment is required, and this practice may be convenient when inoculum is

used regularly. But there are **disadvantages**; the method is labor-intensive. Moreover, the risk of contamination is comparatively high, and most fungi that are kept actively growing will show signs of degeneration sooner or later (Jong and Birmingham 2001; Nakasone et al. 2004). Unwanted selection effects may occur after spontaneous mutations, and fertility and virulence are often lost after multiple transfers. These negative effects can largely be avoided if metabolism is completely suppressed, as is the case in **long-term preservation by freezing and drying** (see below). Some fungi are recalcitrant to these methods, and periodic transfer on agar media is the only feasible way of keeping them.

Cultures are best maintained on agar slants in glass bottles or tubes. The containers can be closed with cotton plugs or various types of commercial cellulose or plastic plugs and metal (screw) caps. When keeping an agar collection, ideally two lines per strain should be maintained and each line alternatingly transferred to two kinds of medium, for example, one nutrient-rich and one nutrient-poor medium, but both should support growth of the species. For a short period after transfer to fresh media, the fungus is **allowed to grow at its optimal temperature for growth, after which it can be stored at a lower temperature to maximize the time between transfers** by reducing metabolism and the evaporation of water.

Most filamentous Ascomycota can be stored at 5 °C in the dark and only need to be transferred every 6–12 months, but at room temperature usually no longer than 3 months, depending on the volume of agar in the container. Other important groups of fungi that should be stored at 17 °C can, as a rule of thumb, be transferred as follows: Oomycota, including *Pythium* and *Phytophthora*, every 9 months; Chytridiomycota, every 2 months; saprotrophic Basidiomycota (e.g., *Coprinus*,*Coprinopsis*, *Conocybe*, *Mycena*, *Mycenella*), every 2 months; mycorrhizal Basidiomycota (*Amanita*, *Boletus*, *Cortinarius*, *Lactarius*, etc.), every 6 weeks.

Compact, refrigerated storage of cotton-plugged containers should be carefully inspected regularly because cultures may be prone to contamination by the growth of cold-tolerant fungi on the outside of containers, in plugs and refrigerator walls, especially when humidity is high.

Smith and Onions (1994) and Jong and Birmingham (2001) discussed the problems related to **mite infestations** and provided suggestions for control. Natural plant and soil samples received in the laboratory are a potential source of mites and should be kept separate from cultures at all times. Mites introduced with incoming cultures are the most difficult to control because they are adapted to living on cultured fungi. If the unwanted intruders are not stopped immediately from spreading and reproducing, an entire collection can be destroyed by contaminations within a surprisingly short period of time. An **effective way of protecting actively growing cultures from mites** while allowing normal air exchange is by the use of tubes that are closed with **cotton plugs soaked in a substance that is highly toxic to mites** (Crous et al. 2009; Smith and Onions 1994), for example, 25 % benzyl benzoate with a colored dye added for visibility.

Caps designed to allow air exchange are inadequate, and parafilm does not block the passage of mites. Plates are also impossible to seal completely from mite infestations. If fungi need to be grown on plates, these cultures should always be stored away from the tubes (preferably on islands in water or oil baths that block mite migration) and never kept longer than absolutely necessary.

2. Storage on Agar Under Mineral Oil and Distilled Water

The workload of maintaining an agar collection can be considerably reduced by adding an overlay of **high-quality mineral oil or liquid paraffin** (Paraffinum perliquidum, 60–80 mPa.s) to the agar tubes. By this method most filamentous Ascomycota and Basidiomycota can be kept viable for at least 10 years without having to transfer to fresh media, and mite infestations can be prevented. Results for preserving Oomycota by the oil overlay technique are generally poor. Metabolism is reduced considerably with this method but not completely stopped, so negative effects such as those described earlier

or unwanted selection for conditions imposed on the fungus may still occur.

After **transfer to fresh agar slants** the culture should have a chance to develop sufficient mycelium (1–2 weeks), after which all mycelium and agar should be completely submerged in autoclaved oil and the tube stored in an upright position at room temperature (15–18 °C). Especially in the first 1 or 2 weeks the oil level may drop, and extra oil should be added if necessary. **Reviving cultures** requires special attention. A first attempt is performed by aseptically taking some mycelium from the culture with a long sterile needle, allowing the oil to drain, and inoculating on a fresh slant of the same agar medium. In cases where no growth occurs using this method, the old oil tube is turned upside down and the oil allowed to drain for 24 h, after which a new transfer is attempted in the same way. If the fungus still does not grow, the old agar in the original oil tube can be overlaid with a thin, fresh layer of hand-warmed medium of the same type and checked for growth after several days.

Storage in distilled water has been applied to many different fungi, including oomycetes, ascomycetes, and basidiomycetes, commonly with good recovery after 2 years (Johnson and Martin 1992; Smith and Onions 1994). An economical method often used is to store the fungi at 4 °C in screw-cap cryovials (1.5–2.0 mL) containing a few small discs of freshly grown cultures topped with sterile distilled water (Nakasone et al. 2004).

C. Metabolically Inactive Preservation

This section highlights the methods that are used widely for the **preservation of fungi for periods longer than 5 years.** Although preferable in regards to preserving fungi, these methods require more equipment and are more labor-intensive than those described earlier.

1. Cryopreservation (Cryogenic Storage)

Cryogenic storage is the most universally applicable and one of the most reliable methods of long-term preservation for living cultures of fungi (Stalpers et al. 1987). It can be applied to both sporulating and nonsporulating cultures. A large proportion of fungal taxa obtained in pure culture today can survive optimized procedures for subsequent freezing and thawing and **will retain most of their properties if stored at temperatures below −139 °C as provided by liquid nitrogen**. Below this temperature, water activity is zero, no ice-crystal dynamics occur, and all cell metabolism is stopped. Assuming that all other potentially harmful influences, such as radiation energy, can be ruled out completely, this would mean that these cultures could be stored indefinitely (Jong 1989). For these reasons, storage in mechanical freezers at −80 or −20 °C is less optimal. **Some fungi are recalcitrant to cryopreservation**, and several Basidiomycota and Ascomycota may lose some morphological characteristics, for example, showing a waxy or reduced growth after preservation, or will no longer sporulate. Only relatively small numbers of fungal taxa do not survive cryopreservation at all. Oomycota or water molds, which are not fungi but funguslike Straminipila, need to be treated with special care (see below).

The disadvantages of cryopreservation include the high costs of storage equipment and liquid nitrogen for cooling. The availability of liquid nitrogen may not always be guaranteed under all circumstances. An additional disadvantage for service collections is that cultures need to be reactivated and grown on agar medium before they can be shipped, in contrast to freeze-dried ampoules, which can be sent immediately. Although shipment of frozen cultures is not impossible in most situations, it is generally considered too expensive.

Cryogenic storage can be in liquid nitrogen at ca. −196 °C or in the **vapor phase** generated by liquid nitrogen. The disadvantages of placing cryovials, particularly glass vials, in the liquid phase have been addressed by Stalpers et al. (1987) and Jong and Birmingham (2001). In storage tanks with a classic design (a certain level of liquid nitrogen at the bottom over which the samples are stored), a temperature gradient may develop in the vapor phase, and if the storage tank is not opened for a long time,

vapor temperatures can even rise as high as −130 °C just below the lid. Therefore, special storage systems have been designed in which the vapor storage compartment is surrounded by a mantle of liquid nitrogen at a high level. The liquid is contained between the inner wall of the tank and outer wall of the storage compartment, and the coldest vapor is only allowed to drop over the top of this wall, creating a dynamic vapor phase by continuous mixture of the cold and warmer vapor anywhere in the storage compartment. At CBS, fungal cultures are stored in storage tanks (Taylor-Wharton, K-series) of such design, where all samples can be kept constantly below −175 °C in the dynamic vapor phase.

To survive cooling and freezing, the cells must be protected from the formation of large intracellular ice crystals, which would cause lethal damage to the cell membranes and organelles (Calcott 1978; Heckly 1978). Most procedures developed for fungi make use of chemical **cryoprotectants** that partly penetrate the living cells where they **prevent or reduce the growth of intracellular ice crystals**. They can be added to the medium used for growing the fungus or added to the harvested mycelium shortly before freezing. Most widely used cryoprotectants are **glycerol and dimethyl sulfoxide (DMSO)** (Hoffmann 1991; Jong 1989; Smith 1983), usually at final concentrations of 10–20 % (w/v) in the case of glycerol and 10–12.5 % for DMSO.

Mixtures of 10 % DMSO and 8 % glucose have proved more effective in protecting fungi than 10 % glycerol, including some fungi that seem to be more sensitive to damage during cooling than most other fungi (Smith 1983), but since DMSO is toxic to humans, glycerol is usually preferred for standard cryopreservation procedures, and most fungi are not less well preserved with glycerol than with DMSO. For some groups of fungi these cryoprotectants still give insufficient survival, and other chemicals can be used, for example, ethylene glycol for the preservation of zoospores of rumen chytridiomycetes (Sakurada et al. 1995).

Chemicals that are more effective in entering living fungal cells generally also show an increased **toxicity**, and exposure time to the cryoprotectant can affect postpreservation survival (Souzu 1987). When toxic cryoprotectants are used, it is advisable to determine the maximum period and temperature of handling prior to storage at ultralow temperatures (Tan 1997).

Vials used for cryopreserving fungi include glass tubes, plastic screw-cap cryotubes (1–2 mL), or straws. Vials should be stabile at storage temperatures and during freezing or warming and closed tightly to avoid any leakage or contamination. Most labs use **plastic cryotubes or drinking straws**, but these vials are less suitable for storage in the liquid phase (Elliot 1976) because of the risk of leaking of liquid nitrogen and splitting of the straws. The choice for a particular type of cryovial may further depend on available storage space and properties of the organisms. Several procedures using polyvinylchloride or polypropylene straws have been described (Elliot 1976; Elliott and Challen 1979; Hoffmann 1991; Stalpers et al. 1987). Straws may be completely sealed and used as the sole container, or several straws may be partially or completely sealed and kept in a sterile cryotube (Hoffmann 1991). At CBS, fungal cultures are preserved mainly in polypropylene drinking straws. Because of their small size, eightfold storage per strain is realized using much less space and consuming much less liquid nitrogen than would be required for storing the same amount of samples in glass vials.

Cultures are inoculated on solid agar media on plates or in liquid cultivation medium in tubes (yeasts). After incubation under optimal conditions for growth, the cultures are checked for purity and identity. The fungal material is preserved in commercial drinking straws 4 mm in diameter (cut to pieces approximately 45 mm long with a standard office paper cutter) that are closed at one end using an industrial heat sealer (Audion Electro, the Netherlands) with adjustable sealing temperature and pressure (Stalpers et al. 1987). The straws are then put into a bacteriological screw-cap bottle and autoclaved at 121 °C for 20 min. Under aseptic conditions in a safety cabinet, straws are half filled with sterilized 10 % aqueous glycerol as the standard, but for some fungi 10 % aqueous DMSO is used as the cryoprotectant. Pieces of agar with mycelium are punched out of the culture plate with a metal cork borer with a pin and transferred to the straws. For each

strain 10–12 straws are thus filled, after which they are labeled and sealed completely. Sealing quality is crucial and should always be checked by squeezing the straws firmly with a pair of flat-beaked forceps. For yeast, straws are filled with 60 % glycerol, and using a pipette the volume is supplemented with ca. 1.5 mL of the gently mixed yeast culture.

In a programmed freezer (e.g., ice cube), the filled straws are **frozen from room temperature to −40 °C at a rate of approximately −1 °C/min, then more rapidly to −80 °C**. Alternatively, the samples can be frozen in a plasma freezer to −40 °C and then transferred to −80 °C in a mechanical freezer or placed in (the vapor of) liquid nitrogen. The plasma freezer should be checked regularly for cooling rates using an external calibrated thermometer. When the frozen material is placed in storage or taken out for use later, it should always be adequately protected from excessive warming.

Sometimes precooled blocks are used to handle vials, but at CBS racks and drawers are taken from the cryogenic system and transferred to a mechanical −80 °C freezer with a modified interior, where the material remains at temperatures below −65 °C while vials are collected or stored.

To check the viability, identity, and purity of the cryopreserved strains, one straw is opened a minimum of 7 days after preservation, and a second check after 3–5 years is advisable.

Frozen straws need to be **thawed sufficiently rapidly to avoid cell damage by recrystalization of water.** This can be done by placing them in warm water at around 35 °C for approximately 5 min until all contained material has thawed. Caution should be taken not to overheat the material. Oomycota are thawed in water at 20 °C because they are very sensitive to overheating and normally do not survive temperatures above 25 °C. Before opening, the surface of the straws is sterilized in 70 % ethanol. Then the straws are cut open with sterile scissors aseptically in a safety cabinet, and the content is taken out with a sterile needle and placed on fresh agar medium suitable for growth. After incubation under the right conditions, the strain is checked for viability, identity, and purity. Many strains start growing within 48 h after opening the straw, but some only do so after a lag of up to 7 days.

The straw technique is less suitable for some fungi, particularly ectomycorrhizal basidiomycetes, which may be explained by the fact that these fungi are more sensitive to the physical damage impacting on the hyphae during preparation. Stielow et al. (2012) describe a new technique in which mycelia are allowed to grow over charcoal filter paper strips (CFSs). Inoculum can be placed centrally on the CFSs, and in the case of extremely slow growing fungi the entire colonies will be used. After the fungus has sufficiently grown, the strips are carefully removed with forceps and placed in a sterile Petri dish and flooded with 10 % (v/v) glycerol solution and incubated for 1–2 min. Then the strips are taken out, with the excess liquid being allowed to drip off, and transferred to cryovials and directly placed in the vapor phase of liquid nitrogen for freezing. For reactivation, a strip can be taken out aseptically and placed on a fresh Petri dish with suitable medium for growth. If the remaining straws in the cryovial are kept frozen during this procedure, they can be used later.

If a programmed freezer is not an option, another convenient technique for cryopreserving fungi that sporulate readily is freezing in a −80 °C freezer using Nalgene Cryo 1 °C freezing containers (Nalgene, Rochester, NY). Agar slices cut from actively growing colonies are placed in sterile plastic cryogenic vials in 20 % glycerol. After 1 or 2 days at room temperature, which allows the glycerol to penetrate the fungus, the cryovials in cryogenic containers are placed in a −80 °C freezer. To have some control over the freezing process in cryovials when placed in the −80 °C freezer, these vials are placed in Nalgene Cryo 1 °C freezing containers that contain a reservoir for isopropyl alcohol providing the controlled cooling. Once frozen, the cryovials can then be transferred to trays for storage in liquid nitrogen storage facilities where available.

2. Freeze Drying

Freeze drying, or lyophilization, is a widely used method for long-term preservation of fungi that has several **advantages over cryopreservation**. Adequately freeze-dried cultures generally maintain their morphological, physiological, and metabolic properties and can remain viable for more than 40 years (Hesseltine et al. 1960). Storage of cultures can be economical with regard to space and equipment, and the ampoules containing the freeze-dried product can be quickly and more easily dispatched than cultures that need to be reactivated before shipment or kept at low temperatures during transport, and this is an obvious advantage for service collection. However, not all fungi can be freeze dried successfully. Although nonsporulating mycelia can be freeze dried with some success (Pertot et al. 1977; Tan et al. 1991), the method is **predominantly applied to yeast cells and propagules of filamentous fungi** and bacteria (Tan 1997, 2011).

Critical parameters for the survival of lyophilized fungal propagules are the composition of the lyoprotectant (Berny and Hennebert 1991; Tan et al. 1995), the cooling rate during the cooling phase (Smith et al. 1986), the size of the propagules, and the thickness of the cell wall (Tan et al. 1994). Residual moisture and storage temperature also influence the shelf life of the dry product.

The use of **lyoprotectants protects** the living cells from possible damage caused by ice-crystal formation during cooling and destabilization of membranes and cellular proteins during subsequent stages of the freeze-drying process. Various media have been tested for their suitability as lyoprotectants in fungal spore suspensions, including sodium glutamate, peptone, various mixtures of saccharides, and skim milk (Berny and Hennebert 1991; Tan et al. 1995).

An aqueous solution of **skim milk** (10 or 20 %) has proved very suitable and has been used widely to prepare spore suspensions for freeze drying. **Saccharides** in the milk protect cellular membranes and membranes of organelles during cooling and drying and play a role in stabilizing dried cellular proteins (Carpenter and Crowe 1988; Carpenter et al. 1991). Lactose and other disaccharides have been found to be very good protectants. **Trehalose,** which is known to be produced naturally by some drought-tolerant filamentous fungi and yeasts (Thevelein 1984; Wiemken 1990), has been found to be optimal, and this disaccharide can therefore be added to the suspension of skim milk (Berny and Hennebert 1991; Tan et al. 1995). At CBS, trehalose is a standard additive in skim milk suspensions used in freeze drying large or multicellular spores.

An optimal freeze-drying process involves (1) **dehydration** of the propagules in the cooling phase prior to desiccation, (2) **sublimation of ice** from the frozen suspension during a phase of primary drying, followed by (3) **removal of chemically bound water** from the cells as well as from the protectant in a secondary drying phase (Tan 1997; Tan et al. 1994). The freeze-drying process can only be controlled sufficiently in a commercial freeze dryer. The optimal **cooling rate** for phase 1 depends on the properties of the spores (see below). As the extracellular water is frozen into ice crystals, the remaining lyoprotectant becomes increasingly viscose, leading to the removal of unbound water from the cells. In the **primary drying phase** that follows, the viscosity of the lyoprotectant solution is so high that it is a glass, viz., a liquid in which the molecules are immobilized (Franks 1990; Tan 1997). To keep a formulation stable, it should be maintained at a temperature below the **glass transition temperature** (Tg). The Tg of a formulation depends on the composition of the protectant and the residual moisture content (rmc) of the glass (Hatley 1990); the drier the glass, the higher the temperatures at which it can be stable. The Tg of a formulation can be determined by differential scanning calorimetry (DSC) (Hatley 1990). A glass will melt at the Tg, and the product will rapidly deteriorate. In the primary drying phase, usually ca. −20 °C for skim milk, the ice crystals are evaporated under a vacuum of ca. 0.30 mbar, leaving a glass with numerous channels. In the **secondary drying phase**, the temperature can be slowly increased to +25 °C, depending on the lyoprotectant, and the vacuum brought to 0.03 mbar

so that bound water can be removed more quickly from the cells and the surrounding cake in order to obtain a sufficiently stable product at the desired storage temperature.

Cultures for freeze drying are **inoculated** on fresh media that favor good sporulation and incubated under optimal conditions. The CBS homepage and strain catalog provide information on media and conditions for sporulation for many fungal species (http://www.cbs.knaw.nl). For yeast and most filamentous fungi, agar slants or plates are suitable. Spores should be harvested at the right moment and be sufficiently mature but not too old. After the culture has been checked for sporulation, purity, and identity, spores can be harvested by aseptically and slowly pouring 5 mL of tyndalized skim milk into the culture tube or plate and gently scraping the colony surface with a pipette or needle. With the pipette the suspension can be transferred back to a tube with skim milk and mixed carefully. Aliquots (0.05–0.2 mL) are then transferred to **glass ampoules** for freeze drying. A concentration of ca. 10^6 spores/mL is generally required. Filled ampoules must be refrigerated (5–7 °C) and processed within 1–2 h to avoid germination. CBS uses two types of ampoules and two basic methods of cooling, depending on the properties of the fungal spores. Thin-walled, one-celled spores dehydrate quickly and can therefore be instantly **cooled** by plunging the ampoules (simple cylindrical ampoules 110 × 6 mm in diameter, non-prescored) into a bath of acetone cooled to −80 °C with a cooling device (e.g., Cryocool, Neslab). Multicellular or thick-walled spores dehydrate less quickly and should be frozen at a lower rate, optimally around −1 °C/min, in order to avoid intracellular ice-crystal formation, which is lethal. For cooling of these spores a plasma freezer or programmed freezer (e.g., ice-cube) can be used.

For these more sensitive spores CBS uses prescored flat-bottom glass "vampoules" (Wheaton, 86 × 12 mm), which can be half closed with rubber stoppers during the drying phases and shut airtight before taking them out of the freeze dryer at the end of the secondary drying phase.

To **start the freeze-drying process**, frozen ampoules are placed in a freeze dryer on a shelf precooled to −40 °C, or they can be stored in a mechanical freezer (−80 °C) for some time if freeze drying cannot be started immediately. Shelf (sample) temperature and pressure can be optimized for the primary and secondary drying phase. For example, in the primary drying phase, the frozen water can be sublimated at ca. −20 °C under high vacuum (0.30–0.40 mbar), while in the secondary phase the samples can be warmed to ca. 25 °C under an ultrahigh vacuum (0.10–0.02 mbar) to remove the bound water from the cells and the surrounding protectant (ice condenser temperature −60 to −80 °C). It is important to document the main process parameters during drying (shelf and condenser temperatures, pressure), which should be fully automated in modern commercial freeze dryers.

The best types of **vials** are those that can be stoppered with cotton plugs (with or without silicon rubber stoppers), minimizing risk of contamination (of the vial content and the equipment used!). The size and shape of the vials matter; in particular, the surface of the pellet in relation to its volume influences the efficacy of the drying process and, ultimately, the viability of the end product.

Trace amounts of oxygen or water introduced when sealing the ampoules after drying can cause the deterioration of samples during storage. **The ampoules therefore need to be sealed under high vacuum or filled with a dry inert gas (nitrogen) before closure.** Vials made of borosilicate glass are easiest to heat-seal securely. Freeze-dried samples survive **storage** in the dark at room temperature (20 °C) for periods of up to 25 years when dried to a rmc below 5 % (Tan et al. 1991). Shelf life is extended considerably if the samples are drier (2–3 % rmc) and can be stored under controlled temperature, preferably 4–6 °C. One ampoule of each series of dried spores should be opened immediately after freeze drying **to check viability, purity, and identity.**

Reactivation of freeze-dried material is simple, but the procedures recommended by

the supplier of the material should always be carefully followed, particularly when rehydrating more sensitive fungal spores. Freeze-dried cells should be given sufficient time to rehydrate before being transferred to a solid medium. After disinfecting the outer surface of the ampoule with 70 % ethanol, it should be opened aseptically (preferably in a safety cabinet), suspending the freeze-dried material by pouring the full content into a tube containing 1–2 mL of sterile water or sterile malt-peptone solution. The tube is then shaken gently and left at room temperature for 4–12 h before the suspension is poured onto a solid agar medium suitable for (vegetative) growth. The remainder of the suspension can be stored in a refrigerator for up to 2 days for use in case the first attempt fails. Optimal conditions for growth should be applied, noting that not all media suitable for sporulation are equally fit for reactivation of freeze-dried material. Most fungi will start growing within 1–2 days, but some, particularly certain slow-growing fungi, need more time to grow. Yeasts cells that are suspended in water or malt peptone can normally be poured immediately onto solid agar medium, and suspensions in malt peptone can be stored in a refrigerator for up to 14 days. If survival is expected to be low, it is advisable to revive cells in 1.2 M sucrose to dilute the protectants and stimulate the efflux of the protectants from the cell while minimizing osmotic expansion (Tan 1997; Tan and Stalpers 1996).

Even if the crucial parameters for freeze drying are carefully optimized and controlled, some fungi will still show loss of viability immediately after drying or considerable decline during later storage. This may be caused by the lesser condition of the strain, which may have deteriorated from having been kept in active growth for a longer time before being first freeze-dried or because of unknown properties of the spores that render them recalcitrant to freeze drying.

IV. Conclusions

Fungal reference specimens kept in herbaria and living cultures permanently preserved in culture collections not only provide the necessary basis for fungal systematics and biodiversity research but are equally important for documenting research in ecology, genetics, and plant pathology. For example, phytosanitary regulations for the protection of food crops against fungal pathogens depend on properly documented reference material in order to be effective.

The value of herbarium specimens and cultures as reference material depends strongly on the quality of the preservation methods and management of the associated data. Mycologists who have performed DNA sequencing on herbarium specimens and cultures from culture collections have demonstrated repeatedly that they provide a rich source of undescribed taxa. To fix the application of already published names in the future, living cultures and vouchered herbarium material have been selected by taxonomists as epitypes, demonstrating again the importance of these resources.

Early fungal genome sequencing projects have relied sometimes completely on isolates kept in laboratories lacking adequate procedures for quality control and preservation. Fungal cultures accessioned in public culture collections that have methods in place for long-term preservation, state-of-the-art authentication, and dispatch will form an indispensable resource of material for global initiatives, such as DNA barcoding and genome sequencing projects. Herbarium specimens associated with these cultures will be of equal importance for future reference.

Acknowledgements Collections Manager Shannon Dominick is thanked for her useful comments on best herbarium practices as well as her conscientious management of the U.S. National Fungus Collections (BPI). Pedro W. Crous is kindly acknowledged for commenting on the manuscript.

References

Baker B (2011) New push to bring U.S. biological collections to the world's online community. Bioscience 61:657–662

Barnes CW, Szabo LJ, Bowersox VC (2008) Identifying and quantifying *Phakopsora pachyrhizi* spores in rain. Phytopathology 99:328–338

Bebber DP, Carine MA, Wood JRI, Wortley AH, Harris EJ, Prance GT, Davidse G, Paige J, Penninton TD, Robson NKB, Scottland RW (2010) Herbaria are a major frontier for species discovery. Proc Natl Acad Sci U S A 107:22169–22171

Berny J-F, Hennebert GL (1991) Viability and stability of yeast cells and filamentous fungus spores during freeze-drying: effects of protectants and cooling rates. Mycologia 83:805–815

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG (2011) The dawn of symbiosis between plants and fungi. Biol Lett 7(4):574–577

Blackwell M (2011) The fungi: 1, 2, 3 … 5.1 million species? Am J Bot 98:426–438

Brock PM, Döring H, Bidartondo MI (2009) How to know the unknown fungi: the role of the herbarium. New Phytol 181:719–724

Calcott PH (1978) Freezing and thawing microbes. Meadowfield Press, Shildon

Carpenter JF, Crowe JH (1988) The mechanism of cryoprotection of proteins by solutes. Cryobiology 25:244–255

Carpenter JF, Arakawa T, Crowe JH (1991) Interactions of stabilizing additives with proteins during freeze-thawing and freeze-drying. Dev Biol Stand 74:225–239

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Wingfield MJ (2003) Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. Mycologia 95:285–293

Cooper A, Poinar HN (2000) Ancient DNA: do it right or not at all. Science 289:1139

Crouch JA, Szabo LJ (2011) Real-time PCR detection and discrimination of the southern and common corn rust pathogens *Puccinia polysora* and *Puccinia sorghi*. Plant Dis 95:624–632

Crouch J, Tomaso-Peterson M (2012) Anthracnose disease of centipedegrass turf caused by *Colletotrichum eremochloae*, a new fungal species closely related to *Colletotrichum sublineola*. Mycologia. doi:10.3852/11-317

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009) Fungal biodiversity, vol 1, CBS Laboratory manual series. CBS, Utrecht

Cubero OF, Crespo A, Fatehi J, Bridge PD (1997) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. Plant Syst Evol 216:243–249

Eberhardt U (2012) Methods for DNA barcoding fungi. In: Kress JW, Erickson DL (eds) DNA barcodes: methods and protocols. Humana Press, New York

Elliot TJ (1976) Alternative ampoule for storing fungal cultures in liquid nitrogen. Trans Br Mycol Soc 67:545–546

Elliott TJ, Challen MP (1979) The storage of mushroom strains in liquid nitrogen. Glasshouse Crops Res Inst Annu Rep 1979:194–204

Farr DF, Farr ER (2004) Electronic information resources. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam, pp 49–57

Forman L, Bridson D (eds) (1989) The herbarium handbook. Royal Botanic Gardens, Kew, Surrey

Franks F (1990) Freeze-drying: from empiricism to predictability. The significance of glass transition. Dev Biol Stand 74:9–19

Fredericks DN, Smith CA, Meier A (2005) Comparison of six DNA extraction methods for the recovery of fungal DNA as assessed by quantitative PCR. J Clin Microbiol 43:5122–5128

Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington, DC

Gilbert MTP, Cuccui J, White W, Lynnerup N, Titball RW, Cooper A, Prentice MB (2004) Absence of *Yersinia pestis*-specific DNA in human teeth from five European excavations of putative plague victims. Microbiology 150:341–354

Hall DV (1981) Microwave: a method to control herbarium insects. Taxon 30:818–819

Hatley RHM (1990) The effective use of differential scanning calorimetry in the optimization of freeze-drying processes and formulations. Dev Biol Stand 74:105–122

Hawksworth DL (1974) Mycologist's handbook. An introduction to the principles of taxonomy and nomenclature in the fungi and lichens. Commonwealth Mycological Institute, Kew, Surrey

Hawksworth DL (2004) Fungal diversity and its implications for genetic resource collections. Stud Mycol 50:9–18

Heckly RJ (1978) Preservation of microorganisms. Adv Appl Microbiol 24:1–53

Hesseltine CW, Bradle BJ, Benjamin CR (1960) Further investigations on the preservation of molds. Mycologia 52:762–774

Hill SR (1983) Microwave and herbarium specimens: potential dangers. Taxon 32:614–615

Hoffmann P (1991) Cryopreservation of fungi. DSM Technical information sheet no. 5. World J Microbiol Biotechnol 7:92–94

Hofreiter M, Serre D, Poinar HN, Kuch M, Pääbo S (2001) Ancient DNA. Nat Rev Genet 2:353–359

Johnson GC, Martin AK (1992) Survival of wood-inhabiting fungi stored for 10 years in water and under oil. Can J Microbiol 38:861–864

Jones MDM, Forn I, Gadelha C, Egan MJ, Bass D, Massana R, Richards TA (2011) Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474:200–203

Jong SC (1989) Prevention and control of mite infestations in fungal cultures. ATCC Q Newslett 7:1127

Jong SC, Birmingham JM (2001) Cultivation and preservation of fungi. In: McLaughlin DJ, McLaughlin EG, Lemke P (eds) The mycota, vol 7B. Springer, Berlin, pp 193–202

Kirk PM, Cannon PF, Minter DW, Stalpers JA (eds) (2008) Ainsworth and Bisby's dictionary of the fungi, 10th edn. CAB International, Wallingford

Lambert DM, Ritchie PA, Millar CD, Holland B, Drummond AJ, Baroni C (2002) Rates of evolution in ancient DNA from Adélie penguins. Science 295:2270–2273

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362:709–715

Lister DL, Bower MA, Howe CJ, Jones MK (2008) Extraction and amplification of nuclear DNA from herbarium specimens of emmer wheat: a method for assessing DNA preservation by maximum amplicon length recovery. Taxon 57:254–258

Lumbsch HT, Huhndorf SH (2010) Myconet, vol 14. Part One. Outline of Ascomycota—2009. Fieldiana Life Earth Sci 1:1–42

May KJ, Ristaino JB (2002) The mitochodrial DNA haplotype of *Phytophthora infestans* in 19th century herbarium specimens revealed. Phytopathology 92:s53

May KJ, Ristaino JB (2004) Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish potato famine. Mycol Res 108:471–479

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (eds) (2006) International code of botanical nomenclature (Vienna Code). A. R. G. Gantner, Ruggell

Metsger DA, Byers SC (1999) Managing the modern herbarium, an interdisciplinary approach. Society of the Preservation of Natural History Collections, Washington, DC

Moore S (1999) Comments on freeze-drying and storing entire mycological specimens. In: Metsger D, Byers S (eds) Managing the modern herbarium: an interdisciplinary approach. Society for the Preservation of Natural History Collections and the Royal Ontario Museum, Washington, DC, pp 368–371

Mueller GM, Bills GF, Foster MS (2004) Biodiversity of fungi. Inventory and monitoring methods. Elsevier, Burlington

Nakasone KK, Peterson SW, Jong S-C (2004) Preservation and distribution of fungal cultures. In: Mueller G, Bills GF, Foster MS (eds) Biodiversity of fungi. Inventory and monitoring methods. Elsevier, Burlington, pp 37–47

O'Gorman DT, Sholberg PL, Stokes SC, Ginns J (2008) DNA sequence analysis of herbarium specimens facilitates the revival of *Botrytis mali*, a postharvest pathogen of apple. Mycologia 100:227–235

OECD (2007) OECD best practice guidelines for biological resource centres. OECD Publications, Paris

Pääbo S, Pinar H, Serre D, Jaenicke-Després V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M (2004) Genetic analyses from ancient DNA. Annu Rev Genet 38:645–679

Pertot E, Puc A, Kremser M (1977) Lyophilisation of non-sporulating strains of the fungus *Claviceps*. Eur J Appl Microbiol 4:289–294

Pyke GH, Ehrlich PR (2010) Biological collections and ecological/environmental research: a review, some observations and a look to the future. Biol Rev 85:247–266

Ristaino JB (1998) The importance of archival and herbarium materials in understanding the role of oospores in late blight epidemics of the past. Phytopathology 88:1120–1130

Ristaino JB (2006) Tracking the evolutionary history of the potato late blight pathogen with historical collections. Outlooks Pest Manage 17:228–232

Ristaino JB, Groves CT, Parra GR (2001) PCR amplification of the Irish potato famine pathogen from historic specimens. Nature 411:695–697

Rossman AY (1987) The Tubeufiaceae and similar Loculoascomycetes. Mycol Pap 157:1–71

Rossman AY (1996) The evolution of fungal diversity—past, present and future. In: Colwell RR, Simidu U, Ohwada K (eds) Microbial diversity in time and space. Plenum, New York, pp 33–39

Rossman A, Palm-Hernandez ME (2008) Systematics of plant pathogenic fungi: why it matters. Plant Dis 92:1376–1386

Rossman A, Simmons E (1999) A modified technique for making dried cultures as voucher specimens. Inoculum 50(4):3

SAFRINET (1999) Collecting and preserving fungi. A manual for mycology. NCF Biodiversity Division, ARC, Pretoria

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sakurada M, Tsuzuki Y, Morgavi DP, Tomita Y, Onodera R (1995) Simple methods for cryopreservation of an anaerobic rumen fungus using ethylene glycol and rumen fluid. FEMS Microbiol Lett 127:171–174

Savile DBO (1962) Collection and care of botanical specimens, vol 1113. Canada Department of Agriculture Research Publication, Ottawa, pp 1–124

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of novel fungal lineages in tundra soils. Science 301:1359–1361

Seifert KA, Rossman AY (2010) How to describe a new fungal species. IMA Fungus 1:109–116

Smith D (1983) Cryoprotectants and the cryopreservation of fungi. Trans Br Mycol Soc 80:360–363

Smith D, Coulson GE, Morris GJA (1986) A comparative study of the morphology and viability of hyphae of *Penicillium expansum* and *Phytophthora nicotiana* during freezing and thawing. J Gen Microbiol 132:2013–2021
Smith D, Onions AHS (1994) The preservation and maintenance of living fungi, 2nd edn. CAB International, Wallingford
Sokolski S, Piché Y, Bérubé JA (2004) *Lophodermium macci* sp. nov., a new species on senesced foliage of five-needle pines. Mycologia 96:1261–1267
Souzu H (1987) Freezing damage in microorganisms. In: Sakai A (ed) Cryopreservation. Asakura Shoten, Tokyo, pp 23–29
Spooner B, Cannon P (2010) World's largest collection of fungi held at Kew Gardens. Mycol News 2010 (1):8–9
Stalpers JA, De Hoog A, Vlug IJ (1987) Improvement of the straw technique for the preservation of fungi in liquid nitrogen. Mycologia 79:82–88
Stielow JB, Vaas LAI, Göker M, Hoffmann P, Klenk H-P (2012) Charcoal filter paper improves the viability of cryopreserved filamentous ectomycorrhizal and saprotrophic Basidiomycota and Ascomycota. Mycologia 104:324–330
Tan CS (1997) Preservation of fungi. Crypt Mycol 18:157–163
Tan CS (2011) Freeze-drying of filamentous fungi and yeasts. PhD thesis, Utrecht University, the Netherlands
Tan CS, Stalpers JA (1996) Vitrification of fungi. In: Cimerman A, Gunde-Cimerman N (eds) Biodiversity. International biodiversity seminar ECCO XIV. Meeting. National Institute of Chemistry, Ljubljana, pp 189–193
Tan CS, Stalpers JA, van Ingen CW (1991) Freeze-drying of fungal hyphae. Mycologia 83:654–657
Tan CS, Vlug IJA, Stalpers JA, van Ingen CW (1994) Microscopical observations on the influence of the cooling rate during freeze-drying of conidia. Mycologia 86:281–289
Tan CS, van Ingen CW, Talsma H, Miltenburg JC, Steffensen CL, Vlug IJA, Stalpers JA (1995) Freeze-drying of fungi: influence of composition and glass transition temperature of the protectant. Cryobiology 32:60–67
Taylor JW, Swann EC (1994) Dried samples: soft tissues, DNA from herbarium specimens. In: Herrmann B, Hummel S (eds) Ancient DNA. Springer, Berlin, pp 166–181
Telle S, Thines M (2008) Amplification of *cox2* (~620 bp) from 2 mg of up to 129 year old herbarium specimens, comparing 19 extraction methods and 15 polymerases. PLoS One 3:e3584
Thevelein JM (1984) Regulation of trehalose mobilization in fungi. Microbiol Rev 48:42–59
Wiemken A (1990) Trehalose in yeasts, stress protectant rather than reserve carbohydrate. Antonie Van Leeuwenhoek 58:209–217
Wood EW, Eriksson T, Donoghue MJ (1999) Guidelines for the use of herbarium materials in molecular research. In: Metsger DA, Byers SC (eds) Managing the modern herbarium, an interdisciplinary approach. Society of the Preservation of Natural History Collections, Washington, DC, pp 265–275
Wu Q, Thiers BM, Pfister DH (2004) Preparation, preservation, and use of fungal specimens in herbaria. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam, pp 23–36
Zischler H, Hoss M, Handt O, von Haeseler A, van der Kuyl AC, Goudsmit J (1995) Detecting dinosaur DNA. Science 268:1192–1193

Evolution

9 Subcellular Structure and Biochemical Characters in Fungal Phylogeny

David J. McLaughlin[1], T.K. Arun Kumar[2], Meredith Blackwell[3,4], Peter M. Letcher[5], Robert W. Roberson[6]

CONTENTS

I. Introduction 229
II. The Role of Subcellular Structure in Fungal Phylogeny 230
III. The Role of Biochemical Characters in Fungal Phylogeny 233
IV. Character and Character State Definition and Refinement........................... 237
V. Structural and Biochemical Database.... 238
VI. Structural and Biochemical Database in Phylogenetic Analysis 239
VII. Development of the Fungal Subcellular Ontology 240
VIII. Specimen Preparation and Evaluation for Subcellular Structure..................... 242
A. Specimen Preparation and Evaluation . 242
1. Fixation.............................. 242
2. Standard Infiltration and Embedding 245
3. Microwave Fixation and Embedding.. 246
4. Section Staining and Microscopy Methods 246
B. Methods for Selected Cellular Features.. 247
1. Nuclear Division Studies 247
2. Apical Organization Studies......... 248
3. Flagellated Fungi Studies 249
IX. Conclusions............................... 251
References................................. 252

[1]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA; e-mail: davem@umn.edu
[2]Department of Botany, The Zamorin's Guruvayurappan College, Calicut, Kerala 673014, India; e-mail: tkakumar@gmail.com
[3]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA; e-mail: mblackwell@lsu.edu
[4]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA; e-mail: mblackwell@lsu.edu
[5]Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA; e-mail: letch006@bama.ua.edu
[6]School of Life Sciences, Arizona State University, Tempe, AZ, 85287, USA; e-mail: robby2@asu.edu

I. Introduction

Structural and biochemical characters have played an important role in determining phylogenetic relationships among Fungi since the beginning of their modern classification in the mid-nineteenth century. It was only with the introduction of ultrastructural methods and improved biochemical analyses in the 1950s that a clearer understanding of structural variation and its phylogenetic implications became possible (McLaughlin et al. 2009). Subcellular structural data complemented biochemical studies on cell walls and enzymatic pathways that began to delimit a monophyletic kingdom Fungi from organisms that are similar to Fungi in form and ecology (Bartnicki-Garcia 1970, 1987). Early subcellular studies of Fungi resulted in an extensive literature in the 1960s through the 1980s, but the number of studies declined with the introduction of more demanding cell preparation procedures developed in the 1980s (Hoch 1986; Howard and O'Donnell 1987). These new cryofixation methods also added confusion as to which methods provided reliable data and discouraged researchers who worked with taxa that were not suitable for preparation by cryofixation methods. As a result, **the number of fungal taxa studied ultrastructurally is still very limited and does not provide a meaningful understanding of subcellular structural variation in Fungi.**

Molecular phylogenetics and phylogenomic data are currently providing a better understanding of fungal relationships and revealing a large number of fungal clades for

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

which little or no subcellular or biochemical data exist (Hibbett et al. 2007; James et al. 2006a; McLaughlin et al. 2009). The AFTOL **Structural and Biochemical Database (SBD)** is intended to clarify the limits of our knowledge of subcellular characters and provide a ready reference for comparison of structural data to reach a meaningful level of understanding of structural evolution in Fungi (Kumar et al. 2013). **Molecular phylogenies have permitted a reevaluation of both biochemical and subcellular characters and provided a new understanding of their evolution** (McLaughlin et al. 2009; Weete et al. 2010). This chapter aims to give the reader guidance on how to acquire and evaluate subcellular characters and on the role of the SBD and Fungal Subcellular Ontology (FSO) in fungal systematics and phylogeny. The structural data also have functional significance and provide guidance in cell and developmental studies on character diversity and evolution within clades and across the Fungi.

II. The Role of Subcellular Structure in Fungal Phylogeny

The relatively simple structure of Fungi masks many instances of homoplasy (e.g., convergent evolution). Light microscopy may suggest that two organisms are not closely related as, for example, in the organization of zoospores, or specialized cell types, such as meiosporangia and spores, but light microscopic images are best understood after the vastly improved resolution of ultrastructural examination. Although Fungi contain a broad array of subcellular structures (Beckett et al. 1974; Bracker 1967; McLaughlin et al. 2001), relatively few provide synapomorphies for kingdom Fungi. Exceptions include flattened mitochondrial cristae, reduced Golgi bodies in most and in certain forms, motile cells with a single posterior flagellum, and similar flagellar apparatus (Baldauf et al. 2004). **Motile cells, septa, and cytological features of dividing nuclei have provided clues to taxonomic relationships within the Fungi** (Fig. 9.1).

Character states for structural characters in Fig. 9.1:

Motile Cell

1. *Centriole* absent, 0; present, 1.
2. Nonflagellated centriole absent, 0; present, 1.
3. *Kinetosome* absent, 0; short, <0.5 µm, 1; long, >0.5 µm, 2.
4. *Organization of microbody lipid complex* (*MLC*) absent, 0; MLC cisterna backs lipid, 1; MLC cisterna backs microbody, 2.
5. *Rhizoplast cap* absent, 0; present, 1.
6. *Flagellar props* absent, 0; present, 1.

Nuclear Division and Spindle Pole Body

7. *Basic spindle pole body form* centriole-associated extranuclear and intranuclear components with intact nuclear envelope, 0; centriole-associated material with open nuclear envelope, 1; small amounts of extranuclear and intranuclear material with intact nuclear envelope, 2; notched ring with persistent half middle piece plus intranuclear component, 3; plaque or disc, 4; globular, 5; ring(s) containing microtubules but lacking centriole nine-fold symmetry, 6; bar, 7.
8. *Metaphase nuclear envelope* intact, 0; intact with small polar fenestrae plugged by the spindle pole bodies, 1; loose polar fenestrae, including extensions of nuclear envelope into the cytoplasm at the spindle pole, but mainly intact, 2; partially dispersed, 3; nearly or entirely dispersed, 4; intact with polar fenestrae adjacent to centrosome containing paired centrioles, 5.

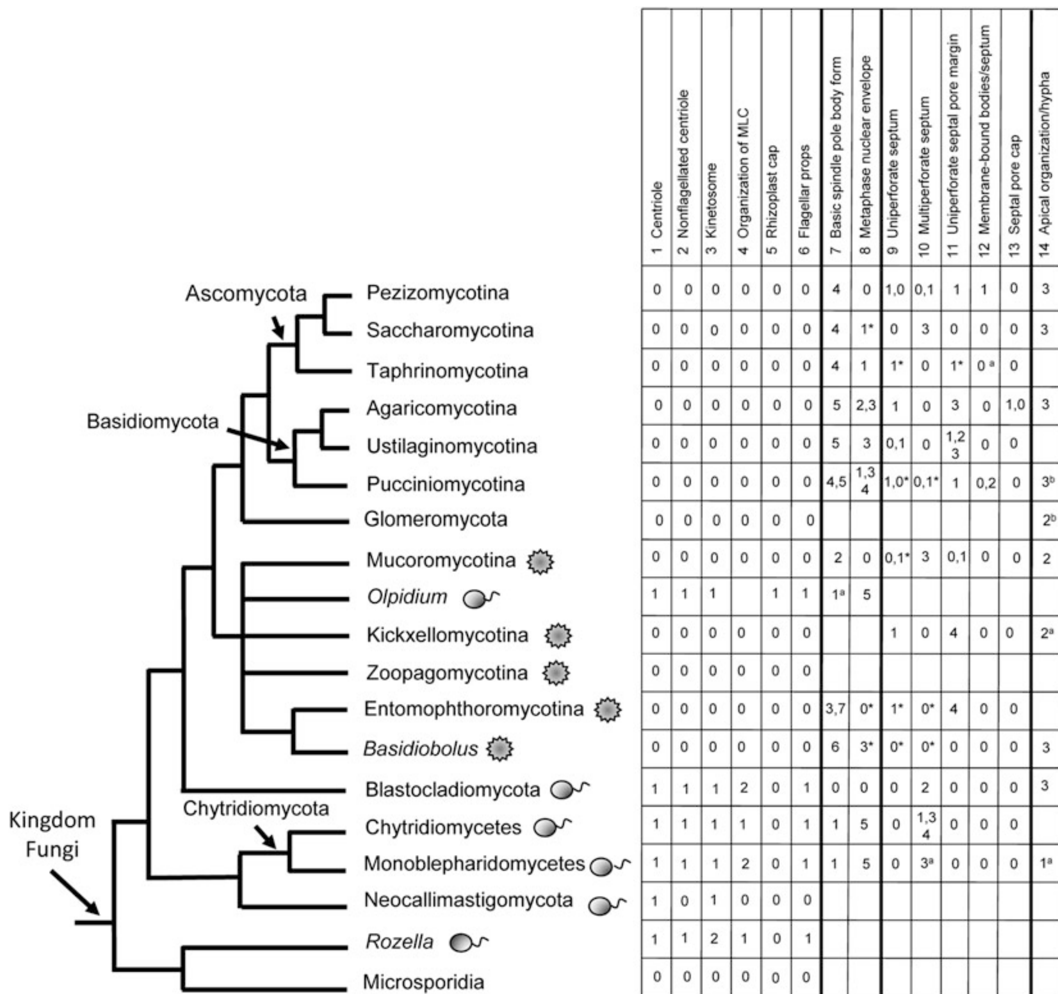
Septum

9. *Uniperforate septum* absent, walled-off pore, or apparently walled-off pore, 0; a single central pore, 1.
10. *Multiperforate septum* absent, 0; simple pores with uniform pores not solely adjacent to hyphal wall, 1; simple pores with variable-sized large pores adjacent to hyphal wall, 2; plasmodesmata, 3; thickened septum with central pore closed by plasmodesmata, 4.
11. *Uniperforate septal pore margin* uniperforate septal pore absent, 0; unelaborated margin, 1; uniperforate septal pore absent but with some type of discontinuity within septum, for example, disruption of central layer of cross wall, wall swelling, or deposits within cross wall, suggestive of a blocked or disrupted pore, 2; septal pore swelling, 3; with lenticular cavity (bifurcate), 4.
12. *Membrane-bound bodies associated with septal pore(s)* absent, 0; Woronin bodies, 1; microbodies, 2.
13. *Septal pore cap* absent, 0; present, 1.

Apical Organization

14. *Apical organization of hypha or germ tube* absent, 0; loose vesicle cluster, 1; apical crescent, 2; Spitzenkörper, 3.

Light microscopy initially revealed the diversity in zoospores of Fungi and fungus-like organisms, and zoospores subsequently became the focus of ultrastructural studies (Barr 1981; Fuller 1977). The structure of the transition zone of the flagellar apparatus (Barr 1992) and of the flagellar



Taxon	1 Centriole	2 Nonflagellated centriole	3 Kinetosome	4 Organization of MLC	5 Rhizoplast cap	6 Flagellar props	7 Basic spindle pole body form	8 Metaphase nuclear envelope	9 Uniperforate septum	10 Multiperforate septum	11 Uniperforate septal pore margin	12 Membrane-bound bodies/septum	13 Septal pore cap	14 Apical organization/hypha
Pezizomycotina	0	0	0	0	0	0	4	0	1,0	0,1	1	1	0	3
Saccharomycotina	0	0	0	0	0	0	4	1*	0	3	0	0	0	3
Taphrinomycotina	0	0	0	0	0	0	4	1	1*	0	1*	0[a]	0	
Agaricomycotina	0	0	0	0	0	0	5	2,3	1	0	3	0	1,0	3
Ustilaginomycotina	0	0	0	0	0	0	5	3	0,1	0	1,2 3	0	0	
Pucciniomycotina	0	0	0	0	0	0	4,5	1,3 4	1,0*	0,1*	1	0,2	0	3[b]
Glomeromycota	0	0	0	0	0	0								2[b]
Mucoromycotina	0	0	0	0	0	0	2	0	0,1*	3	0,1	0	0	2
Olpidium	1	1	1		1	1	1[a]	5						
Kickxellomycotina	0	0	0	0	0	0			1	0	4	0	0	2[a]
Zoopagomycotina	0	0	0	0	0	0								
Entomophthoromycotina	0	0	0	0	0	0	3,7	0*	1*	0*	4	0	0	
Basidiobolus	0	0	0	0	0	0	6	3*	0*	0*	0	0	0	3
Blastocladiomycota	1	1	1	2	0	1	0	0	0	2	0	0	0	3
Chytridiomycetes	1	1	1	1	0	1	1	5	0	1,3 4	0	0	0	
Monoblepharidomycetes	1	1	1	2	0	1	1	5	0	3[a]	0	0	0	1[a]
Neocallimastigomycota	1	0	1	0	0	0								
Rozella	1	1	2	1	0	1								
Microsporidia	0	0	0	0	0	0								

Fig. 9.1 Distribution of 14 characters used in differentiating subphyla and classes of Fungi. Taxa with motile cells are indicated by flagellated cell diagrams, and zygosporic fungi by zygospore diagrams. Characters *1–6* associated with flagellum apparatus of motile cells; characters *7* and *8* associated with the nucleus; characters *9–13* associated with the septum; character *14* associated with the hyphal apex. Data from AFTOL Structural and Biochemical Database https://aftol.umn.edu/. *Asterisk* (*) indicates that the character state is based on information from a single taxon. See text for character state descriptions. Fungal phylogenetic tree data are based on James et al. (2006a), White et al. (2006), and Hibbett et al. (2007). [a]Unpublished. [b]Germ tubes only

roots within the zoospore (Barr 1981) separated early diverging Fungi from other fungus-like groups with motile cells that are more closely related to stramenopiles or Rhizaria (Braselton 2001; Cavalier-Smith 2001; Dick 2001; Fuller 2001) (see Beakes et al., Chap. 3, and Bulman and Braselton, Chap. 4, Vol. VII. Part A). The **systematic importance of zoospore structure has been supported by recent studies** that revealed multiple monophyletic lineages within basal Fungi, although relatively few taxa have been studied within each lineage (James et al. 2006b).

The septal pore structure was first observed with light microscopy, but **septum diversity was not fully understood until septa were examined ultrastructurally** (Bracker 1967). Hemispherical pads at the septal pore were first reported by light microscopy in Basidiomycota (Buller 1933). The pads, termed dolipores when

examined ultrastructurally (Moore and McAlear 1962), helped to distinguish some Basidiomycota from the Ascomycota. It should be noted, however, that the flared dolipore septum may be an artifact consistently seen with chemical fixation and is not pronounced with cryofixation (Hoch and Howard 1981). The **septal pore apparatus**, i.e., the septum and cytoplasmic organization around the pore (Bracker and Butler 1963), lacked dolipores in some Basidiomycota, and these simple septate taxa share plesiomorphic septal characters with Ascomycota (Celio et al. 2006). Similarly, in some zygosporic fungi, light and the earliest electron microscopy revealed a distinctive pore with a lenticular cavity (Benjamin 1959; Benny 1972; Reichle and Lichtwardt 1972), which was subsequently shown to be synapomorphic for the Kickxellomycotina (Celio et al. 2006; Hibbett et al. 2007).

The fundamental nature of mitosis and meiosis to eukaryotic cells made the microtubule organizing center at the spindle pole, i.e., **the centriole and its associated material or the spindle pole body (SPB) in Fungi that lack motile cells, and changes in the nucleoplasm, nucleolus, and nuclear envelope during division, a focus of study for phylogenetic characters** that were used to separate Fungi from fungus-like organisms (Heath 1980, 1986). Cryofixation resolved concerns about the validity of using membrane fragmentation during nuclear division as a structural character. Septa, nuclear division, and SPB characters provide synapomorphies for some clades within Fungi (Celio et al. 2006).

Other subcellular features also may have phylogenetic significance. The growing hyphal apex forms a Spitzenkörper in many Fungi, but it appears to be absent among most zygomycete taxa (Roberson et al. 2010). The **hyphal apical organization** is currently being examined in unstudied major fungal clades (Bentivenga et al. 2013). Septa in ascogenous hyphae and the ascus have been the source of subcellular characters, as has the ascus apex (Bellemere 1994; Cole 1979; Kimbrough 1994). Structural variations in the developmental stages of basidiospores are potential sources of phylogenetic characters, but additional studies are needed to assess their phylogenetic potential (McLaughlin et al. 1985; Miller 1988; Yoon and McLaughlin 1986). Specialized cell types, such as cystidia and paraphyses, are also potential sources of characters, but homoplasy may be a problem with interpretation at certain taxonomic ranks (Bellemere 1994; Jenkinson et al. 2008; Padamsee et al. 2008; Pfister and Kimbrough 2001) but are incompletely surveyed. Host–parasite interactions show remarkable structural variations in some fungal groups and have been used for taxonomic purposes (Bauer et al. 2001; Bergerow et al. 2006). Dictyosomes, consisting of stacked cisternae, are restricted to a few basal fungi and replaced by less morphologically complex Golgi equivalents, comprised of only a single cisterna, in other fungi including many flagellated taxa (Roberson et al. 2010). Other subcellular features may also be of phylogenetic interest at the ordinal or lower taxonomic ranks, such as hyphal branching involving the disruption of the outer wall (McLaughlin et al. 1995b), the microscala (McLaughlin 1990) or symplechosome (Bauer and Oberwinkler 1991; Oberwinkler and Bauer 1989), which consists of endoplasmic reticulum or mitochondria cross-linked by regularly spaced filaments, or unusual vesicles, such as those with tubular invaginations (McLaughlin et al. 2008). The continual addition of new cell structures, such as a new septal pore cap type (Padamsee et al. 2012) and type of septal pore organization (Healy et al. 2013), suggests that the Fungi are still poorly understood ultrastructurally.

Many of the structural studies by systematic mycologists interpret cellular components on a morphological basis only. Cell biological analyses are needed to understand these cell components. Differentiating Woronin bodies in Ascomycota, a specialized form of peroxisome in Pezizomycotina, from microbodies at the septal pore in other Ascomycota and Basidiomycota required cytochemical localization for HEX-1 protein (Dhavale and Jedd 2007; Roberson et al. 2010). Similar studies are needed for other cell structures, such as the atractosome, which exhibits a morphology that varies among species (Oberwinkler et al. 2006; Weiss et al. 2004). This microbody-like structure is found around the septal pore in some Pucciniomycotina and contains peripheral electron-opaque material or curved cisternae.

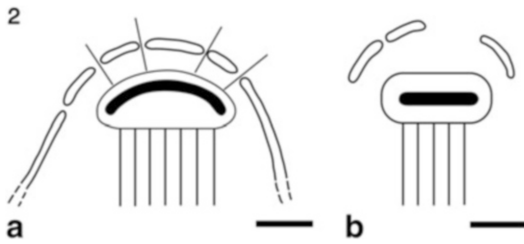


Fig. 9.2 Drawings comparing spindle pole body–nuclear envelope relationship at metaphase–anaphase in smut fungi: (**a**) *Ustilago maydis* (Ustilaginomycotina) at meiosis and (**b**) *Microbotryum violaceum* (Pucciniomycotina) at mitosis. In *U. maydis* the SPB is subglobular with a convex internal layer, while in *M. violaceum* it is subglobular with a flat internal layer and resembles a modified disc. Scale bar = 0.2 μm. *U. maydis* after O'Donnell and McLaughlin (1984); *M. violaceum* after Poon and Day (1976)

There are limitations to the use of structural characters for phylogenetic reconstruction without guidance from an independent and data set such as molecular sequences. While nuclear division and SPB and/or septal characters can be used independently to produce phylogenetic trees (Lutzoni et al. 2004; McLaughlin et al. 1995a), guidance from molecular data is desirable to recognize structural homoplasy and to determine character polarity, i.e., its plesiomorphic or apomorphic status. Many unexpected taxonomic relationships were revealed with molecular sequence data and led to a new understanding of structural characters, for example, motile cells of Blastocladiomycota and Chytridiomycota (James et al. 2006b) (see Powell and Letcher, Chap. 6, and James et al., Chap. 7, Vol. VII, Part A), and SPB form among certain smut taxa, which are now classified in different subphyla (Celio et al. 2006; McLaughlin et al. 1995b) (Fig. 9.2) (see Aime et al., Chap. 10, Vol. VII, Part A). Thus, sequence data aid in polarizing often autapomorphic structural characters.

III. The Role of Biochemical Characters in Fungal Phylogeny

Before effective microscopic methods were available to search out distinctive features of fungi, biochemical and physiological characters were used to distinguish among taxa. Nineteenth-century mycologists used stains and dyes that yielded color reactions that were used to obtain information for lichens and other ascomycetes (Nylander 1865; Rolland 1887). For lichens, simple spot tests based on reactions of metabolites to KOH and $Ca(ClO)_2$, at first used without knowledge of the basis for the reaction, helped to differentiate the products produced by specimens with similar appearance (Nylander 1867). In the early nineteenth century, however, chemists were interested in determining the nature of the compounds. For example, Pereira (1854) discussed using lichens such as species of *Roccella* to obtain litmus. Fluorescence and thin-layer chromatography were more sophisticated means developed later to obtain similar information.

Color tests continue to be used in identification keys for many fungi, including lichens, yeasts, other ascomycetes, and basidiomycetes (Ammirati et al. 1985). The well-known Melzer's reagent (Melzer 1924), a modification of earlier formulations of iodine, produces a blue staining (amyloid reaction due to fungal starch) of certain basidiospores and ascus tips of apothecial fungi, such as *Peziza* by Melzer's iodine, and the red staining (dextrinoid reaction due to glycine betaine in some fungi) of hyphidia of *Vararia* and other members of the Lachnocladiaceae (Baral 1987; Dodd and McCraken 1972; Kuo 2006; McCracken and Dodd 1971). A more recently developed spot test, Diazonium Blue B (DBB), has been used to distinguish basidiomycete (red staining) from most ascomycete (yellowish or nonstaining) yeasts (Kurtzman et al. 2011) but is of more limited use in distinguishing filamentous fungi (Hutchison and Summerbell 1990). DBB apparently links to tyrosine and histidine residues of wall proteins of basidiomycetes (Tevayanond 1981).

In addition to DBB, a wide array of biochemical and physiological tests have traditionally been used in yeast taxonomy. Yeasts have few morphological characters for distinguishing taxa, so bacterial methods were adapted for distinguishing them. Approximately 80 tests for determining the assimilation and fermentation

Table 9.1 Major chemical characters used in distinguishing Fungi from other major groups of organisms (Vogel 1960, 1965; Alexopoulos et al. 1996; Prillinger 2002; Torruella et al. 2009)

Group	Lysine pathway[a]	Storage compound	Cell wall	Sterol
Plants	Diaminopimelic (DAP) (Vogel 1960, 1965)	Starch	Cellulose, hemicellulose, lignin	Stigmasterol, brassicasterol, others
Animals	–	Glycogen, lipids, trehalose	–	Cholesterol
Fungi	∂-aminoadipate (AAA)	Glycogen, lipids, trehalose	β-1,3 and 1,6 glucans + chitin (β-1,4-*N*-acetylglucosamine polymer), chitosan in zygomycete fungi (β-1,4-glucosamine polymer), mannoprotein or polysaccharide matrix	Ergosterol, others including cholesterol, 24-methylene cholesterol, 24-ethyl cholesterol, brassicasterol
Oomycota	DAP	Mycolaminarins, soluble β-1,3-glucans	β-glucan, hydroxy proline and cellulose	Fucosterol or acquired
Myxomycetes	DAP	Glycogen	β-galactose	Campesterol [(24R)-24-Methylcholestan-3β-ol]
Dictyostelids	DAP	Glycogen, trehalose in mature spores?	Cellulose	∂-22-stigmasten-3β-ol

[a]See text for discussion of genes involved in lysine synthesis

potential of carbon and nitrogen sources, water potential, and vitamin production are commonly used. Ubiquinone (CoEnzyme Q) also has been used in yeasts, as well as for some filamentous fungi, but is variable within many lower-rank taxa. Although such characters are no longer used solely to identify yeasts, they are used in conjunction with other characters and are important in determining the potential for substrate utilization in the natural environmental (Kurtzman et al. 2011).

In addition to using biochemical attributes to distinguish taxa, they also were used as some of the only characters that extended across taxa for use in hypothesizing higher relationships or distinguishing Fungi from other major groups of organisms (Table 9.1). Linnaeus (1753) considered fungi to be plants and placed them in class Cryptogamia with ferns, bryophytes, and algae based on the presence of a cell wall. Later the chemical composition of the wall became a main character to distinguish among the wide variety of unrelated organisms with cell walls. As late as the mid-twentieth Century, G. W. Martin, a prominent mycologist, asked, “Are fungi plants?” He thought that biologists would answer with “an unqualified affirmative” (Martin 1955). Alexopoulos (1952), however, had already accepted the “strong and growing opinion among mycologists that the fungi may have originated from animal-like forms.” As more was learned about biochemistry, other characters, such as the fungal storage product glycogen, lysine synthesis pathway, and the ergosterol membrane sterol (Weete et al. 2010), were used to distinguish fungi from other walled organisms (Alexopoulos et al. 1996) (Table 9.1).

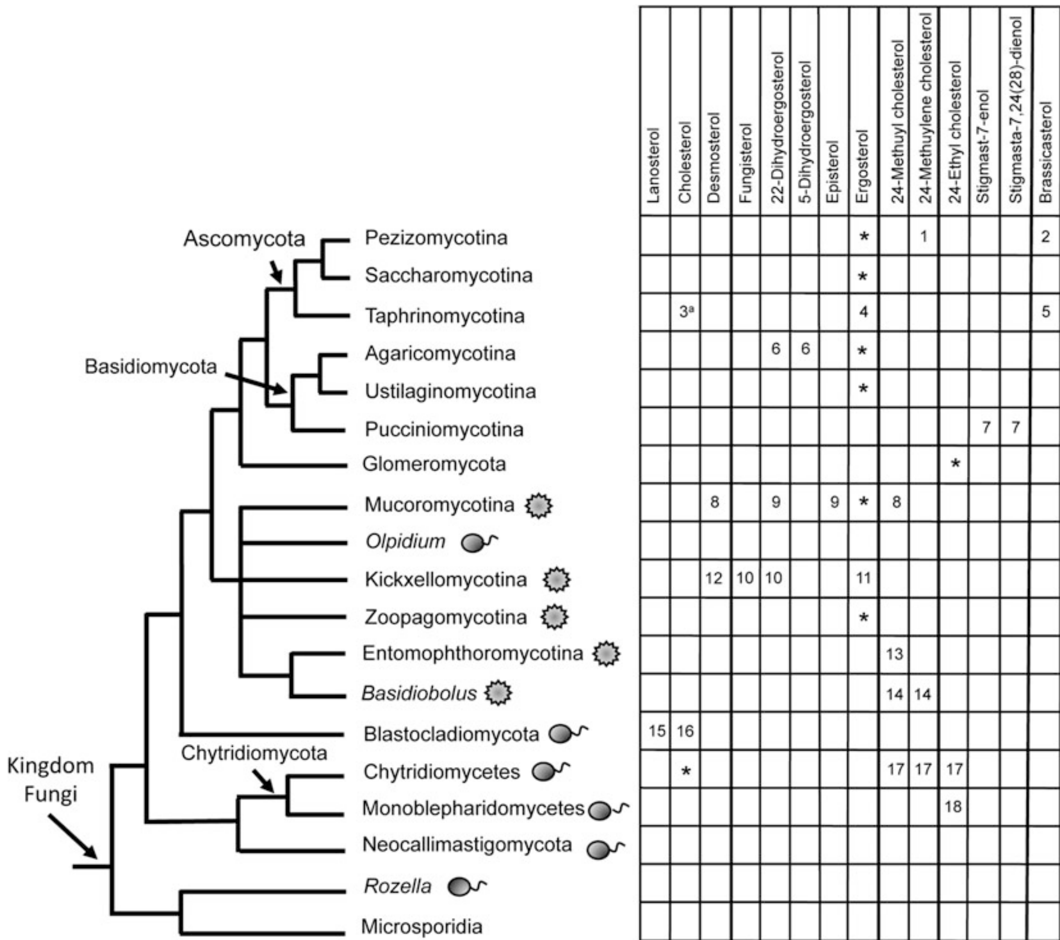
Walls of fungi in particular have been used successfully in distinguishing Fungi from the other major filamentous organisms, for example the Oomycota, and later were to become important as a target for the action of antibiotics. The classic studies of Bartnicki-Garcia firmly established the differences between the cell wall chemistry of oomycetes and Fungi,

including uniflagellated forms (e.g., Bartnicki-Garcia 1970, 2006), and this work has been tested many times by DNA analyses to show the distance between the two groups. **Cell wall chemistry also has been used to support differences among fungal groups.** Prillinger and his colleagues (e.g., Prillinger et al. 1993, 2002) used a gas chromatography method (Prillinger et al. 1990) to analyze the cell wall carbohydrate composition and compare 250 species of ascomycete and basidiomycete yeasts. The differences in neutral sugars of the groups indicated that several patterns of wall carbohydrates were consistent with phylogenetic analyses based on DNA as well as septal pore margin and septal pore cap characters. Ascomycetes with simple pores and no associated membranes typically have a predominance of glucose with some mannose and galactose. Pucciniomycotina with simple septal pores and lacking a septal pore cap had cell walls that were predominantly mannose but also with glucose, galactose, and fructose. Ustilaginomycotina with septal pore margin flared or not and no septal pore cap had walls that were predominantly glucose but also often containing mannose and galactose and lacking xylose. Members of Agaricomycotina had cell walls predominantly of glucose with mannose and xylose, and these findings also corresponded with the presence of dolipore septa and a septal pore cap.

More recently, **the predominant type of sterol present in fungal membranes has been surveyed to compare sterol types and pathways with recent phylogenetic information** that was not available when much of the sterol data were collected (Weete et al. 2010). Most of the fungi in the Ascomycota and Basidiomycota have membranes that are distinctive from other groups of organisms in that they contain ergosterol, long considered to be the "fungal sterol." Among the early diverging fungal lineages, however, there is variation in the membrane sterols (Fig. 9.3). Membranes of Glomeromycota tested contain 24-ethyl cholesterol, and this finding has implications for previously used research protocols based on mycorrhizal biomass measurements by the presence of ergosterol in soils (Grandmougin-Ferjani et al. 1999). Exceptions to ergosterol membranes also exist within the Ascomycota and Basidiomycota, although most phylum members have ergosterol membranes. For example, Pucciniomycotina have distinctive membranes of either 24-ethyl-cholesta-7,24(28)-dienol (stigmasta-7,24(28)-dienol) or 24-ethyl-cholest-7-enol (stigmast-7-enol) intermediates in the 24-ethyl-cholesterol pathway. The synthesis of these sterols is separated by a single step in their pathway (Weete et al. 2010). Among the ascomycetes, the Erysiphales differ from other members of the Pezizomycotina by production of 24-methyl-cholesta-5,24(28)-dienol (24-methylene cholesterol) (Fig. 9.3).

A major problem inherent in using the presence or absence of biochemical and physiological characters such as sterols is whether a feature is absent or merely not expressed. Certain examples need clarification to fully utilize the information from the expression of sterol membranes as a phylogenetic character. There is some indication that certain parasitic fungi closely associated with plants and animals scavenge membrane sterols from their hosts. Such information is vital beyond its use in evolutionary studies because of the common treatment of many fungal infections using antifungal drugs that target sterol synthesis. *Pneumocystis carinii* has been reported to obtain membrane sterols, i.e., cholesterol, from its host. Other reports indicate that *P. carinii* has genes for the synthesis of several sterols based in part on genome scans (Giner et al. 2002; Joffrion and Cushion 2010; Kaneshiro 2002). Is the major membrane sterol, brassicasterol, in the membranes of other symbiotic fungi including *Tuber*, *Terfezia*, and the Taphrinales accumulated or is it synthesized?

Lysine synthesis has been used as a major distinguishing feature between Fungi and all other major groups of organisms. The method of lysine synthesis has become murky because it has recently been found that the ∂-aminoadipate reductase (AAR) gene, a core gene of the AAA pathway, and *lysA* gene, a core gene of the DAP pathway, are both sometimes present



Taxon	Lanosterol	Cholesterol	Desmosterol	Fungisterol	22-Dihydroergosterol	5-Dihydroergosterol	Episterol	Ergosterol	24-Methyl cholesterol	24-Methylene cholesterol	24-Ethyl cholesterol	Stigmast-7-enol	Stigmasta-7,24(28)-dienol	Brassicasterol
Pezizomycotina								*		1				2
Saccharomycotina								*						
Taphrinomycotina		3[a]						4						5
Agaricomycotina					6	6		*						
Ustilaginomycotina								*						
Pucciniomycotina												7	7	
Glomeromycota											*			
Mucoromycotina			8		9		9	*	8					
Olpidium														
Kickxellomycotina			12	10	10			11						
Zoopagomycotina								*						
Entomophthoromycotina									13					
Basidiobolus									14	14				
Blastocladiomycota	15	16												
Chytridiomycetes		*							17	17	17			
Monoblepharidomycetes											18			
Neocallimastigomycota														
Rozella														
Microsporidia														

Fig. 9.3 Sterol distribution in Fungi. Taxa with motile cells are indicated by flagellated cell diagrams, and zygosporic fungi by zygospore diagrams. *Asterisks* indicate the predominant sterol present in a terminal taxon (usually a phylum). Distinctive sterols are present in some clades within phyla: *1* Erysiphales; *2 Tuber* and *Terfezia*; *Tuber* also contains mostly ergosterol; *3 Pneumocystis*; *4 Schizosaccharomyces*; *5 Taphrina* and *Protomyces*; *6* several Polyporaceae; *7* Pucciniales; *8* members of Mortierellales; *9* some Mucorales; *10* Kickxellales; *11* Dimargaritales; *12 Smittium* spp.; *13* most members of Entomophthorales contain 24-methyl cholesterol, although several are known to have cholesterol or 24-methylene cholesterol or mixtures of 24-methyl cholesterol and 24-methylene cholesterol; *14 Basidiobolus*, one species only; *15 Catenaria anguillulae*; *16 Allomyces macrogynus* and *Blastocladiella emersonii*; *17 other chytrids*; *18* only *Monoblepharella* sp. was sampled. Basal fungal groups and fungal sister taxa are poorly sampled or completely unsampled. Fungal phylogenetic tree based on James et al. (2006a), White et al. (2006), and Hibbett et al. (2007). Sterol data after Weete et al. (2010). [a]*Pneumocystis* spp. may scavenge membrane cholesterol from hosts, although recent studies indicate that it has genes to synthesize a variety of Δ7 and Δ8 24-alkylsterols (Kaneshiro 2002; Giner et al. 2002; Joffrion and Cushion 2010)

in the same organisms, including all fungi that have completed or nearly completed genomes (Torruella et al. 2009). In another example, xylose fermentation is a rare ability of fewer than 20 of approximately 1,000 described yeasts, determined by the presence of three genes. Urbina and Blackwell (2012) compared several species of yeast that assimilated D-xylose and xylitol as sole carbon sources. Some of the yeasts fermented xylose, but others did not. Fungi competent in xylose fermentation had three intact genes [xylose reductase

(XYL1), xylitol dehydrogenase (XYL2), and xylulose kinase (XYL3)] that functioned in xylose fermentation. In the yeasts that had the ability to assimilate but not ferment xylose, however, the xylose reductase gene (XYL1) had a modified motif that may prevent the correct 3-D configuration and functioning of the gene. A new era in the interpretation of characters of organisms, comparative genomics (Stajich et al. 2009), allows one to determine whether genes controlling certain factors are present or absent or nonfunctional and, perhaps, even why they are nonfunctional (see Stajich, Chap. 11, this volume).

IV. Character and Character State Definition and Refinement

A broad range of subcellular and biochemical characters are potential phylogenetic markers within the Fungi at a variety of taxonomic levels. The major focus for structural characters for the AFTOL Structural and Biochemical Database (SBD, see Sect. V) thus far has been those characters associated with nuclear division and the SPB, septa, and motile cell. Additional characters currently being assessed for their phylogenetic utility include hyphal apical organization, and some meiosporangium and cystidial features, but other sources of potential characters include other sterile cell types and haustoria. Several promising biochemical characters, including membrane sterols and cell wall components, are being developed with the aid of genomics.

To determine whether structural characters are evolutionarily significant and have phylogenetic application, the **homology** of characters and their states must be assessed (Celio et al. 2006). Comparative methods in which the same or similar character states occur in related organisms at the same developmental stage have been the most direct way to support hypotheses of homology. **A variety of data sources, such as molecular phylogenies, new structural data, and cell biological data, can aid in clarifying homologies.** The advent of molecular phylogenetic data has permitted testing homologies based on comparative methods and has been especially useful in reevaluating conclusions drawn from comparative methods. For example, the determination, using molecular methods, that smut fungi consisted of two distantly related groups prompted a reevaluation of SPB characters that revealed subtle structural differences between SPBs of smuts in two subphyla of Basidiomycota (Fig. 9.2) (McLaughlin et al. 1995b). Although structural characters provide additional strong support for phylogenies, a stable phylogeny based on DNA sequences is required to determine relationships among taxa and to polarize the structural and biochemical characters.

A variety of structures are associated with the septal pore, including variations in pore plugs and several types of vesicles. These characters have presented problems in determining homologies (Celio et al. 2006). Pore plugs may vary with the developmental stage of the cell and with functional changes in vegetative and reproductive hyphae. The characters associated with pore plugs usually have homologies limited to specific taxonomic groups. When they were applied across phyla, as in the type of pore plug observed in some Pezizomycotina and Basidiomycota, new structural data revealed differences between the pore plugs that permitted a redefinition of the character state (Fig. 9.4) (Kumar et al. 2012). Similarly, it was unclear whether Woronin bodies (Ascomycota) occur within Pucciniomycotina where microbodies are associated with the septal pore; however, the determination that the Woronin-body-specific protein HEX-1 is restricted to Pezizomycotina clarified the character state definitions (Dhavale and Jedd 2007). The isolation of specific proteins that localize at septal pore caps and pore plugs (van Driel et al. 2008; van Peer et al. 2010) may provide a more precise understanding of the septal pore apparatus and character evolution.

Character homology requires continued reassessment as additional data become available to accurately trace character evolution within and among fungal clades and unravel the structural changes that accompany functional evolution. Cell biological approaches, involving protein isolation and localization within cells, hold promise

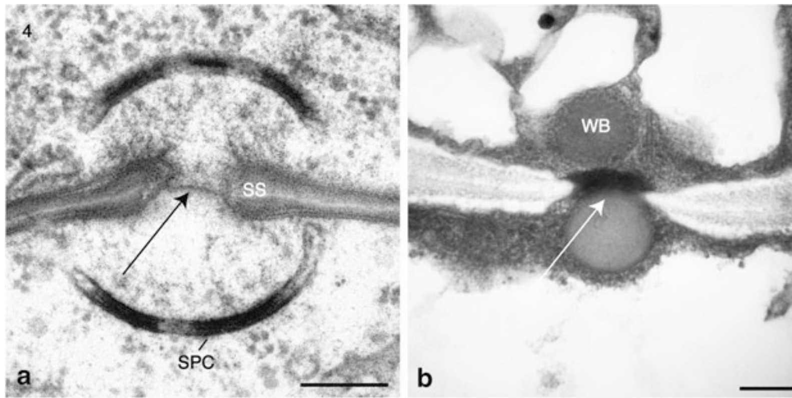


Fig. 9.4 Comparison of septal pore plugs in (**a**) hypha of *Exidia crenata* (G. Thorn, pers. comm.) (Agaricomycotina) and (**b**) excipulum of *Orbilia* sp. (Pezizomycotina) reveals a thin plate in the former and a thickened plate in the latter (*arrows*). These pore plug differences are consistent and clarify the structural differences between these subphyla. *SPC* septal pore cap, *SS* septal pore swelling, *WB* Woronin body. Scale bar = 0.2 μm. (**a**) from Lü and McLaughlin (1991) published as *Auricularia auricula-judae*; (**b**) from Kumar et al. (2012). Figures used with permission of Mycological Society of America

in gaining an understanding of character states and their relationships.

V. Structural and Biochemical Database

The SBD, developed and maintained by the Assembling the Fungal Tree of Life (AFTOL) consortium, is a searchable Internet resource publicly available at https://aftol.umn.edu. The **SBD compiles new and published subcellular and biochemical data on Fungi**, supplemented by images and literature links, **to serve as a phyloinformatics tool** (Kumar et al. 2013).

The development of a comprehensive repository that provides subcellular and biochemical data on Fungi was initiated during the first phase of the AFTOL project (AFTOL1), and additions and improvements to the database are being made during AFTOL2. The main goals were to bring together data widely scattered in the literature, thereby **enhancing information retrieval, provide standardized data interpretation and uniformity in terminology for detailed comparison, evaluate image quality, identify phylogenetically informative characters and assign character state coding, and provide illustrated character states** to guide users unfamiliar with subcellular data interpretation. **The** production of **data matrices in NEXUS format** is a key feature for analyses, including **tree reconstructions and ancestral state reconstructions**, thereby enabling independent morphological or combined morphological and molecular phylogenetic analyses.

The SBD consists of a number of data tables for characters and their states; ancillary information including bibliographic information, fixation methods, and data quality; and voucher information. The focus of the database is the table linking species, cell type, and character state. See Celio et al. (2006) for a diagram of the database design.

During database development, the following data mining and entry steps were followed: subcellular and biochemical data were collected from published studies and new research and their quality and phylogenetic informativeness were assessed and recorded in Microsoft Excel files (Microsoft Corp., Redmond, WA, USA) prior to entry in the database. Simultaneously, characters and character states were defined, modified as data were accumulated, and recorded in accompanying Microsoft Word files. Quality published/unpublished images (with copyright permissions) supporting the character states were scanned at 1,200 dpi, saved in

JPEG/TIFF formats, and uploaded to the database. Characters and character states illustrated with photographs and interpretive line drawings for nuclear division and SPB, septum/pore cap, motile cells, and sterols were assembled, coded, and compiled as lists in downloadable PDF format. The line drawings are either from published literature or generated from hand drawings using Adobe Photoshop CS3 and Adobe Illustrator CS2 programs (Adobe Systems, San Jose, CA). The nonproprietary Ruby on Rails Web framework and the Ruby programming language were used to rebuild the database application and Web site in AFTOL2.

The data in the SBD are continuously checked and modified as required. New data submissions pass through a review process and are checked and approved by the database administrator before they appear online. Images deposited in the database eventually may be linked to and stored in a central repository, such as Morphbank, from which they can be retrieved. Acquiring copyright permission for reuse of images from journals with predominantly commercial interests, however, remains a major impediment to providing access to images for the scientific community. This brings up an important issue that authors of scientific works need to consider carefully. We appeal to authors who wish their data to be made available to the scientific community to choose journals with appropriate copyright policies on reuse of images or to retain copyright to their images.

VI. Structural and Biochemical Database in Phylogenetic Analysis

The SBD provides sets of character states in NEXUS files, which can be analyzed in various ways to study character evolution or to perform phylogenetic analyses. Structural characters, for example, spindle pole organization during nuclear division, presumably represent the expression of multiple genes under evolutionary constraint and thus may suggest alternate phylogenies to those derived from phylogenies based on a limited gene data set (Celio et al. 2007). Many studies of character evolution have employed **PAUP*** (Swofford 2002), **MacClade** (Maddison and Maddison 2000), and, more recently, **Mesquite** (Maddison and Maddison 2007) and either a summary phylogenetic tree, combining the results of several analyses (Celio et al. 2006), or a single tree, for example, Kumar et al. (2012). The phylogenetic programs **aid in determining phylogenies or ancestral or derived character states**, but the **limited structural and biochemical data sets available for Fungi** can present difficulties in drawing conclusions (Celio et al. 2006; Kumar et al. 2012).

The phylogenetic analysis programs have different uses and limitations. PAUP* constructs phylogenetic trees using structural or biochemical characters alone or in combination with molecular characters. It has a full range of parsimony options, such as irreversible or Dollo characters, that can be used to code characters for analyses. MacClade 4 and Mesquite 2 are not designed to infer phylogenetic trees; instead, they use an imported tree or trees in studies of character evolution. MacClade is the easier of these programs to use, and it provides some parsimony options not available in Mesquite. Mesquite is being developed as a platform to allow many different types of analysis, including experimental ones. It is more complex to use but includes likelihood reconstructions not available in MacClade.

Incorporation of structural characters into molecular phylogenetic studies is little used because it is not clear how to weight the structural characters for a combined analysis. If structural characters represent multiple genes under evolutionary constraint, they may provide a strong phylogenetic signal, which can be swamped out when treated as the equivalent of a single character in a molecular data set. Some structural characters provide a very strong phylogenetic signal, for example, some meiosporangium types (ascus, basidium), or the septal pore apparatus, which act as stable markers at phylum or lower taxonomic ranks. For character evolution studies the best approach at present appears to be to use well supported molecular phylogenies to trace structural and biochemical evolution while the database of these characters is gradually assembled, and to

be alert to clues provided by new data that may challenge current phylogenetic interpretations.

VII. Development of the Fungal Subcellular Ontology

The reconstructed SBD aims to provide a major resource that manages morphological and biochemical information on Fungi and serves as a phyloinformatics tool. However, the extent to which data deposited in the SBD are utilized by other biological and genetic databases is currently limited because of the lack of defined logical relationships between the characters and terms and because of terminology problems that are associated with many fungal subcellular terms (Kumar et al. 2011). Uniformity in the use of terms, along with defining them in ways that are meaningful and represented by class/subclass relations, thereby revealing the hierarchical connections between terms, is now increasingly used to help software programmers and researchers in other specialties establish links between diverse databases. Such a "controlled vocabulary that describes objects and the relations between them in a formal way" (Hughes et al. 2008) is termed an ontology and is now being used by biologists who wish to make their data accessible to researchers in other related disciplines.

The Fungal Subcellular Ontology (FSO) provides a controlled vocabulary describing subcellular structures unique to Fungi. The FSO establishes a full complement of terms that provide an operating ontological framework for the SBD. **The FSO was developed using a phylogenetic perspective for character selection and definition and includes characters from across kingdom Fungi.** Characters included in the FSO have inherent phylogenetic signal and reveal the extensive character state diversity within the Fungi. In the existing, mostly gene-centric, public ontologies registered with Open Biological and Biomedical Ontology (OBO) Foundry, a consortium that provides coordination among the ontologies registered with it, the terms that describe the structural diversity found among Fungi have definitions limited to model organisms and do not reveal the full range of diversity among the Fungi, and many terms that are evolutionarily significant are absent. Hence, an ontology developed by fungal evolutionary biologists will be of use to fungal systematists and can be easily adapted by others, especially the genomics community. The FSO covers both model and nonmodel fungi and is freely downloadable in OBO-Edit format at http://aftol.umn.edu/ontology/fungal_subcellular.obo.

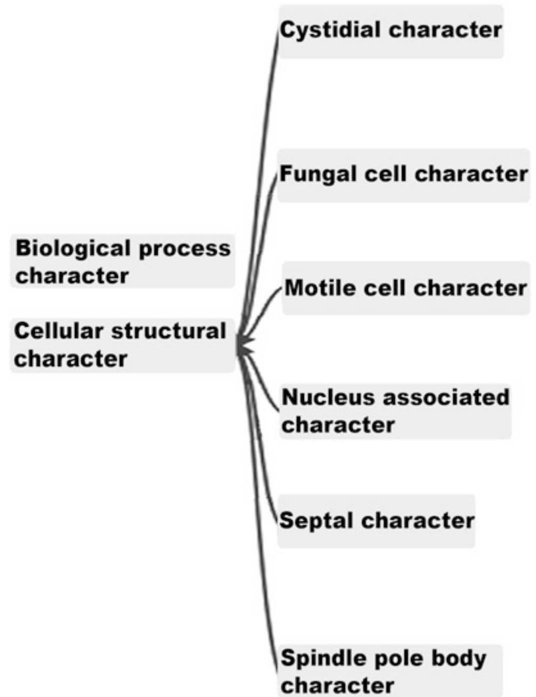


Fig. 9.5 Graphical representation of part of fungal subcellular ontology, displaying subclass relationships under higher-level class terms, biological process, and cellular structure character

Character data for (1) septum and septal pore organization, (2) nuclear division, (3) SPB form and development, and (4) the motile cell structure of Fungi have been included in the SBD and form the primary source of terms for the FSO. The ontology terms were categorized under two higher-level classes: biological process character and cellular structural character (Figs. 9.5 and 9.6). The FSO covers characters from across eight phyla (Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Entomophthoromycota, Glomeromycota, Monoblepharidiomycota, Neocallimastigo-

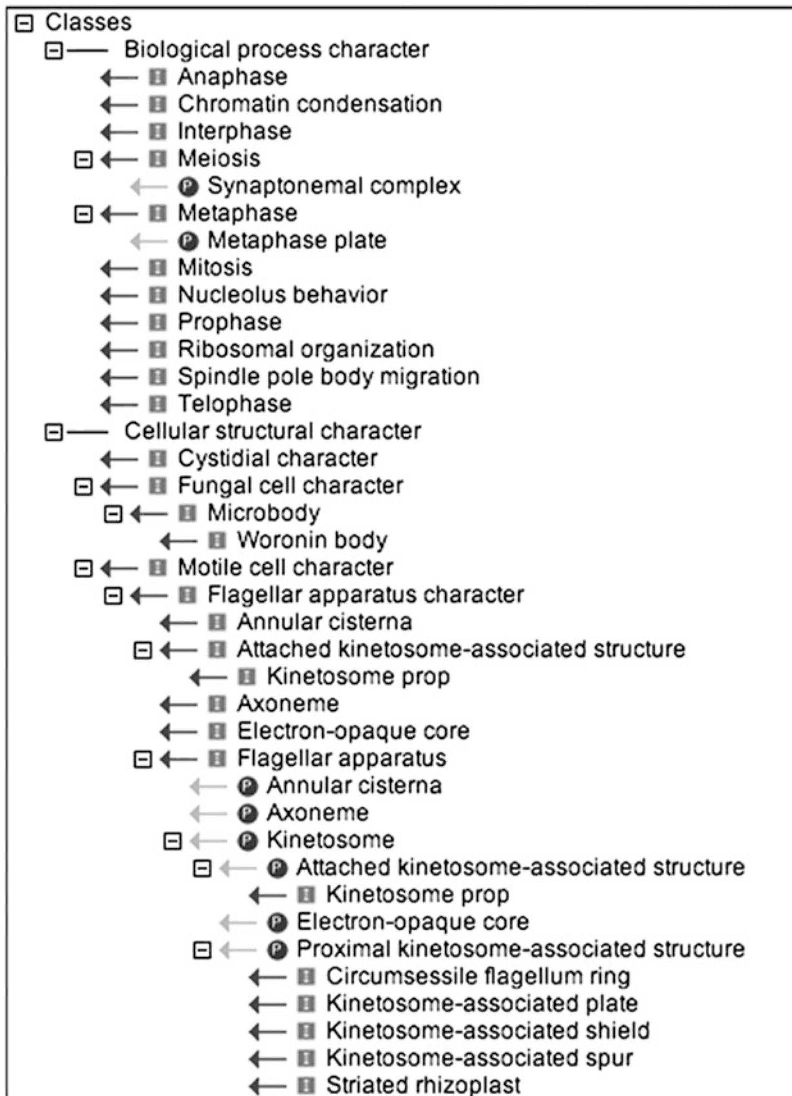


Fig. 9.6 Ontology tree in OBO-Edit format showing part of fungal subcellular ontology structure with expanded nodes showing is_a [**I**] and part_of (**P**) biological relationships

mycota) and four unplaced subphyla (Kickxellomycotina, Mortierellomycotina, Mucoromycotina, Zoopagomycotina). The highly derived Microsporidia have not been included. For details of the methodology of FSO development, see Kumar et al. (2011).

The FSO complements existing biological ontologies, such as the Gene Ontology (GO), which is the most popular of the biological ontologies. GO is an organism-independent ontology that was developed to describe the attributes of plant, animal, and microbial genes and gene products (Barrell et al. 2009; Camon et al. 2004; Gene Ontology Consortium 2000, 2004, 2006, 2008). The GO provides a comprehensive platform for cellular, molecular, and biological process-related terms for model organisms across the living world. The Fungal Anatomy Ontology (FAO; http://yeastgenome.org/fungi/fungal_anatomy_ontology/) and Ascomycete Phenotype Ontology (APO) are other publicly available fungal-oriented ontolo-

gies. The FAO describes the gross structural anatomy of model fungal species. The APO is a specialized ontology for the phenotypes of ascomycetes.

VIII. Specimen Preparation and Evaluation for Subcellular Structure

Knowledge of fungal cell structure and how to interpret it has declined with the emphasis on DNA analyses and the improvements in molecular methodologies that have permitted the rapid expansion of sequence data. Therefore, **to provide guidance on fungal cell studies**, we provide an overview of the **methods and specialized techniques used to obtain ultrastructural data for selected cell features** or traits.

A. Specimen Preparation and Evaluation

1. Fixation

To examine subcellular structures using transmission electron microscopy (TEM), regardless of what organism is being studied, samples must be fixed (to kill rapidly and preserve), embedded (to stabilize and support), thin-sectioned (to provide a thin slice of specimen allowing electrons to pass), and stained (to enhance cellular contrast). **Two fixation approaches** commonly used in the preparation of cells are the **chemical and cryogenic (i.e., low temperature) preparations**. Both protocols use multiple steps and chemicals that together work to achieve the common goal of microscopists for sample preparation: the maintenance of cellular structure in as close to the living state as possible, i.e., normal in structure and free from artifacts of preparation (see below). This goal is, strictly speaking, unattainable (Bracker 1967). Thus, it is the responsibility of microscopists to recognize preparation artifacts and to minimize or eliminate them when present by protocol modification or, if unavoidable, to interpret the data in light of them. It should be noted that there are many approaches to preparing samples for electron microscopic investigation that differ depending on the goals of the study (e.g., structural or cytochemical) and type of sample one is studying. It should also be noted that using both chemical and cryogenic procedures in a study may be beneficial because some components of the cell may be better preserved with one procedure than the other (Heath and Rethoret 1982; Mims et al. 1988). Here we limit our discussion to those protocols that are designed to maximize the structural integrity and resolution of fungal cells and tissues.

A critical step that is often overlooked is to maintain a specimen under optimal conditions up to the point of fixation. If the tissue is harvested directly from the environment, it must be held under appropriate physiological and environmental conditions during transportation back to the laboratory for fixation or, in some situations, specimens may be fixed on site if chemical fixation protocols are used. If cells or tissues are maintained in the laboratory, they must be grown on an appropriate medium under correct environmental conditions.

As a rule, before starting a new study one should consult references on related fungi to find fixation protocols and procedural hints that may work for a species or cell type to be studied. For example, if one is interested in the study of hyphal growth, fusion events, or mycoparasitism, and chemical fixation protocols are appropriate, cultures growing on agar can be quickly and easily fixed with little or no interruption events prior to fixation by simply pouring the fixative directly onto cultures in Petri dishes (Bracker and Grove 1971; Bracker et al. 1996; Grove and Bracker 1970; Hoch and Fuller 1977).

a) Chemical Preparation

Processing fungal tissues for ultrastructural studies using chemical fixation protocols has followed similar procedural milestones, as have plant and animal studies. Seminal articles that represent this development are Hawker (1965), Bracker (1967), Girbardt (1969), and Grove and Bracker (1970). Here we describe briefly the typical stages in the process of chemical fixation.

Primary fixation: The standard procedures for chemical fixation start with an initial (pri-

mary) fixation in glutaraldehyde that is buffered appropriately in either phosphate or cacodylate buffers. Glutaraldehyde is an excellent compound for primary fixation of cells and tissues because of its ability to cross link proteins, forming a stable polymeric complex. Penetration rates of the fixative, however, can be rather slow depending on the cell/tissue type, resulting in moderate to severe artifacts in some cases. Thus, to optimize penetration rates (faster penetration results in better representation of healthy living samples), tissues must be cut into small pieces. Additionally, a mixture of aldehydes can be used such as a combination of glutaraldehyde and formaldehyde that can provide excellent results for dense, hard-to-penetrate samples. Fixation is often carried out for 1–6 h at room temperature or, depending on the sample, at 4 °C. For some difficult-to-fix fungal material such as thick-walled teliospores and haustoria in mature leaf tissue, fixation times of 1–6 days have been used with good results (e.g., Roberson and Luttrell 1987; Roberson et al. 1990; Taylor and Mims 1990). Furthermore, it is at times advisable to add a small amount of detergent (0.1 % Triton X-100) to the primary fixative when working with samples that have a hydrophobic exterior. This helps such tissues to sink beneath the surface of the fixative and facilitates good fixation. After the primary fixation, the tissue is then washed thoroughly in buffer without fixative before proceeding. Aldehydes do not contain a heavy metal and, therefore, do not impart amplitude contrast, or so-called electron density, to the sample.

Secondary fixation: Commonly, after the primary fixation and washing, cells are treated with osmium tetroxide (OsO_4) as a secondary fixative. Osmium tetroxide is a strong oxidizing agent that reacts with and cross links proteins and lipids, especially targeting double bonds. This characteristic makes the fixative important for stabilizing unsaturated fatty acid tails of membranes. Not only does the fixative stabilize the tissue structure, but the reduced osmium resulting from fixation acts as a heavy metal stain to provide electron density. Secondary fixation is commonly carried out with 1 % OsO_4 in either buffer (typically) or distilled water for 2 h at room temperature or 4 °C. The secondary fixative is then washed thoroughly with distilled water, and the sample is processed further.

En bloc staining: The next step, en bloc staining, is often necessary to enhance general cytoplasmic staining (Bracker 1967). Here, the tissue is exposed to 0.5–1 % uranyl acetate in water for 1 or 2 h in the dark at room temperature or overnight at 4 °C. Because of the positive charge of the uranyl ions, they bind tightly to negatively charged elements of membranes, nucleic acids, and proteins to provide enhanced contrast. Uranyl acetate is also thought to stabilize nucleic acids and phospholipids and is considered a specialized fixative for these structures.

Dehydration: After the specimen has been chemically fixed and thoroughly washed, it must be dehydrated using ethanol or acetone. Dehydration is required because the specimens must ultimately be embedded in a solid matrix so that they can be sectioned. The embedding components used for structural studies are often epoxy-based resins that are not soluble in water and will not polymerize, i.e., harden, properly in the presence of any water.

Dehydration of specimens is usually carried out slowly in graded stages to prevent the collapse or extraction of cell components. Even though the tissues are well fixed at this point, they are still susceptible to these artifacts during dehydration. Ethanol is the dehydration agent typically used. To allow a gentle removal of water, the specimen is moved through a graded series of aqueous-based ethanol solutions such as 10, 30, 50, 70, and 90 %, and finally through two changes of 100 % ethanol. The tissues should remain in each solution for 10–15 min. It should be noted that flexibility can be tolerated; for example, some workers will hold tissues at 50 % ethanol overnight at 4 °C or longer with few noticeable effects on the structure. Dehydration is typically performed at room temperature, though dehydrations at cooler temperatures, such as 4 °C, are thought to reduce artifacts. To facilitate the dehydration process, the samples can be placed on a rotating rack that continually mixes the solutions.

At the end of the dehydration steps, the sample is ready to be transitioned into the stages for resin infiltration and embedment (see below).

b) Cryogenic Preparation

Cryogenic methods for preparing tissues for electron microscopy are a major alternative to

chemical preparation protocols. These methods can be more costly, result in lower throughput than chemical preparation, i.e., there can be significant loss of ultrastructural information due to freeze damage, and are not amenable to field-collected samples that cannot be transported back to the lab. However, the benefits can include much improved preservation and the elimination of artifacts associated with chemical fixation (Gilkey and Staehelin 1986; Hoch 1986; Howard and O'Donnell 1987; Moor 1987). Cryogenic methods can be divided into two parts: cryofixation and freeze substitution.

Cryofixation: The initial step of freezing cells and tissues is analogous to the primary fixation step described earlier with respect to chemical preparation methods. A significant obstacle in cryofixation is avoiding the formation of ice crystals during the freezing process. When freezing rates are too slow, ice crystals can grow to sizes large enough to disrupt tissue structure at the resolutions used for electron microscopy. Three common methods to avoid ice crystal growth are to use ultrarapid freezing methods that are so fast that ice crystals do not have time to grow, use high pressure to slow the rate of ice crystal growth, and use cryoprotectants that nucleate small crystals and slow their growth. The first two methods are most desirable and will be discussed in what follows.

There are three ultrarapid freezing methods: immersion (plunge and spray), propane jet, and metal mirror freezing (Chandler and Roberson 2009). For the Fungi, plunge and, to a lesser degree, spray immersion freezing have had well-documented success (Hoch and Howard 1980; Hoch and Staples 1983; Howard 1981; Howard and Aist 1979; Mims et al. 1988; Roberson and Fuller 1988; Roberson et al. 2011; Shields and Fuller 1996; Vargas et al. 1993). Plunge freezing will be discussed here. Ultrarapid freezing rates are achieved when heat is withdrawn from cells at rates of 5,000–10,000 °C/s. At these speeds, cellular motion is stopped within milliseconds, and little or no damaging ice crystals form. However, such rates of heat loss may be obtained only when certain conditions are met. First, samples must be 10–12 μm thick or less, meaning a single cell layer or smaller. Exceptions to this size requirement are rare, but they do occur. For example, in *Auriscalpium vulgare* (Celio et al. 2007) and *Ascodesmis nigricans* (Mims et al. 1990) workers were able to plunge freeze complex tissues with minimal to no detectible ice damage. Second, samples must be rapidly plunged into a cold liquid (i.e., cryogen) that has the characteristics of remaining liquid at very low temperatures (−185 to −190 °C) and absorbing heat rapidly without boiling. Hydrocarbons meet these requirements, and liquid propane is the most commonly used cryogen because it is widely available and inexpensive. Liquid propane or a combination of liquid propane and ethane are also popular alternatives; however, hydrocarbons are explosive.

As mentioned earlier, for plunge freezing the sample must be thin. Hyphae can often be grown over thin, water-permeable membrane supports (e.g., dialysis membrane, cellophane) covering the surface of a semi-solid growth medium. Once growth is established, a selected region of cells and supporting membrane are cut to approximately 4 × 7 mm; cells are then allowed time to recover (typically 30–60 min) from the wounding before freezing. For more details see Hoch (1986), Howard and O'Donnell (1987), and Chandler and Roberson (2009). An air-driven stirrer is positioned in the condensed propane in order to prevent temperature gradients from forming and to aid in heat transfer. It is also advisable that a gentle stream of nitrogen gas be directed across the propane surface to provide an insulating barrier to oxygen. As with chemical preparation, the specimen to be frozen is kept in appropriate growth conditions at all times just prior to the instant of freezing to insure that the cells are as normal in function and structure as possible. Just prior to freezing, the specimen is grasped with forceps and, with a smooth quick motion, plunged into the liquid propane. The frozen specimen is then transferred to a temporary holding basket, kept in the propane bath. Once 15–20 samples have been frozen, the basket is quickly transferred to the freeze-substitution solution.

Freezing samples at high pressure is useful because ice crystal growth is significantly reduced, which permits large tissues and other difficult to freeze cells, for example, spores, to be frozen successfully. The pressures required for this to occur are around 2,100 atm, which represents a technical challenge to achieve and requires the use of a hydraulic piston and sophisticated delivery mechanism that function

within fractions of a second. Equipment such as the Baltec HPM 010 High-Pressure Freezer (Baltec Products, Middlebury, CT) or Leica EM HPM High-Pressure Freezer (LEICA Microsystems Inc., Buffalo Grove, IL) is required for this mode of freezing. For this procedure, the specimen is typically positioned in a double planchet or specimen platform that is 3 mm in diameter. The planchet and specimen are inserted and locked into the ballistic chamber, which is rapidly pressurized, followed milliseconds later by a burst of liquid nitrogen. At these pressures, liquid nitrogen is a good cryogen (unlike at atmospheric pressure), and freezing is accomplished within 100 ms. High-pressure freezing has been used successfully in freezing fungal spores (Bonfante et al. 1994; Mims and Richardson 2005; Roberson 1993), host–pathogen associations (Celio et al. 2000; Harding et al. 1999; Mendgen et al. 1991; Mims et al. 2002, 2003), septal pore complexes (van Driel et al. 2009), and zoosporangia (Fisher et al. 2000).

Freeze substitution: After cryofixation, the samples can be stored in liquid nitrogen or processed in one of a number of ways. Freeze substitution is the approach followed if the researcher wishes to examine the material in thin section using TEM. The objectives of freeze substitution are to gently dehydrate the sample while simultaneously exposing it to secondary chemical fixation and en bloc staining at low temperature prior to embedding in resin at room temperature. The advantages of this strategy are that many of the artifacts caused by chemical fixation and dehydration at room temperature, for example, shrinkage and membrane wrinkling, are eliminated. What follows is a brief description of a freeze-substitution protocol used for persevering fungal structure.

The frozen specimen is first placed in the cold (−85 to −90 °C) substitution fluid, which is typically composed of acetone, 1 % osmium tetroxide, and 0.05 % uranyl acetate. The specimen is held at these low temperatures for 24–48 h while the ice in the specimen is slowly and gently replaced by substitution fluid and dehydration is complete. The specimen is then warmed to −20 °C for 2 h, during which time the osmium tetroxide becomes increasingly more chemically active. The specimen is then warmed to 4 °C for 2 h, and then to room temperature for 1 h, after which it is washed in 100 % acetone.

These freeze-substitution protocols can be carried out automatically by dedicated freeze-substitution units or by hand moving the sample from one cold chamber to the next. The washed specimen is then infiltrated and embedded in epoxy resin, sectioned, stained, and viewed.

There are numerous ways in which the freeze-substitution process can be modified to meet the needs of a study. The more common modifications center on changes in the chemistry of the freeze-substitution solutions. For structural studies, for example, the use of tannic acid, glutaraldehyde, or potassium permanganate have been reported to provide good results (Giddings 2003; Howard and Aist 1979; McDaniel and Roberson 2000; Winey et al. 1995). When studies call for the localization of proteins or carbohydrates using antibodies, lectins, or other cytochemical approaches, additional changes in protocol are typically required, both in the freeze-substitution makeup and type of embedding resin used (e.g., use of acrylic-based instead of epoxy-based resin) in order to preserve the chemical nature of the cell (Bourett and Howard 1991, 1994; Bourett et al. 1993; Buser and McDonald 2010; Roberson and Vargas 1994).

2. Standard Infiltration and Embedding

Prior to obtaining thin sections for TEM analysis, the sample must first be thoroughly infiltrated with liquid components of an embedding resin followed by the polymerization (hardening) of the resin (typically by heat), creating a highly cross-linked matrix of which the sample is a part. If these procedures are not performed adequately, the sample may suffer from severe distortion artifacts during sectioning or may be unsectionable.

Resins that are used most commonly for structural studies in the Fungi are low-viscosity epoxy-based, for example Spurr's, or epoxy/ethylene glycol diglycidyl ether-based, for example Quetol 651, resins. These low-viscosity resins polymerize into a hard matrix that is very well suited for infiltrating and sectioning cells bound by walls. Nonetheless, extended

resin infiltration schedules are recommended to ensure complete resin penetration into most fungal cells and tissues (see below). Typically, four liquid components are mixed together in certain ratios to achieve the desired hardness. For most fungal samples hard resins are favored because they provide the best characteristics for sectioning through cell walls.

Resin infiltration begins immediately after dehydration when the samples are transferred from absolute ethanol into 100 % acetone. Samples are exchanged two to three times and held in each change for no longer than 15 min at room temperature. For infiltration of resin, the sample is incubated in resin:acetone mixtures at ratios of 1:3, 1:1, 3:1, and then 100 % resin. Incubation times are typically 8–12 h for each step. It is advisable to place the specimen vials on a rotating rack for slow continuous mixing throughout the infiltration steps. Several changes of 100 % resin are desirable to ensure that no traces of acetone remain in the solution, which could prevent complete polymerization and make sectioning difficult or impossible.

Embedment is the next step and involves placing the specimens in molds that will determine the shape and size of the final resin block. The final selection of the type of mold (usually flat sided or cylindrical) is dependent on the specimen and its desired specimen orientation during sectioning. The molds are placed into an oven at the temperature and time specified for the resin.

3. Microwave Fixation and Embedding

Microwave-assisted processing of biological specimens for electron microscopy has recently attracted attention as a tool to achieve rapid specimen fixation and embedding. Microwave-processing protocols have also been used for specimen staining for electron microscopy, immunolabeling, antigen retrieval, and in situ hybridization. Reduced processing time, improved preservation of morphology, and the antigenicity of specimens subjected to microwave processing have been highlighted as significant benefits over the conventional specimen-preparation methods (Webster 2007). Specially designed laboratory microwave processors for handling hazardous materials are used to carry out the protocols. Better knowledge of the variables involved in the process that give reproducible results and modified protocols, like vacuum microwave processing (Giberson et al. 1997), have been reported to save more time and improve overall quality (Giberson and Demaree 1995; Giberson et al. 1997).

Microwave fixation and embedding for fungi is relatively new. The Vacuum Microwave Processing Protocol (Ted Pella, Inc., Redding, CA, USA) has been used to process samples in a specially designed laboratory microwave with a vacuum chamber and a temperature probe to monitor and control heat inside the embedding solution. Infiltration of samples (e.g., thin fruit body pieces, mycelium, germinating spores on agar) is quick and requires relatively small amounts of chemicals. It works fairly well with fungal samples; however, reproducibility of results is a concern with thick tissue samples and cells with very thick walls.

4. Section Staining and Microscopy Methods

Because standard TEM requires sections ranging from 60 to 80 nm thick in order to allow electrons to pass, they are referred to as thin or ultrathin sections and are cut on an instrument called an ultramicrotome.

Epoxy embedded tissues (or blocks) are hard and must be cut using a glass or diamond knife. A diamond knife is preferred because of its constancy in cutting characteristics, ease of use, and high-quality cutting edge. Diamond knives are extremely hard and will remain sharp through thousands of cuttings if they receive proper care. These knives, however, are expensive, and once the edge is damaged, it must be professionally sharpened. After the block has been trimmed, it is mounted into an ultramicrotome and the knife is secured in place. Cutting thin sections is a time-consuming and tedious job, but given the proper training, patience, and good modern equipment, the process is relatively easy to carry out.

After thin sections are cut, they are collected on grids. Grids come in many patterns and materials, but all are 3 mm in diameter and stamped out of a thin metallic foil (copper, gold, nickel). The sections must now be stained (poststained) with heavy metals such as ura-

nium or lead. This is accomplished by floating the grid, section side down, on a drop of 2 % uranyl acetate (wt/vol) in water or ethanol (50 %) for approximately 10 min, followed by a thorough washing in water. This is followed by staining on a drop of aqueous lead citrate for 5 min and another washing. Depending on the resin and tissue, other poststaining schedules may be needed. Grids are then allowed to dry and are stored in a grid box until imaged with TEM.

B. Methods for Selected Cellular Features

1. Nuclear Division Studies

Analysis of nuclear division and SPB cycles requires capturing specific division stages to fully understand the changes that occur **during meiosis or mitosis**, but **finding dividing nuclei can be challenging. The number of nuclear division studies is limited**; the best-studied group is the Basidiomycota, with the Ascomycota seriously understudied for a phylum that constitutes more than half of the known Fungi. The **gaps in our knowledge make generalizations about character distribution and evolution speculative** (Fig. 9.1). **Some phyla/subphyla present great difficulties in handling cells for cytological study**, for example, Glomeromycota, or do not respond well to the usual methods for cell preparation and selection, for example, zygomycete fungi, and remain unstudied or little studied. **Meiotic studies have advantages over mitotic ones** in that the SPB may be larger and more differentiated and goes through a series of changes involving SPB fusion and separation that results in distinctive structural features of potential phylogenetic significance (McLaughlin et al. 1995b).

Choosing places in the life cycle where nuclear divisions are known to occur optimizes the study of nuclear division and SPB cycles. **Analysis** of fixed cells **with a DNA-specific fluorochrome can aid in finding sites of dividing nuclei and can be combined with cell selection,** either during the initial fixation process (Fig. 9.7) or after embedding, to select specific cells for serial sectioning, thereby maximizing data acquisition (Bourett and McLaughlin 1986; McLaughlin et al. 1996; Swann et al. 1999). Very **small nuclei and those in which chromatin condensation is not easily observed** present

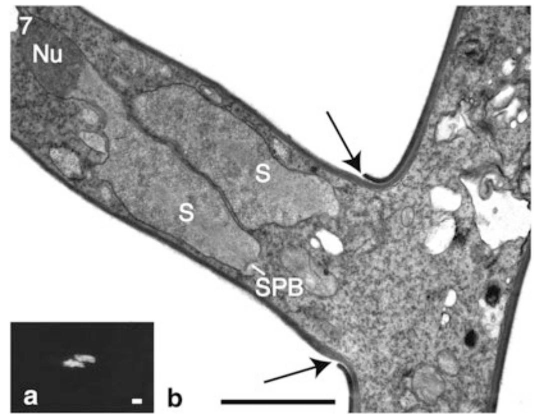


Fig. 9.7 (**a**) Light micrograph of dikaryotic nuclei at base of a branching hypha of *Helicobasidium mompa* (Basidiomycota) at mitotic metaphase–anaphase stained with a DNA-specific fluorochrome. (**b**) Transmission electron micrograph of same cell selected with fluorescent microscopy during primary fixation and serial sectioned after processing for TEM. Nuclei divide at the base of new branches. Note the wall break on branching (*short arrows*), a characteristic of some Pucciniomycotina clades. *Nu* nucleolus, *S* spindle, *SPB* spindle pole body. Scale bar = 2 μm. From Bourett and McLaughlin (1986), used with permission of Canadian Journal of Botany

challenges in nuclear division studies, although in yeasts and germinating spores, concentrating large numbers of cells for sectioning helps to overcome this impediment (e.g., Frieders and McLaughlin 1996; Heath and Rethoret 1982). The method developed by Taylor (1984) and modified by Bourett and McLaughlin (1986) is applicable to **cultured hyphae.** It involves growing mycelium on specially prepared cover slips in a thin layer of agar, carrying out an initial chemical fixation, staining with a DNA-specific fluorochrome, and photographing and selecting nuclei by scribing the cover slip with a diamond scribe. The cover slip is then processed to complete the postfixation and flat embedding between layers of Teflon-coated Mylar (Kleven and McLaughlin 1989). Selected cells are recovered by relocating the scribed cells and mounting them for sectioning. This method works well for Fungi that will grow within the agar layer and that form limited aerial hyphae.

Methods involving dispersal of **meiosporangia** such that individual cells can be selected

at different stages of meiosis can also be very effective, for example, germinating rust teliospores (O'Donnell and McLaughlin 1981), and these methods have been applied to asci. Alternatively, intact hymenia can be used by cutting thick sections and staining them with toluidine blue O to locate desired areas with the light microscope for thin sectioning. Finding dividing nuclei is especially aided by morphological markers, such as branching or budding, in cells under study that allow prediction of where to find dividing nuclei in fixed and flat-embedded cells (Fig. 9.7) (Bourett and McLaughlin 1986; Swann et al. 1999). No single procedure can be applied to all organisms. Each organism or group needs preliminary study to ascertain the best way to proceed.

2. Apical Organization Studies

It is desirable when possible to **utilize both light microscopy and TEM in studies of hyphal apical organization**. The major advantage of using light microscopy is that hyphae can be examined in their living state, and thus the dynamics of growth can be coupled with structure. However, this can also result in challenges for those fungi with hyphae that are susceptible to the stress of being grown and examined for light microscopy studies. Furthermore, the small diameter of some hyphae makes light microscopy examination less valuable. Girbardt (1957), Grove and Bracker (1970), and López-Franco and Bracker (1996) have demonstrated the utility of phase-contrast light microscopy in studies of the hyphal apex. Studies using the membrane-selective fluorescent vital dye FM4-64 have also been shown to be useful in studies of hyphal apices (Fischer-Parton et al. 2000; Roberson et al. 2011).

Many components of the apical cytoplasm in actively growing hyphae (vesicle and cytoskeletal organization and appearance) are quite delicate and **susceptible to disruption from prefixation handling and fixation artifacts when chemical fixation protocols are used** to prepare samples for TEM. Fortunately, most fungal hyphae are well suited for **cryofixation** by plunging because they can be grown easily as monolayers over cellulose membranes on the surface of nutrient agar medium and are often thin enough (<10–12 μm) to allow the rapid freezing rates required to achieve high-quality ultrastructural preservation (Hoch 1986; Howard and O'Donnell 1987). Other freezing methods are less suitable primarily because of the prefixation handling of the sample that is required. Over the past several decades, the use of cryofixation protocols has become the method of choice for preserving the ultrastructure of the hyphal tip, as well as other structures when applicable. The reasons for this are clear when the results obtained with cryofixation/freeze substitution are compared with those obtained with chemical fixation procedures (Hoch and Howard 1980, 1981; Howard and Aist 1979; Mims et al. 1988). When using cryopreparation methods the cytoplasm is preserved with increased clarity, cytoskeletal elements are well maintained, and membrane-associated structures (e.g., vesicles, vacuoles, mitochondria, Golgi apparatus, endoplasmic reticulum) appear rounded or with smooth profiles. In contrast, similar structures prepared using chemical fixations may appear disorganized and distorted as a result of the effects of postfixation rearrangements, osmotic incompatibilities, or harsh dehydration. Because well-frozen cells are immobilized in milliseconds and dehydration occurs slowly at low temperatures, the cytoplasmic structure is preserved at a high level of fidelity.

Although there are significant advantages to using cryomethods for studies of hyphal apices, the issue of inconsistent freezing rates resulting in artifacts, such as minor to severe ice-crystal damage and fracturing of cells, is common to all methods of freezing. The best way **to avoid sectioning poorly frozen hyphal-tip cells** is to **carefully screen and select cells before sectioning.** For this, the hyphae must be flat embedded between a Teflon-coated microscope slide and Mylar strip (Kleven and McLaughlin 1989). After polymerization of the resin, the Mylar strip is removed and the cells now embedded in a thin layer of resin are imaged with phase contrast light microscopy using an oil-immersion 100× objective. Severely damaged cells are quite easy to detect

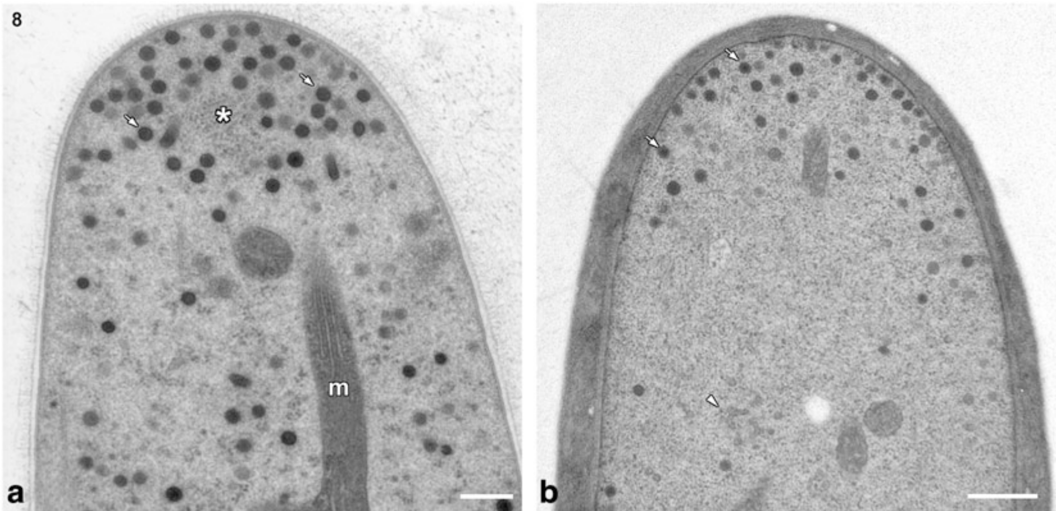


Fig. 9.8 Transmission electron micrographs of near median sections through hyphae illustrating apical organization. Hyphae fixed using plunge-freezing and freeze-substitution methods. (**a**) *Fusarium solani* (Ascomycota) with organized Spitzenkörper composed, in part, of spherically arranged apical vesicles (*arrows*) and cluster of ribosomes (*asterisks*). Central core region, typical of the ascomycete Spitzenkörper, is not present in this section. Tangential section through mitochondrion (m). Scale bar = 0.3 µm; micrograph by Matt Garret. (**b**) *Coemansia reversa* (Kickxellomycotina) illustrating apical vesicles (*arrows*) arranged in crescent pattern beneath apical plasma membrane. Such a vesicular arrangement at the hyphal apex represents a significant difference from that observed in ascomycete and basidiomycete hyphae. Membrane network of Golgi equivalent at *arrowhead*. Scale bar = 0.35 µm

and avoid, while mild freeze damage is more difficult to distinguish at the light microscope level (Hoch 1986). This screening method is critical not only for avoiding cells that contain obvious freeze damage but also for selecting cells that show no evidence of prefixation growth stress due to handling of the samples just prior to cryofixation. Stress is most easily identified as a swelling of the apical region or lack of a well-organized vesicle cluster, i.e., Spitzenkörper, in basidiomycetes and ascomycetes. Well-frozen cells appear as they would if imaged in their living state, for example, well-defined organelles in a clear cytosolic matrix. After hyphae are selected for sectioning, the small region containing the cells and surrounding resin is removed using a sharp razor blade and glued to the top of a blank resin stub using superglue (Howard and O'Donnell 1987). After the block is trimmed, cells are ready for sectioning.

The methods of cryofixation and freeze substitution have been used successfully in describing the fundamental organization of the hyphal apex in septate and nonseptate fungi (Fig. 9.8) (Amicucci et al. 2010; Harris et al. 2004; Hoch and Howard 1980; Hoch and Staples 1983; Hohmann-Marriott et al. 2006; Howard 1981; Howard and Aist 1979; Köhli et al. 2008; Newhouse et al. 1983; Roberson and Fuller 1988; Roberson et al. 2011; Srijayanthi et al. 1994; Vargas et al. 1993) and the effects of growth inhibitors and mutations on the hyphal apex organization (Howard and Aist 1980; McDaniel and Roberson 2000; Riquelme et al. 2002; Roberson and Fuller 1990).

3. Flagellated Fungi Studies

The vast majority of taxa in Chytridiomycota are microscopic with relatively simple thallus morphology. **Subcellular characters associated with posteriorly uniflagellate zoospores are informative at higher taxonomic ranks.** Chytridiomycota phylogeny is currently based

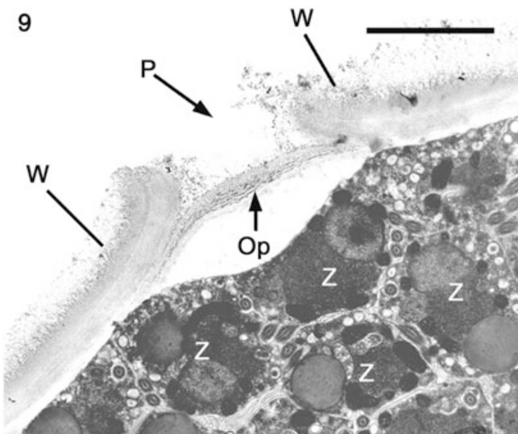


Fig. 9.9 Longitudinal section through mature sporangium of an unidentified member of Rhizophydiales (Chytridiomycota), illustrating cleaved zoospores (Z), sporangial wall (W), discharge pore (P), and endo-operculum (Op). Scale bar = 5 μm

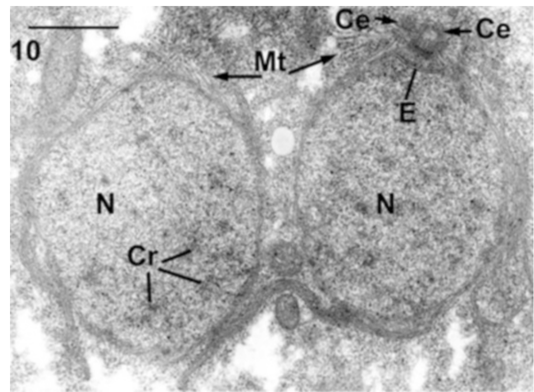


Fig. 9.10 Daughter nuclei (N) following a mitotic division in a sporangium of *Angulomyces argentinensis* (Chytridiomycota). Nuclei contain dispersed chromatin (Cr); nucleus on right illustrates invagination of nuclear envelope (E) with adjacent paired centrioles (Ce) and cytoplasmic microtubules (Mt). Scale bar = 0.5 μm

on molecular sequences, while support from common features of higher-rank taxonomy utilizes zoospore ultrastructure. For example, until recently the order Chytridiales was the largest order in Chytridiomycetes, but it was considered to be polyphyletic. Combined molecular and ultrastructural studies have resulted in establishing the limits of a monophyletic Chytridiales (Vélez et al. 2011) and in the circumscription of the orders Rhizophydiales (Letcher et al. 2006), Cladochytriales (Mozley-Standridge et al. 2009), and Lobulomycetales (Longcore and Simmons 2012; Simmons et al. 2009). Caution is recommended, however, because relatively few species within each higher taxon have been examined. Additional taxa at the generic and species levels have also been delineated on the basis of combined molecular and ultrastructural analyses (Letcher et al. 2008; Picard et al. 2009; Powell et al. 2011). Thus, one aspect of obtaining robust chytrid phylogenies is reliable preservation, fixation, and observation of biological specimens to complement molecular data.

Because of the microscopic nature and size limits of most chytrids, obtaining information regarding aspects of thallus morphology and zoospore ultrastructure is enhanced by electron microscopic analyses.

TEM provides thallus ultrastructure information related to wall construction and ornamentation, discharge apparatus (Fig. 9.9), septal characteristics, mitosis (Fig. 9.10), zoospore development, and rhizoid structure. TEM is also the primary tool for revealing zoospore ultrastructural characters and character states. An adequate quantity of thalli and zoospores must be obtained for subcellular studies. A number of Petri dishes with a suitable nutrient agar are inoculated with either chytrid thalli or a zoospore suspension obtained from pure culture and incubated at room temperature. Within 1–5 days, mature thalli that cover the dishes may be harvested, or the dishes may be flooded with distilled water or dilute salts (Fuller and Jaworski 1987) to obtain zoospore release. Thalli or zoospores are concentrated in an aqueous suspension and then preserved through chemical fixation.

Because the procedure is relatively straightforward and reliable, **chemical fixation** is the preferred method for specimen preparation for both chytrid thalli and zoospores. Vehicles for fixatives include phosphate, cacodylate, and collidine buffers (Glauert 1991). Phosphate buffers are nontoxic and most closely mimic extracellular fluids, although precipitates often occur during fixation and preservation of microtu-

bules may be less than optimal. Cacodylate buffers are easily prepared, although the buffers contain arsenic, which may act as a fixative. Sym-collidine buffers, although toxic, are easily prepared, stable at room temperature, and exist at pH 7.4 when neutralized with strong acid (Glauert 1991).

Glutaraldehyde is the primary fixative of choice, and specimen fixation and preparation for TEM analysis follow procedures described in Letcher and Powell (2005). Although ethanol and acetone are widely used for dehydration and embedding, for zoosporic fungi, acetone is preferred. Dehydration and specimen embedding follow procedures described in Letcher and Powell (2005). After thorough drying, grid-mounted sections are stained to obtain sufficient contrast (Lewis and Knight 1977).

If nickel rather than copper grids are used, sections are initially treated by being floated on a drop of 1 % periodic-acid solution for 5 min to demonstrate the presence of glycogen and then washed twice with filtered deionized (FDI) water. A periodic-acid stain cannot be used with copper grids because the acid will etch the copper. The periodic-acid oxidation is followed by immersion of sections in saturated uranyl acetate in 70 % ethanol for 8–10 min to enhance contrast (Silva et al. 1968), followed by a wash with 70 % ethanol to dissolve residual uranyl acetate crystals and a wash with FDI water. Sections are then stained for 4–6 min in lead citrate (Reynolds 1963) in the presence of sodium hydroxide pellets and then washed once with 0.1 M sodium hydroxide and once with FDI water.

When thoroughly dry, sections may be examined by TEM at 60–80 kV. Random sections on mesh grids are most commonly observed for obtaining images of randomly oriented features (Fig. 9.11). Serial sections mounted on slot grids are observed to obtain progressive imaging through selected structures such as nuclei undergoing mitosis or the flagellar apparatus (Fig. 9.12).

Culturing methods and zoospore fixation for electron microscopic analysis for Blastocladiomycota are similar to those for Chytridiomycota (e.g., Chong and Barr 1974; Dewel and Dewel 1990). Culturing methods for the anaerobic Neocallimastigomycota have been summarized by Rezaeian et al. (2004). Treatment of Neocallimastigomycota motile cells for electron microscopy is similar to that for other groups (e.g., Gold et al. 1988; Heath et al. 1983; Li and Heath 1991). Members of the genus *Olpidium* (*O. brassicae* and *O. bornovanus*), although now indicated to be affiliated with the nonmotile zygomycete fungi, have a motile spore, and treatment of those organisms for ultrastructural analysis is similar to treatment of other organisms with motile spores (e.g., Barr and Hartmann 1977; Lange and Olson 1978).

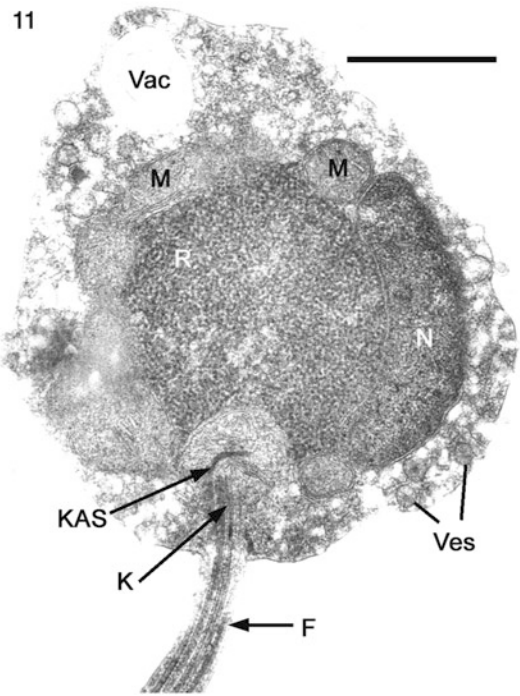


Fig. 9.11 Longitudinal section through zoospore of an unidentified member of Rhizophydiales (Chytridiomycota) illustrating organization of ultrastructural components. *F* flagellum, *K* kinetosome, *KAS* kinetosome-associated electron-dense structure, *M* mitochondrion, *N* nucleus, *R* aggregated ribosomes, *Vac* vacuole, *Ves* vesicles in peripheral cytoplasm. Scale bar = 1 µm

IX. Conclusions

Structural and biochemical characters have a great deal to tell us about the evolution and diversification of the Fungi. Our appreciation for how these characters have evolved is chang-

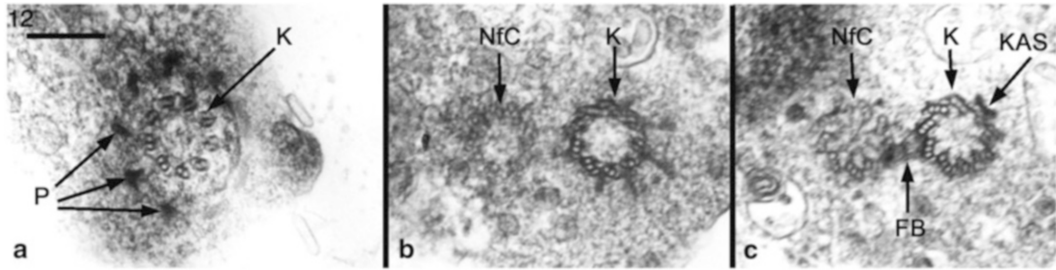


Fig. 9.12 Transverse serial sections through kinetosomal region of a zoospore of *Terramyces* sp. (Chytridiomycota). (**a**) Posterior end of kinetosome (K) with flagellar props (P). (**b**) Kinetosome and adjacent nonflagellated centriole (NfC). (**c**) Kinetosome and nonflagellated centriole connected by a fibrillar bridge (FB), and a kinetosome-associated electron-dense structure (KAS) adjacent to the kinetosome. Scale bar in (**a**) =0.25 μm

ing dramatically as molecular phylogenetic data provide a well-supported Fungal Tree of Life. We are entering a new era of scientific understanding with the advent of phylogenomic analyses based on whole genomes of a large number of Fungi. These genomic data will need to be linked to structural and biochemical information, much of which still needs to be obtained, to characterize genome function. The SBD and FSO can contribute to the orderly assembly of these data and to a better understanding of fungal cells biologically, genetically, and ecologically.

Acknowledgements This work was supported by the National Science Foundation Assembling the Fungal Tree of Life award (DEB-0732550) to D.J.M., (DEB-0732599) to P.M.L., (DEB-0732671) and the Boyd Professor Research Fund to M.B., (DEB-0732503) to R.W. R., College of Biological Sciences and Imaging Center, University of Minnesota, and the Minnesota Supercomputing Institute for support of the Structural and Biochemical Database development. The authors wish to thank Mahajabeen Padamsee and Maritza Abril for their contributions to the AFTOL2 project and Rosanne Healy for assistance with figures and helpful discussion. M.B. acknowledges a Radcliffe Institute Fellowship that provided the time and environment for writing.

References

Alexopoulos CJ (1952) Introductory mycology. Wiley, New York, NY

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory mycology. Wiley, New York, NY

Amicucci A, Balestrini R, Kohel A, Barbieri E, Saltarelli R, Faccio A, Roberson RW, Bonfante P, Stocchi V (2010) Hyphal and cytoskeleton polarization in *Tuber melanosporum*: a genomic and cellular analysis. Fungal Genet Biol 48:561–572

Ammirati JF, Traquair JA, Horgen PA (1985) Poisonous mushrooms of the northern United States and Canada. University of Minnesota Press, Minneapolis, MN

Baldauf SL, Battacharya D, Cockrill J, Hugenholtz P, Pawlowski J, Simpson AGB (2004) The tree of life: an overview. In: Cracraft J, Donoghue MJ (eds) Assembling the tree of life. Oxford University Press, Oxford, pp 43–75

Baral H-O (1987) Lugol's solution/IKI versus Melzer's reagent: hemiamyloidity, a universal feature of the ascus wall. Mycotaxon 29:399–450

Barr DJS (1981) The phylogenetic and taxonomic implications of flagellar rootlet morphology among zoosporic fungi. Biosystems 14:359–370

Barr DJS (1992) Evolution and kingdoms of organisms from the perspective of a mycologist. Mycologia 84:1–11

Barr DJS, Hartmann VE (1977) Zoospore ultrastructure of *Olpidium brassicae* and *Rhizophlyctis rosea*. Can J Bot 55:1221–1235

Barrell D, Dimmer E, Huntley RP, Binns D, O'Donovan C, Apweiler R (2009) The GOA database in 2009—an integrated Gene Ontology Annotation resource. Nucleic Acids Res 37:D396–D403

Bartnicki-Garcia S (1970) Cell wall composition and other biochemical markers in fungal phylogeny. In: Harborne JB (ed) Phytochemical phylogeny. Academic Press, London, pp 81–103

Bartnicki-Garcia S (1987) The cell wall: a crucial structure in fungal evolution. In: Rayner ADM, Brazier CM, Moore D (eds) Evolutionary biology of the fungi. Cambridge University Press, London, pp 389–403

Bartnicki-Garcia S (2006) Chitosomes: past, present and future. FEMS Yeast Res 6:957–965

Bauer R, Oberwinkler F (1991) The symplechosome: a unique cell organelle of some basidiomycetes. Bot Acta 104:93–97

Bauer R, Bergerow D, Oberwinkler F, Piepenbring M, Berbee ML (2001) Ustilaginomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7B, Systematics and evolution. Springer, Berlin, pp 57–83

Beckett A, Heath IB, McLaughlin DJ (1974) An atlas of fungal ultrastructure. Longman Group, London

Bellemere A (1994) Asci and ascospores in ascomycete systematics. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, NY, pp 111–125

Benjamin RK (1959) The merosporangiferous Mucorales. El Aliso 4:321–433

Benny GL (1972) Histochemistry of the cell wall and septum of vegetative cells of selected species of Dimargaritaceae and Kickxellaceae. Mycologia 64:854–862

Bentivenga SP, Kumar TKA, Kumar L, Robeson RW, McLaughlin DJ (2013) Cellular organization in germ tubes of *Gigaspora*. Mycologia 105:1087–1099. doi:10.3852/12-291

Bergerow D, Stoll M, Bauer R (2006) A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analysis and morphological data. Mycologia 98:900–916

Bonfante P, Balestrini R, Mendgen K (1994) Storage and secretion processes in the spore of *Gigaspora margarita* Becker & Hall as revealed by high-pressure freezing and freeze substitution. New Phytol 128:93–101

Bourett TM, Howard RJ (1991) Ultrastructural immunolocalization of actin in a fungus. Protoplasma 163:199–202

Bourett TM, Howard RJ (1994) Enhanced labelling of concanavalin A binding sites in fungal endomembranes using a double-sided, indirect method. Mycol Res 98:769–775

Bourett TM, McLaughlin DJ (1986) Mitosis and septum formation in the basidiomycete *Helicobasidium mompa*. Can J Bot 64:130–145

Bourett TM, Picollelli MA, Howard RJ (1993) Postembedment labeling of intracellular concanavalin A-binding sites in freeze-substituted fungal cells. Exp Mycol 17:223–235

Bracker CE (1967) Ultrastructure of fungi. Annu Rev Phytopathol 5:343–372

Bracker CE, Butler EE (1963) The ultrastructure and development of septa in hyphae of *Rhizoctonia solani*. Mycologia 55:35–58

Bracker CE, Grove SN (1971) Continuity between cytoplasmic endomembranes and outer mitochondrial membranes in fungi. Protoplasma 73:15–34

Bracker CE, Morré DJ, Grove SN (1996) Structure, differentiation, and multiplication of Golgi apparatus in fungal hyphae. Protoplasma 194:250–274

Braselton JP (2001) Plasmodiophoromycota. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7A, Systematics and evolution. Springer, Berlin, pp 81–91

Buller AHR (1933) The translocation of protoplasm through septate mycelium of certain pyrenomycetes, discomycetes, and hymenomycetes. In: Researches on fungi, vol 5. Longmans, Green, London, pp 75–167

Buser C, McDonald K (2010) Correlative GFP-immunoelectron microscopy in yeast. Method Enzymol 470:603–618

Camon E, Magrane M, Barrell D, Lee V, Dimmer E, Maslen J, Binns D, Harte N, Lopez R, Apweiler R (2004) The Gene Ontology Annotation (GOA) database: sharing knowledge in UniProt with gene ontology. Nucleic Acids Res 32:D262–D266

Cavalier-Smith T (2001) What are fungi? In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7A, Systematics and evolution. Springer, Berlin, pp 3–37

Celio GJ, Richardson EA, Mims CW (2000) Ultrastructure of the infection of poinsettia by *Oidium* sp. using high pressure freezing and freeze substitution. Microsc Microanal Proc 6:684–685

Celio GJ, Padamsee M, Dentinger BTM, Bauer R, McLaughlin DJ (2006) Assembling the fungal tree of life: constructing the structural and biochemical database. Mycologia 98:850–859

Celio GJ, Padamsee M, Dentinger BTM, Josephsen KA, Jenkinson TS, McLaughlin EG, McLaughlin DJ (2007) Septal pore apparatus and nuclear division of *Auriscalpium vulgare*. Mycologia 99:644–654

Chandler DE, Roberson RW (2009) Current concepts in light and electron microscopy. Jones and Bartlett, Boston, MA

Chong J, Barr DJS (1974) Ultrastructure of the zoospores of *Entophlyctis confervae-glomeratae*, *Rhizophydium patellarium* and *Catenaria anguillulae*. Can J Bot 52:1197–1204

Cole GT (1979) Contributions of electron microscopy to fungal classification. Am Zool 19:589–608

Dewel RA, Dewel WC (1990) The fine structure of the zoospore of *Sorochytrium milnesiophthora*. Can J Bot 68:1968–1977

Dhavale T, Jedd G (2007) The fungal woronin body. In: Howard RJ, Gow NAR (eds) The mycota, vol 8, Biology of the fungal cell, 2nd ed. Springer, Berlin, pp 87–96

Dick MW (2001) The peronosporomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7A, Systematics and evolution. Springer, Berlin, pp 39–72

Dodd JL, McCracken DA (1972) Starch in fungi. Mycologia 64:1341–1343

Fischer-Parton S, Parton RM, Hickey PC, Dijksterhuis J, Atkinson HA, Read ND (2000) Confocal microscopy of FM4-64 as a tool for analyzing endocytosis and vesicle trafficking in living fungal hyphae. J Microsc 198:246–259

Fisher KE, Lowry DS, Roberson RW (2000) Cytoplasmic cleavage in zoosporangia of *Allomyces macrogynus*. J Microsc 198:260–270

Frieders EM, McLaughlin DJ (1996) Mitosis in the yeast phase of *Agaricostilbum pulcherrimum* and its evolutionary significance. Can J Bot 74:1392–1406

Fuller MS (1977) The zoospore, hallmark of the aquatic fungi. Mycologia 69:1–20

Fuller MS (2001) Hyphochytriomycota. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7A, Systematics and evolution. Springer, Berlin, pp 73–80

Fuller MS, Jaworski A (1987) Zoosporic fungi in teaching and research. Southeastern Publishing, Athens, GA

Gene Ontology Consortium, Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM et al (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29

Gene Ontology Consortium (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32:D258–D261

Gene Ontology Consortium (2006) The Gene Ontology (GO) project in 2006. Nucleic Acids Res 34:D322–D326

Gene Ontology Consortium (2008) The Gene Ontology (GO) project in 2008. Nucleic Acids Res 36:D440–D444

Giberson RT, Demaree RS Jr (1995) Microwave fixation: understanding the variables to achieve rapid reproducible results. Microsc Res Tech 32:246–254

Giberson RT, Demaree RS, Nordhausen RW (1997) Four-hour processing of clinical/diagnostic specimens for electron microscopy. J Vet Diagn Invest 9:61–67

Giddings TH (2003) Freeze-substitution protocols for improved visualization of membranes in high-pressure frozen samples. J Microsc 212:53–61

Gilkey JC, Staehelin LA (1986) Advances in ultrarapid freezing for the preservation of cellular ultrastructure. J Electron Micr Tech 3:177–210

Giner JL, Zhao H, Beach DH, Parish EJ, Jayasimhulu K, Kaneshiro ES (2002) Comprehensive and definitive structural identities of *Pneumocystis carinii* sterols. J Lipid Res 43:1114–1124

Girbardt M (1957) Der Spitzenkörper von *Polystictus versicolor*. Planta 50:47–59

Girbardt M (1969) Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma 67:413–441

Glauert AM (1991) Fixation, dehydration and embedding of biological specimens. North-Holland, Amsterdam

Gold JJ, Heath IB, Bauchop T (1988) Ultrastructural description of a new chytrid genus of caecum anaerobe, *Caecomyces equi gen. nov, sp. nov.*, assigned to the Neocallimasticaceae. Biosystems 21:403–415

Grandmougin-Ferjani A, Dalpé Y, Hartmann MA, Laruelle F, Sancholle M (1999) Sterol distribution in arbuscular mycorrhizal fungi. Phytochemistry 50:1027–1031

Grove SN, Bracker CE (1970) Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. J Bacteriol 104:989–1009

Harding MW, Stutz JC, Roberson RW (1999) Host-parasite relationships in bean cultivars of varying susceptibility to bean rust. Can J Bot 77:1551–1559

Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, Plamann M, Momany M (2004) Spitzenkörper meets polarisome: microscopy, genetics, and genomics converge. Eukaryot Cell 4:225–229

Hawker LE (1965) Fine structure of fungi as revealed by electron microscopy. Biol Rev Camb Philos Soc 40:52–92

Healy RA, Kumar TKA, Hewitt DA, McLaughlin DJ (2013) Functional and phylogenetic implications of septal pore ultrastructure in the ascoma of *Neolecta vitellina*. Mycologia 105:802–813. doi:10.3852/12-347

Heath IB (1980) Variant mitoses in lower eukaryotes: indicators of evolution of mitosis? Int Rev Cytol 64:1–80

Heath IB (1986) Nuclear division: a marker for protist phylogeny. Prog Protistol 1:115–162

Heath IB, Rethoret K (1982) Mitosis in the fungus *Zygorhynchus moelleri*: evidence for stage specific enhancement of microtubule preservation by freeze substitution. European J Cell Biol 28:180–189

Heath IB, Bauchop T, Skipp RA (1983) Assignment of the rumen anaerobe *Neovcallimastix frontalis* to the Spizellomycetales (Chytridiomycota) on the basis of its polyflagellate zoospore ultrastructure. Can J Bot 61:295–307

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James TY, Kirk PM, Lucking R, Lumbsch HT, Lutzoni FL, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai Y-C, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljag U, Kurtzman CP, Larsson K-H, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo J-M, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schußler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao Y-J, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Hoch HC (1986) Freeze-substitution of fungi. In: Aldrich HC, Todd WJ (eds) Ultrastructural techniques for microorganisms. Plenum, New York, NY, pp 183–212

Hoch HC, Fuller MS (1977) Mycoparasitic relationships I. Morphological features of interaction between *Pythium acanthicum* and several fungal hosts. Arch Microbiol 111:207–224

Hoch HC, Howard RJ (1980) Ultrastructure of freeze substituted hyphae of the basidiomycete *Laetisaria arvalis*. Protoplasma 103:281–297

Hoch HC, Howard RJ (1981) Conventional chemical fixations induce artifactual swelling of dolipore septa. J Exp Mycol 5:167–172

Hoch HC, Staples RC (1983) Ultrastructural organization of the non-differentiated uredospore germling of *Uromyces phaseoli* variety *typica*. Mycologia 75:795–824

Hohmann-Marriott MF, Uchida M, van de Meene AML, Garret M, Hjelm BE, Kokoori S, Roberson RW (2006) Electron tomography and its application to revealing fungal ultrastructure: Tansley review. New Phytol 172:208–220

Howard RJ (1981) Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze substitution. J Cell Sci 48:89–103

Howard RJ, Aist JR (1979) Hyphal tip cell ultrastructure of the fungus *Fusarium*: improved preservation by freeze-substitution. J Ultrastruct Res 66:224–234

Howard RJ, Aist JR (1980) Cytoplasmic microtubules and fungal morphogenesis: ultrastructure effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells. J Cell Biol 87:55–64

Howard RJ, O'Donnell KL (1987) Freeze substitution of fungi for cytological analysis. Exp Mycol 11:250–269

Hughes LM, Bao J, Hu Z-L, Honavar V, Reecy JM (2008) Animal trait ontology: the importance of a unified trait vocabulary for animal species. J Anim Sci 86:1485–1491

Hutchison LJ, Summerbell RC (1990) Studies on the systematics of ectomycorrhizal fungi in axenic culture. Reactions of mycelia to diazonium blue B stain. Mycologia 82:36–42

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüssler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DH, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006a) Reconstructing the early evolution of the fungi using a six gene phylogeny. Nature 443:818–822

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R (2006b) A molecular phylogeny of the flagellated fungi (Chytridiomycota) and a proposal for a new phylum (Blastocladiomycota). Mycologia 98:860–871

Jenkinson TS, Celio GJ, Padamsee M, Dentinger BTM, Meyer ML, McLaughlin DJ (2008) Conservation of cytoplasmic organization in the cystidia of *Suillus* species. Mycologia 100:539–547

Joffrion TM, Cushion MT (2010) Sterol biosynthesis and sterol uptake in the fungal pathogen *Pneumocystis carinii*. FEMS Microbiol Lett 311:1–9

Kaneshiro ES (2002) Sterol biosynthesis in *Pneumocystis*: unique steps that define unique targets. Drug Resist Updat 5:259–268

Kimbrough JW (1994) Septal ultrastructure and ascomycete systematics. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, NY, pp 127–141

Kleven NL, McLaughlin DJ (1989) A light and electron microscopic study of the developmental cycle in the basidiomycete *Pachnocybe ferruginea*. Can J Bot 67:1336–1348

Köhli M, Galati V, Boudier V, Roberson RW, Philippsen P (2008) Growth-speed correlated localization of exocyst and polarisome components in growth zones of *Ashbya gossypii* hyphal tips. J Cell Sci 121:3803–3814

Kumar TKA, Crow JA, Wennblom TJ, Abril M, Letcher PM, Blackwell M, Roberson RW, McLaughlin DJ (2011) An ontology of fungal subcellular traits. Am J Bot 98:1504–1510

Kumar TKA, Healy R, Spatafora JW, Blackwell M, McLaughlin DJ (2012) *Orbilia* ultrastructure, character evolution and phylogeny of Pezizomycotina. Mycologia 104:462–476. doi:10.3852/11-213

Kumar TKA, Blackwell M, Letcher PM, Roberson RW, McLaughlin DJ (2013) Research and teaching with the AFTOL SBD: an informatics resource for fungal subcellular and biochemical data. IMA Fungus 4:259–263. doi:10.5598/imafungus.2013.04.02.11

Kuo M (2006) Using a microscope: equipment. MushroomExpert.Com website. http://www.mushroomexpert.com/microscope_equipment.html

Kurtzman CP, Fell JW, Boekhout T (eds) (2011) The yeasts: a taxonomic study, 5th edn. Amsterdam, Elsevier

Lange L, Olson LW (1978) The zoospore of *Olpidium radicale*. Trans Brit Mycol Soc 71:43–55

Letcher PM, Powell MJ (2005) *Kappamyces*, a new genus in the Chytridiales. Nova Hedwigia 80:115–133

Letcher PM, Powell MJ, Churchill PF, Chambers JG (2006) Ultrastructural and molecular delineation of a new order, the Rhizophydiales. Mycol Res 110:898–915

Letcher PM, Powell MJ, Viusent MC (2008) Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales. Mycologia 100:325–334

Lewis PR, Knight DP (1977) Staining methods for sectioned material. North-Holland, Amsterdam
Li J, Heath IB (1991) The development and zoospore ultrastructure of a polycentric chytridiomycete gut fungus, *Orpinomyces joyonii* comb. nov. Can J Bot 69:580–589
Linnaeus C (1753) Species plantarum. Laurentius Salvius, Stockholm
Longcore JE, Simmons DR (2012) The Polychytriales ord. nov. contains chitinophilic members of the rhizophlyctoid alliance. Mycologia 104:276–294
López-Franco R, Bracker CE (1996) Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. Protoplasma 195:90–111
Lü HS, McLaughlin DJ (1991) Ultrastructure of the septal pore apparatus and early septum initiation in *Auricularia auricula-judae*. Mycologia 83:322–334
Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R (2004) Where are we in assembling the fungal tree of life, classifying the fungi, and understanding the evolution of their subcellular traits? Am J Bot 91:1446–1480
Maddison WP, Maddison DR (2000) MacClade, version 4, Analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, MA
Maddison WP, Maddison DR (2007) Mesquite: a modular system for evolutionary analysis, version 2.0. http://mesquiteproject.org
Martin GW (1955) Are fungi plants? Mycologia 47:779–792
McCracken DA, Dodd JL (1971) Molecular structure of starch-type polysaccharides from *Hericium ramosum* and *Hericium coralloides*. Science 174:419
McDaniel DP, Roberson RW (2000) Intracellular motility and mechanisms of control during hyphal tip growth in *Allomyces*. Fungal Genet Biol 31:223–234
McLaughlin DJ (1990) A new cytoplasmic structure in the basidiomycete *Helicogloea*: the microscala. Exp Mycol 14:331–338
McLaughlin DJ, Beckett A, Yoon KS (1985) Ultrastructure and evolution of ballistosporic basidiospores. Bot J Linn Soc 91:253–271
McLaughlin DJ, Berres ME, Szabo LJ (1995a) Molecules and morphology in basidiomycete phylogeny. Can J Bot 73(Suppl 1):S684–S692
McLaughlin DJ, Frieders EM, Lü HS (1995b) A microscopist's view of heterobasidiomycete phylogeny. Stud Mycol 38:91–109
McLaughlin DJ, Frieders EM, Berres ME, Doublès JC, Wick SM (1996) Immunofluorescence analysis of the microtubule cytoskeleton in the yeast phase of the basidiomycete *Kriegeria eriophori* with a brief note on the yeast phase of *Septobasidium carestianum*. Mycologia 88:339–349
McLaughlin DJ, McLaughlin EG, Lemke PA (eds) (2001) The mycota. Systematics and evolution, vol 7A, B. Springer, Berlin
McLaughlin DJ, Celio GJ, Padamsee M, Dentinger BTM (2008) Cystidial structure in two genera of the Russulales. Botany 86:545–550
McLaughlin DJ, Hibbett DS, Lutzoni F, Spatafora JW, Vilgalys R (2009) The search for the Fungal Tree of Life. Trends Microbiol 17:488–497
Melzer MV (1924) L'ornementation des spores de Russules. Bull Soc Myc de Fr 40:78–81
Mendgen K, Welter K, Scheffold F, Knauf-Beiter G (1991) High pressure freezing of rust infected leaves. In: Mendgen K, Leseman DE (eds) Electron microscopy of plant pathogens. Springer, Berlin, pp 31–42
Miller SL (1988) Early basidiospore formation in *Lactarius lignyotellus*. Mycologia 80:99–107
Mims CW, Richardson EA (2005) Light and electron microscopy of teliospores and teliospore germination in the rust fungus *Coleosporium ipomoeae*. Can J Bot 83:451–458
Mims CW, Roberson RW, Richardson EA (1988) Ultrastructure of freeze-substituted and chemically fixed basidiospores of *Gymnosporangium juniperi-virginianae*. Mycologia 80:356–364
Mims CW, Richardson EA, Kimbrough JW (1990) Ultrasturcture of ascopore delimitation in freeze substituted samples of *Ascodesmis nigricans* (Pezizales). Protoplasma 156:94–102
Mims CW, Rodriguez-Lother C, Richardson EA (2002) Ultrastructure of the host–pathogen interface in daylily leaves infected by the rust fungus *Puccinia hemerocallidis*. Protoplasma 219:221–226
Mims CW, Celio GJ, Richardson EA (2003) The use of high pressure freezing and freeze substitution to study host–pathogen interactions in fungal diseases of plants. Microsc Microanal Proc 9:522–531
Moor H (1987) Theory and practice of high pressure freezing. In: Steinbrecht RA, Ziehold K (eds) Cryotechniques in biological electron microscopy. Springer, New York, NY, pp 175–191
Moore RT, McAlear JR (1962) Fine structure of Mycota 7. Am J Bot 49:86–94
Mozley-Standridge SE, Letcher PM, Longcore JE, Porter D, Simmons DR (2009) Cladochytriales - a new order in Chytridiomycota. Mycol Res 113:498–507
Newhouse JR, Hoch HC, MacDonald WL (1983) The ultrastructure of *Endothia parasitica*. Comparison of a virulent with a hyphovirulent isolate. Can J Bot 61:389–399
Nylander W (1865) Ad historiam reactionis iodi apud Lichenes et Fungos notula. Flora 48:465–468
Nylander W (1867) Hypochlorite of lime and hydrate of potash, two new criteria in the study of lichens.

J Linn Soc Bot 9:358–365, Translated and communicated by Leighton

O'Donnell KL, McLaughlin DJ (1981) Ultrastructure of meiosis in the hollyhock fungus, *Puccinia malvacearum* I. Prophase I – prometaphase I. Protoplasma 108:225–244

O'Donnell KL, McLaughlin DJ (1984) Ultrastructure of meiosis in *Ustilago maydis*. Mycologia 76:468–485

Oberwinkler F, Bauer R (1989) The systematics of gasteroid, auricularioid heterobasidiomycetes. Sydowia 41:224–256

Oberwinkler F, Kirschner R, Arenal F, Villarreal M, Rubio V, Bergerow D, Bauer R (2006) Two new pycnidial members of the Atractiellales: *Basidiopycnis hyalina* and *Proceropycnis pinicola*. Mycologia 98:637–649

Padamsee M, Celio GJ, McLaughlin DJ (2008) Reciprocal illumination: using phylogeny and ultrastructure to study cystidia of two *Psathyrella* species. Botany 86:1–9

Padamsee M, Kumar TKA, Riley R, Binder M, Boyd A, Calvo AM, Furukawa K, Hesse C, Hohmann S, James TY, LaButti K, Lapidus A, Lindquist E, Lucas S, Miller K, Shantappa S, Grigoriev IV, Hibbett DS, McLaughlin DJ, Spatafora JW, Aime MC (2012) The common food contaminant *Wallemia sebi* has a small genome and reveals mechanisms for surviving an osmotically stressful environment. Fungal Genet Biol 49:217–226

Pereira J (1854) The elements of materia medica and therapeutics. Blanchard and Lea, Philadelphia, PA

Pfister DH, Kimbrough JW (2001) Discomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The mycota, vol 7A, Systematics and evolution. Springer, Berlin, pp 257–281

Picard KT, Letcher PM, Powell MJ (2009) *Rhizidium phycophilum*, a new species in Chytridiales. Mycologia 101:696–706

Poon NH, Day AW (1976) Somatic nuclear division in the sporidia of *Ustilago violacea*. IV. Microtubules and spindle pole body. Can J Microbiol 22:507–522

Powell MJ, Letcher PM, Longcore JE (2011) *Operculomyces* is a new genus in the order Rhizophydiales. Mycologia 103:854–862

Prillinger H, Dörfler C, Laaser G, Hauska G (1990) A contribution to the systematics and evolution of higher fungi: yeast-types in the basidiomycetes. Part III: *Ustilago*-type. Z Mycol 56:251–278

Prillinger H, Oberwinkler F, Umile C, Tlachac K, Bauer R, Dörfler C, Taufratzhofer E (1993) Analysis of cell wall carbohydrates (neutral sugars) from ascomycetous and basidiomycetous yeasts with and without derivatization. J Gen Appl Microbiol 39:1–34

Prillinger H, Lopandic K, Schweigkofler W, Deak R, Henk J, Aarts HJM, Bauer R, Sterflinger K, Kraus GF, Maraz A (2002) Phylogeny and systematics of the fungi with special reference to the Ascomycota and Basidiomycota. In: Breitenbach M, Crameri R, Lehrer SB (eds) Fungal allergy and pathogenicity, vol 81, Chem Immunol. Karger, Basel, pp 207–295

Reichle RE, Lichtwardt RW (1972) Fine structure of the trichomycete, *Harpella melusinae*, from black-fly guts. Arch Mikrobiol 81:103–125

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Rezaeian M, Beakes GW, Parker DS (2004) Methods for the isolation, culture and assessment of the status of anaerobic rumen chytrids in both in vitro and in vivo systems. Mycol Res 34:1215–1226

Riquelme M, McDaniel D, Roberson RW, Bartnicki-García S (2002) The effect of ropy-1 mutation on cytoplasmic organization in mature hyphae of *Neurospora crassa*. Fungal Genet Biol 37:171–179

Roberson RW (1993) Cryofixation and freeze substitution of teliospores of *Gymnosporangium clavipes*: an ultrastructural investigation. Mycol Res 97:195–204

Roberson RW, Fuller MS (1988) Ultrastructural aspects of the hyphal tip of *Sclerotium rolfsii* preserved by freeze substitution. Protoplasma 146:143–149

Roberson RW, Fuller MS (1990) Effects of the sterol biosynthesis inhibitor cyproconazole on hyphal tip cells of *Sclerotium rolfsii*. II. An electron microscopic study. Exp Mycol 14:124–135

Roberson RW, Luttrell ES (1987) Ultrastructure of teliospore ontogeny in *Tilletia indica*. Mycologia 79:753–763

Roberson RW, Vargas M (1994) The tubulin cytoskeleton and its sites of nucleation in *Allomyces macrogynus*. Protoplasma 182:19–31

Roberson RW, Luttrell ES, Fuller MS (1990) Mycoparasitisum of teliospores of the smut fungus *Ustilago bullata* by an oomycete. Can J Bot 68:2415–2421

Roberson RW, Abril M, Blackwell M, Letcher P, McLaughlin DJ, Mouriño-Pérez RR, Riquelme M, Uchida M (2010) Hyphal structure. In: Borkovich K, Ebbole D (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 8–24

Roberson RW, Saucedo B, MacLean D, Propster J, Unger B, O'Neil TA, Parvanehgohar K, Cavanaugh C, Lowry D (2011) The hyphal tip structure of *Basidiobolus* sp.: a zygomycete fungus of uncertain phylogeny. Fungal Biol 115:485–492

Rolland L (1887) De la coloration en bleu développée par l'iode sur divers champignons. Bull Soc Myc de Fr 3:134–137

Shields JP, Fuller MS (1996) Ultrastructure of chytridiomycete and oomycete zoopores using spray-freeze fixation. Protoplasma 191:84–95

Silva MT, Guerra FC, Magalhães MM (1968) The fixative action of uranyl acetate in electron microscopy. Experientia 24:1074

Simmons RD, James TY, Meyer AF, Longcore JE (2009) Lobulomycetales, a new order in the Chytridiomycota. Mycol Res 113:450–460

Srijayanthi S, Vargas M, Roberson RW (1996) Functional, organizational, and biochemical analysis of actin in the hyphal tip cells of *Allomyces macrogynus*. Mycologia 88:57–80

Stajich JE, Berbee ML, Blackwell M, Hibbett DS, James TY, Spatafora JW, Taylor JW (2009) The fungi. Curr Biol 19:R840–R845

Swann EC, Frieders EM, McLaughlin DJ (1999) *Microbotryum*, *Kriegeria*, and the changing paradigm in basidiomycete classification. Mycologia 91:51–66

Swofford DL (2002) PAUP*, Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer Associates, Sunderland, MA

Taylor JW (1984) Correlative light and electron microscopy with fluorescent stains. Mycologia 76:462–467

Taylor J, Mims CW (1990) Fungal development and host cell responses in the rust fungus *Puccinia substriata* var. *indica* in seedlings of mature leaves of susceptible and resistant pearl millet. Can J Bot 69:1207–1219

Tevayanond R (1981) On the nature of the reaction of diazonium blue B with basidiomycetous yeasts. PhD Thesis, University of California, Davis

Torruella G, Suga H, Riutort M, Pereto J, Ruiz-Trillo I (2009) The evolutionary history of lysine biosynthesis pathways within eukaryotes. J Mol Evol 69:240–248

Urbina H, Blackwell M (2012) Multilocus phylogenetic study of the *Scheffersomyces* yeast clade and characterization of the N-terminal region of xylose reductase gene. PLoS ONE 7(6):e39128

Van Driel KGA, van Peer AF, Grijpstra J, Wösten HAB, Verkleij AJ, Muller WH, Boekhout T (2008) Septal pore cap protein SPC18, isolated from the basidiomycetous fungus *Rhizoctonia solani*, also resides in pore plugs. Eukaryot Cell 7:1865–1873

Van Driel KGA, Humbel BM, Verkleij AJ, Stalpers J, Müller WH, Boekhout T (2009) Septal pore complex morphology in the Agaricomycotina (Basidiomycota) with emphasis on the Cantharellales and Hymenochaetales. Mycol Res 113:559–576

Van Peer AF, Wang F, van Driel KGA, de Jong JF, van Donselaar EG, Müller WH, Boekhout T, Lugones LG, Wösten HAB (2010) The septal pore cap is an organelle that functions in vegetative growth and mushroom formation of the wood-rot fungus *Schizophyllum commune*. Environ Microbiol 12:833–884

Vargas M, Aronson JM, Roberson RW (1993) Cytological organization of hyphal tip cells of *Allomyces macrogynus*. Protoplasma 176:43–52

Vélez CG, Letcher PM, Schultz S, Powell MJ, Churchill PF (2011) Molecular phylogenetic and zoospore ultrastructural analyses of *Chytridium olla* establish the limits of a monophyletic Chytridiales. Mycologia 103:118–130

Vogel HJ (1960) Two modes of lysine synthesis among lower fungi: evolutionary significance. Biochim Biophys Acta 41:172–173

Vogel HJ (1965) Lysine biosynthesis and evolution: fungi, gymnosperms and angiosperms. In: Bryson V, Vogel HJ (eds) Evolving genes and proteins. Academic, New York, NY, pp 25–40

Webster P (2007) Microwave-assisted processing and embedding for transmission electron microscopy. In: Kuo J (ed) Electron microscopy methods and protocols. Humana Press, Totowa, NJ, pp 47–65

Weete JD, Abril M, Blackwell M (2010) Phylogenetic distribution of fungal sterols. PLoS One 5(5): e10899. doi:10.1371/journal.pone.0010899

Weiss M, Bauer R, Bergerow D (2004) Spotlights on heterobasidiomycetes. In: Agerer R, Piepenbring M, Blanz P (eds) Frontiers in basidiomycete mycology. IHW, Eching, pp 7–48

White MM, James TY, O'Donnell K, Cafaro MJ, Tanabe Y, Sugiyama J (2006) Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia 98:872–884

Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings TH, McDonald KL, McIntosh JR (1995) Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. J Cell Biol 129:1601–1615

Yoon KS, McLaughlin DJ (1986) Basidiosporogenesis in *Boletus rubinellus* II. Late spore development. Mycologia 8:185–197

10 Fungal Diversity in the Fossil Record

THOMAS N. TAYLOR[1,2], MICHAEL KRINGS[1,2,3,4], EDITH L. TAYLOR[1,2]

CONTENTS

I. **Introduction** ... 259
II. **History of Fossil Fungi** ... 260
III. **Techniques** ... 261
A. Morphologic Comparison ... 261
B. Fungal Spores ... 261
C. Thin Sections and Acetate Peels ... 263
D. Plant Resins (Amber) ... 264
IV. **Major Fungal Lineages** ... 265
A. Chytridiomycota ... 265
B. Blastocladiomycota ... 266
C. Zygomycetous Fungi ... 266
C.1 So-Called Sporocarps ... 266
D. Glomeromycota ... 267
E. Basidiomycota ... 268
F. Ascomycota ... 269
VI. **Lichens** ... 270
VI. **Enigmatic Fossils** ... 271
A. Uncertain Affinities ... 271
B. Plant–Fungal Associations with Uncertain Functions ... 272
VI. **Conclusions** ... 273
References ... 273

[1]Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA; e-mail: tntaylor@ku.edu; m.krings@lrz.uni-muenchen.de; etaylor@ku.edu

[2]Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA; e-mail: tntaylor@ku.edu; m.krings@lrz.uni-muenchen.de; etaylor@ku.edu

[3]Department für Geo- und Umweltwissenschaften, Paläontologie und Geobiologie, Ludwig-Maximilians-Universität, Richard-Wagner-Straße 10, Munich 80333, Germany; e-mail: m.krings@lrz.uni-muenchen.de

[4]Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, Munich 80333, Germany; e-mail: m.krings@lrz.uni-muenchen.de

I. Introduction

Fungi that are both generalists and specialists occur in virtually every ecosystem today, where they colonize a wide variety of (micro-)habitats and provide numerous functions ranging from decomposing organic matter to immobilizing nutrients (Cantrell et al. 2011). Considering the remarkable diversity in types of fungi, the levels of interaction with other components of the biological and physical world, and their role as drivers of many processes in modern ecosystems, it is surprising that fungi and fungal activities have not been consistently studied in the fossil record. **In a very real sense, the study of fossil fungi has "fallen between the cracks" of geologic time because the activities of fungi (e.g., decomposition, parasitism) result in fossil materials that are not especially appealing and, therefore, not normally collected.** This has resulted in an underrepresentation of fossil forms and an underappreciation of both their biodiversity and their distribution in time and space. In addition, fossil fungi continue to present a dilemma to those interested in their geological history and biological features for several reasons. One of these is the fact that, until relatively recently, there has not been a concerted effort to systematically study fungi in the fossil record. A second continuing problem centers on the "proprietary rights" of just who should study fossil fungi. Paleontologists are the most likely scientists to discover fungi or their activities in the fossil record, but in most instances, they either have no interest in these organisms or lack the scientific expertise to evaluate them. At the same time, the mycologi-

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)

© Springer-Verlag Berlin Heidelberg 2015

cal community lacks access to these organisms, and thus there is little collaborative effort to date devoted to exploring questions relating to the fossils or the biodiversity of fungi in time and space.

Despite the preceding comments, there is already scattered historical evidence of fossil fungi that indicates an untapped wealth of information and, more recently, a new emphasis on their paleodiversity and evolutionary history that is beginning to emerge. The following sections provide examples of fossil fungi, as well as some enigmatic fossils that have been at one time or another considered to be fungal. Although there is an increasing body of literature dealing with the importance of fungi in paleoecology, sedimentology, coal geology, geobiology, and geobiochemistry (e.g., Stubblefield and Taylor 1988; Gadd 2008; Hower et al. 2009), we have focused the present review on the more biological dimensions of paleomycology. Finally, we comment on some of the limiting aspects of examining the record of the activities of fossil fungi and suggest examples in which they represent a valuable data source that is unavailable using other scientific tools.

II. History of Fossil Fungi

Systematic analyses of fungi in the fossil record represent a relatively new avenue of research, despite the fact that fossil plants and animals have been studied for more than 250 years. Nevertheless, many paleobotanists and paleozoologists, especially during the nineteenth and early twentieth centuries, have occasionally made reference to or figured (what they believed were) fungi or indications of fungal activity co-occurring with the plant and animal fossils or sediment samples they were studying. Even the most distinguished naturalists of the day, including Charles Darwin, took notice of the presence of fungi associated with certain plant fossils (Smith 1884). In fact, the earliest compendium of fossil fungi was published in 1898 (Meschinelli 1898). Meschinelli's book is a remarkable volume that is lavishly illustrated and demonstrated the interest in fossil fungi of the scientific community of the day. In most cases, however, the presence of fungi was merely noted as a curiosity in early studies, although a few workers provided detailed descriptions of these life forms and discussed how they might have affected host performance and ecosystem functioning in the past.

Two series of historical contributions provide detailed information on fungi and other microorganisms co-occurring with fossil plants. One of these is a series of papers by the French paleobotanist Bernard Renault (e.g., Renault and Bertrand 1885; Renault 1894, 1895a, b, 1896a, 1903) on microorganisms associated with various Carboniferous plants preserved in cherts from France. The renowned American botanist, paleontologist, and sociologist Lester Ward called Renault's studies "a superb work on a very difficult, but at the same time very important subject" [quote in Andrews (1980)]. Renault's observations were summarized in the final section of *Bassin Houiller et Permien d'Autun et d'Epinac, Fascicule IV, Flore Fossile, Deuxième partie* (1896b) and in his volume *Sur Quelques Microorganismes des Combustibles Fossiles* (1900, atlas 1899). A similar approach was used later by Robert Kidston and William H. Lang in their work on the Lower Devonian Rhynie chert; their studies appeared as a series of contributions (*On Old Red Sandstone plants showing structure, from the Rhynie chert Bed, Aberdeenshire*) of the Royal Society of Edinburgh (Kidston and Lang 1917, 1920a, b, 1921a, b). In Part V of this series, Kidston and Lang (1921b) described numerous microorganisms from the Rhynie chert, including cyanobacteria, algae, and a diverse assemblage of fungi and funguslike organisms, many of which they were able to document as being involved in various levels of interactions with other organisms. The studies of Renault and Kidston and Lang, and perhaps a few others today, represent benchmarks in the analysis of the interrelationships between microorganisms and land plants in fossil ecosystems.

For the next 50 years, however, there was relatively little activity with fossil fungi [surveyed in Tiffney and Barghoorn (1974) and Pirozynski (1976)]. In 1975, **Pirozynski and**

Malloch published an influential paper in which they suggested that fungi formed a critical component of the movement of plants onto the land. Their initial thesis postulated that some type of mutualistic relationship between a fungus and a green alga provided the necessary physiological adaptations so that this (new) organism could function in what must have been a very inhospitable environment. Their hypothesis, together with an increasing number of reports of Precambrian microbial life (Taylor et al. 2009), appears to have initiated a more general paleobiological interest in evidence of microbial (including fungal) activities from other, geologically younger, paleoecosystems (Taylor 1993).

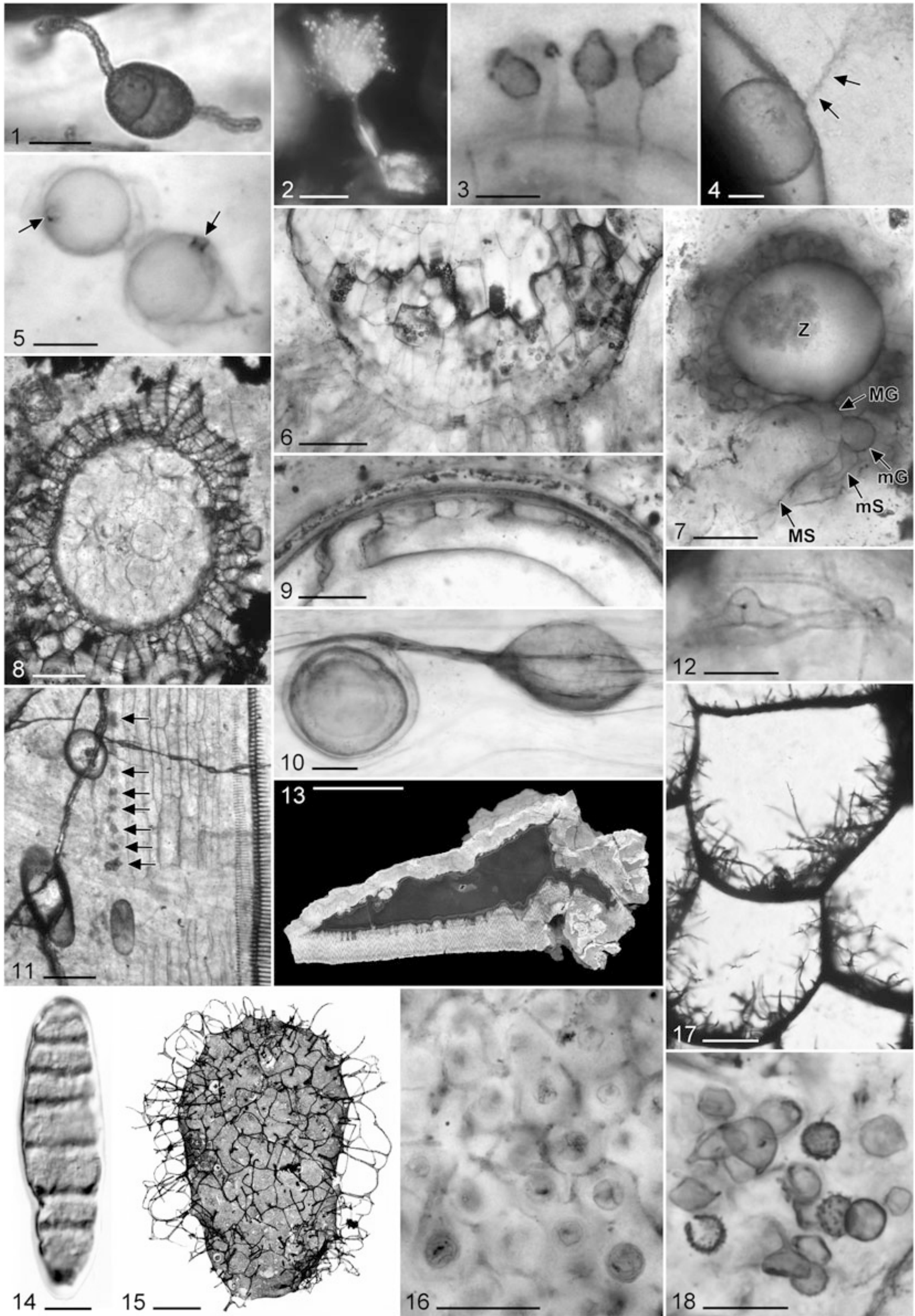
III. Techniques

A. Morphologic Comparison

No doubt the earliest technique used to study fossil fungi simply involved the recognition and description of various spots and other structures on the surface of compressed leaves of angiosperms, especially those from the Cenozoic. There are numerous descriptions of fungal structure related to ascocarps and other reproductive structures in the literature (e.g., Unger 1850; Lesquereux 1877; Meschinelli 1898). These early reports relied on the so-called **picture-matching technique** with living fungi and thus less accurately represented the taxonomy of the fossils than later studies. They did, however, appreciably contribute to the realization that various types of fungi were associated with specific plant parts, as they are today, and that, in some instances, it appears that the same fungus was consistently present on the same type of plant or plant organ. On some specimens it is possible to scrape off part of the fungus from the leaf surface and examine the hyphae and, in some cases, the spores or other parts of the fossil fungal reproductive structures. Compressed leaves, like those from the Eocene Geisel Valley in Saxony-Anhalt, Germany, or the Miocene Clarkia beds in Idaho, USA, have well-preserved cuticles that can be peeled or macerated from the rock; these often contain multiple epiphyllous fungi (e.g., Dilcher 1965; Daghlian 1978; Phipps and Rember 2004). While a number of Cenozoic floras worldwide with cuticular preservation have been extensively studied, most have not been analyzed for their epiphyllous fungi in any systematic way. Epiphyllous fungi on fossil leaves can also serve as additional sources of important information. One relates to the use of such leaf-borne fungi as proxy records for certain climate variables (e.g., temperature limitations) that can be used to help reconstruct paleoclimate or to calibrate paleoclimatic models (Wells and Hill 1993; Phipps 2006; Singh and Chauhan 2008; Ding et al. 2011). A second use may relate to the function of fungi in ancient ecosystems (Tripathi 2009). For example, are there specific types (species) of fossil fungi that are consistently found on certain leaf genera or species, and how do these relationships relate to modern leaf/fungus associations? The answers to questions like these can be found in the extensive and robust data source of compressed fossil leaves, a source that still needs to be exploited.

B. Fungal Spores

The various types of spores, mycelia, and fructifications that are often recovered by acid digestion of sediments and rocks (e.g., Elsik 1996; Kalgutkar and Jansonius 2000) represent another type of fungal fossil that is abundant but is not a major source of information about the biology of ancient fungi. Historically, fungal spores (Fig. 10.1) and occasionally other dispersed fungal remains have been used in stratigraphic studies based on palynomorph assemblages (e.g., Graham 1962) and have been used to suggest extinction and recovery events based on their presence or absence (e.g., Eshet et al. 1995; Vajda and McLoughlin 2004; Visscher et al. 2011). In a limited way, they have also been used to corroborate host–parasite interactions (e.g., Eocene angiosperm leaf type and epiphyllous fungus and spore type) (Dilcher 1965).



Figs. 10.1–10.18 Representatives of fungal fossils (references in text). **Fig. 10.1** Fungal spore; Lower Permian permineralization, Germany; bar = 10 μm. **Fig. 10.2** *Aspergillus collembolorum*, conidial head

One major problem in using fossil fungal spores as a taxonomic tool is their relative uniformity and lack of ornamentation features (Sheffy and Dilcher 1971). This is especially true of conidiospores; however, new imaging systems may hold promise in this regard. In studies of modern fungal spores at the ultrastructural level the focus has generally been on developmental features of conidia (e.g., Mims et al. 1995), with little attention paid to features of the spore wall that might be useful in systematics. Examination of surface ornamentation, even at the nanometer scale, has generally been concerned with questions related to molecular interactions and cell-surface properties (e.g., Dufrêne et al. 1999). As far as we know, no studies have appeared on the wall ultrastructure of any fossil fungal spores. Whether this technique might yield additional information about the biological affinities or development of fossil fungal spores, as has been done for fossil pollen, remains unknown, but it may offer promise in the future.

C. Thin Sections and Acetate Peels

The late 1800s witnessed a burst of activity in paleobotany once structurally preserved (permineralized) plant remains were discovered associated with Carboniferous coals. **Permineralizations** represent a preservation type in which minerals have precipitated in the cell lumina and intercellular spaces but have not replaced the organic matter of the cell walls. Such fossils occur in coal balls—concretions of mineralized (calcium carbonate, pyrite) plant material (peat) of predominantly Carboniferous and Permian age (Phillips et al. 1976). On the other hand, in the preservational process known as **petrifaction**, the intercellular spaces and other voids are also filled with precipitated minerals, typically forms of silica (chalcedony, quartz, opal), but the organic matter in the plant has been replaced by minerals as well, so that very little or no organic matter remains. For example, the organisms in the famous Early Devonian Rhynie chert paleoecosystem are petrified in silica.

To examine the cells and tissue systems of petrified fossils, it is necessary to prepare **thin sections** (=petrographic thin sections), in which a piece of the fossil or the rock containing the fossil is cemented to a microscope slide and then ground thin enough to be examined in transmitted light. This technique was also used for early studies on permineralized plants in coal balls. While the primary focus of the early studies on coal balls was the plants, there were occasional reports of fungal remains in the matrix of plant debris and sometimes within plant tissue (e.g., Williamson 1878; Cash and Hick 1879; Weiss 1904). As an alternative to the

Figs. 10.1–10.18 (continued) with chains of conidia; Eocene amber, Baltic; bar = 25 μm (courtesy A.R. Schmidt). **Fig. 10.3** Chytrid zoosporangia in outer wall of spore; Lower Devonian chert, Scotland; bar = 20 μm. **Fig. 10.4** Chytrid zoosporangium in megaspore wall; *arrows* indicate rhizoidal system; Middle Mississippian chert, France; bar = 20 μm. **Fig. 10.5** Putative chytrid zoosporangia in solitary unicells; *arrows* indicate discharge papillae; Middle Mississippian chert, France; bar = 10 μm. **Fig. 10.6** *Nothia aphylla* prostrate axis, showing host response to fungal attack in the form of secondarily thickened cell walls; Lower Devonian chert, Scotland; bar = 0.5 mm. **Fig. 10.7** Zygosporangium-apposed gametangia complex of *Jimwhitea circumtecta* (Endogonaceae); *Z* mantled zygosporangium, *MG* macrogametangium, *mG* microgametangium, *MS* macrosuspensor, *mS* microsuspensor; Middle Triassic permineralized peat, Antarctica; bar = 20 μm. **Fig. 10.8** So-called fungal sporocarp (type *Sporocarpon* sp.); Lower Pennsylvanian coal ball, Great Britain; bar = 100 μm. **Fig. 10.9** *Scutellosporites devonicus*, germination shield in near median longitudinal section; Lower Devonian chert, Scotland; bar = 30 μm. **Fig. 10.10** Acaulosporoid glomeromycotan spore; Lower Devonian chert, Scotland; bar = 100 μm. **Fig. 10.11** Vesicles and minute arbuscule-like structures (*arrows*) in inner cortex of stigmarian appendage; Lower Pennsylvanian coal ball, Great Britain; bar = 100 μm. **Fig. 10.12** Hypha with clamp connection; Middle Mississippian chert, France; bar = 10 μm. **Fig. 10.13** *Ganodermites libycus*, longitudinal thick section (polished surface) through basidiocarp; lower Miocene permineralization, Libya; bar = 2 cm. **Fig. 10.14** Septate conidium; middle Silurian sandstone, Sweden; bar = 10 μm (from Sherwood-Pike and Gray 1985). **Fig. 10.15** *Tappania* sp.; early Neoproterozoic shale, Canada; bar = 50 μm (courtesy N.J. Butterfield). **Fig. 10.16** *Winfrenatia reticulata*, hyphal net enclosing cyanobacterial unicells; Lower Devonian chert, Scotland; bar = 50 μm. **Fig. 10.17** *Cashhickia acuminata* in calamite rootlet, showing intracellular hyphae arising from host cell walls; Upper Pennsylvanian chert, France; bar = 30 μm. **Fig. 10.18** Fungal propagules in *Sphenophyllum* leaf; Upper Pennsylvanian chert, France; bar = 20 μm

expensive and time-consuming thin-section technique, some early-twentieth-century scholars working on Carboniferous coal-ball fossils started to use a liquid acetate mixture that was poured onto acid-etched surfaces. Once the acetate mixture hardened, a peel of the surface could be removed and examined with light microscopy. A modification of this technique was published in 1956 [Joy et al. (1956); see also Galtier and Phillips (1999)] that affected how coal-ball plants were studied and, at the same time, may have decreased the number of discoveries of fossil fungi in coal balls. In this modification, preformed sheets of cellulose acetate were used to make peels. Using these sheets, the technique was easier to master and less time consuming and produced many peels in the time it took for a single petrographic thin section or liquid peel to be made. As a result, **acetate peels** became the technique of choice in examining permineralized fossil plants. Although there are other reasons for the general lack of information about fossil fungi (as noted earlier), it is now apparent that the classic, time-consuming thin-section technique represents the best method to study fossil fungi, especially features that provide information about the interactions of fungi with other organisms (e.g., Krings et al. 2007a, b; Taylor et al. 2011). As these studies have shown, the etching process used in the peel technique often removes tiny organisms preserved in permineralized peat, including fungi.

Transmitted light microscopy of acetate peels and thin sections represent the primary methods currently being used in the analysis of fossil fungi, but there are others that are specific for various preservation types. For example, scanning electron microscopy (SEM), reflected and transmitted light microscopy of plant cuticles, and other types of imaging systems (Raman spectroscopy) are also in use.

D. Plant Resins (Amber)

Amber is a type of woody seed-plant resin that is a lipid soluble mixture of volatile and non-volatile terpenoid or phenolic secondary compounds. It may be produced and stored in secretory canals/cavities or produced as the result of injury (Langenheim 2003). Fossil fungi preserved in amber can be easily observed and studied in detail using various microscopy techniques (Speranza et al. 2010). It is therefore not surprising that fungi in amber were described as early as the nineteenth and early twentieth centuries (e.g., Goeppert and Berendt 1845; Caspary and Klebs 1907). Only recently, however, have these fossils received wider scholarly attention. Today there are reports of representatives of many different groups of fungi in amber because the translucent nature of the matrix makes it relatively easy to determine even very delicate features useful in systematics, as well as those useful in determining interactions with other organisms. Some examples include basidiocarps (Hibbett et al. 1995, 1997a, 2003), a carnivorous soil fungus that traps nematodes (Schmidt et al. 2007), sooty molds (Rikkinen et al. 2003), a representative of the genus *Aspergillus* growing on a springtail (Fig. 10.2) (Dörfelt and Schmidt 2005), and evidence of animal parasitism by fungi (Sung et al. 2008). Because of the extraordinary preservation potential that amber affords, it will continue to be an important avenue for research on fossil fungi. Amber pieces usually are quite small, however, and thus rarely provide information on the ecological configuration of the community in which the fungi (and their host organisms) lived. Moreover, almost all amber comes from Cretaceous and Cenozoic strata, which is too geologically recent to record the origin or evolution of most major groups of fungi. While amber has been reported from the Carboniferous (approximately 320 million years ago [Ma]) (Bray and Anderson 2009), it has not been found to contain direct evidence of fungi, with one possible exception (Smith 1898). Many woody plants from the Paleozoic are known to have produced resins, however, and if amber of sufficient size and preservation could be discovered, it might afford another source of information about very ancient fungi.

IV. Major Fungal Lineages

A. Chytridiomycota

Modern chytrids occur in diverse habitats from the tropics to the Arctic and are found in almost all forms of terrestrial and aquatic ecosystems, so it is not surprising that they have also been reported in the fossil record. Molecular clock estimates hypothesize that the chytrids are an ancient group that inhabited the Earth at least 1.5 gigayears, or billion years, ago (Ga) or even earlier (Heckman et al. 2001). Unfortunately, the body fossil record of this group in the Precambrian consists of only a few reports (e.g., Belova and Akhmedov 2006), and the interpretation of these discoveries remains controversial (Butterfield 2005). To date, the most convincing chytrid fossils come from the Early Devonian Rhynie chert ecosystem (Taylor et al. 2004a, b), which is dated at approximately 410 Ma (Pragian) (Fig. 10.3). The fossils occur both within the silica matrix (Kidston and Lang1921b) and associated with land plants (Illman 1984), charophytes (Taylor et al. 1992a), degraded plant material (Boullard and Lemoigne 1971), and fungal spores (Hass et al. 1994; Krings et al. 2009a). Some of these chytrids are so well preserved that they can be classified as holocarpic or eucarpic, monocentric or polycentric, and with and without an operculum. Most of the Rhynie chert chytrid fossils represent structures interpreted as zoosporangia because they are the same size and have the same morphology as modern forms. At least one fossil from the Rhynie chert, however, has been interpreted as a chytrid zoospore based on the presence of a single, posteriorly directed flagellum (Taylor et al. 1992b).

Within Carboniferous rocks are several reports of chytridlike organisms associated with pollen grains and spores (Millay and Taylor 1978), seeds (Oliver 1903), and other fungi (Krings et al. 2009b). In the Carboniferous of France are excellent examples of various types of resting spores as well as different forms of zoosporangia (Krings et al. 2009b, c). Some of these are associated with (degraded) plant tissue (e.g., the wood or the periderm of lycopsids), while others occur in unicells (unicellular algae?) within the chert matrix; still others reside on or within land-plant or fungal spores (Fig. 10.4). Some of the chytrids are interpreted as endobiotic, with holocarpic thalli that morphologically compare with zoosporangia of the extant chytrid *Olpidium* (Fig. 10.5). In some forms, there are distinct discharge pores or papillae, while others possess a stalk or an apophysis that attaches the thallus to the substrate.

Almost all of the chytridlike fossils that have been reported to date are of uncertain affinity. In a few instances, however, some fossils of chytridlike organisms have been directly referred to extant genera (e.g., Bradley 1967; García Massini 2007) or to closely related forms (Daugherty 1941). In recent years, the approach has been to describe and illustrate the diversity of chytridlike fossils in time and space and to record the type of host and ecosystem with which they are associated.

One approach that has been informative relative to fossil members of the Chytridiomycota is a focus on the relationship between the chytrid and any existing host response. Within the Rhynie chert are a variety of fungal spores, some of which are comparable to the asexual spores of extant members of the Glomeromycota (Hass et al. 1994). Extending into the spore lumen in many of these fossils are tapering structures termed callosities, lignotubers, or papillae that represent a host response to some invading organism. Many callosities form as a response to microfungi, such as chytrids that penetrate the spore wall. As this occurs the spore protoplast in turn synthesizes new wall material around the invading filament or hyphae. Other forms of Rhynie chert microfungi with possible affinities to the Chytridiomycota are consistently found between particular wall layers of these large spores or completely occupying the lumen of the spore (Hass et al. 1994). Another host response to a parasitic chytrid attack occurs within the charophyte *Palaeonitella cranii* in the Rhynie chert (Taylor et al. 1992a). In this example, the parasitic microfungus causes host cells to greatly expand as a result of the infection, a process known as hypertrophy. What makes this host response so striking is the fact that a similar response occurs in some modern *Chara* species (Karling 1928). In the fossil example, there are several chytrids that have penetrated the host cell wall. Other host responses with chytrids as the causative agents reported in certain Rhynie chert land plants include hyperplasia, a pattern in which there is active cell division (increase in number of cells) associated with the invasion of the parasite (Taylor et al. 2004a, b).

In addition, the Rhynie chert plant *Nothia aphylla* responds effectively to fungal (chytrid?) attacks by thickening of the cell walls that separate uninfected from infected tissue and by controlled cell death in infected areas of the tissue (Fig. 10.6) (Krings et al. 2007a).

B. Blastocladiomycota

This clade of core chytrids was included as an order of the Chytridiomycota until recently when it was elevated to the phylum level based on a life cycle with sporic meiosis and several ultrastructural (James et al. 2006) and molecular features (Porter et al. 2011). One fossil that shows a number of features of this group is the Early Devonian microorganism *Palaeoblastocladia milleri* (Remy et al. 1994a). The fossil includes two types of thalli that are nearly identical in morphology but that differ in the types of reproductive structures they produce. One bears terminal zoosporangia and resting sporangia, whereas the other forms chains of 2–3 gametangia. Based on a complement of features like those in certain extant Blastocladiomycota and the presence of a subgenus *Euallomyces*-type life cycle [alternating diploid sporophytic and haploid gametophytic forms; *Eucladiella* type of Karling (1973)], it is hypothesized that *P. milleri* had an alternation of generations with sporic meiosis like that of some extant species of *Allomyces*. Another interesting microfungus from the Rhynie chert is *Kryphiomyces catenulatus*, which occurs as an endobiotic mycelial thallus in a glomeromycotan spore (Krings et al. 2010a). The fossil consists of catenulate hyphae and terminal spherical reproductive structures or propagules. Hyphal morphology in *K. catenulatus* is reminiscent of that in certain extant Hyphochytridiomycota, Chytridiomycota, Blastocladiomycota (i.e., *Gonapodya*), and even Ascomycota, but specific features that can be used to assign the fossil to any modern group are absent.

C. Zygomycetous Fungi

Molecular clock estimates suggest that the first zygomycetes appeared approximately 1.4–1.2 Ga (Blair 2009); more conservative estimates place the divergence at around 800 Ma (Berbee and Taylor 2001). Nevertheless, documented fossil evidence of zygomycetes is exceedingly rare. Several Precambrian microfossils have been directly compared to life history stages seen in modern zygomycetes (e.g., Hermann and Podkovyrov 2006; Stanevich et al. 2007), but none of these are conclusive. Perhaps the most persuasive pre-Mesozoic fossil interpreted as a member of the zygomycetous fungi is *Protoascon missouriensis* from the Carboniferous (Middle Pennsylvanian) of North America (Batra et al. 1964; Baxter 1975). This fungus consists of a bulblike structure with appendages arising in a whorl from one end. The appendages form a basket-like structure around an ornamented sporangium containing a single spore. Although originally thought to be an ascomycete, Taylor et al. (2005a) reinterpreted *P. missouriensis* as an azygo- or zygosporangium subtended by a suspensor forming appendages.

The record of fossil zygomycetes from the Mesozoic is equally scanty, but the few fossils that have been attributed to this group of fungi are far more informative than the Paleozoic records. The most compelling Mesozoic fossil of a zygomycetous fungus documented to date comes from the Triassic of Antarctica and has been named *Jimwhitea circumtecta* (Krings et al. 2012). This fossil (Fig. 10.7) is interpreted as a zygosporangium-apposed gametangium complex that closely resembles the zygosporangium–gametangium complexes seen in certain extant species of *Endogone*. Other fossils of zygomycetous sexual reproductive structures from the Triassic of Antarctica include several intact sporocarps containing spores, in part sheathed by a hyphal mantle, also suggested as belonging to the Endogonales (White and Taylor 1989, 1991).

C.1 So-Called Sporocarps

Some of the most puzzling microfossils in Carboniferous coal balls and cherts are spherical structures (approximately 0.1–1.5 mm in diameter) composed of a central cavity surrounded by a complex, and in some forms highly ornamented, investment (Fig. 10.8) (e.g., Hutchinson 1955; Baxter 1960; Stubblefield et al. 1983;

Stubblefield and Taylor 1983; Taylor et al. 1994); similar structures have also been found in permineralized peat from the Triassic of Antarctica (e.g., Taylor and White 1989; White and Taylor 1991). These structures, which are today commonly termed sporocarps (but see Krings et al. 2011a), may occur singly, but there are many specimens in which several individuals are clustered together. Sporocarps have been interpreted as fungal in origin based on the investment, which is constructed of interlaced hyphae that form one to several distinct layers. The number and configuration of the individual investment layers, as well as surface ornamentation, may be highly variable and therefore have traditionally been used to distinguish between different sporocarp morphotypes, such as *Dubiocarpon*, *Mycocarpon*, *Sporocarpon*, and *Traquairia* (Taylor et al. 2009).

Many sporocarps contain one to several spherical structures in the cavity, which have led to their interpretation as ascomycete cleistothecia (e.g., Stubblefield and Taylor 1983). In this hypothesis, the larger internal spherical structures would represent asci and the smaller ones, ascospores. An alternative interpretation, however, views the sporocarps as belonging to the zygomycetous fungi (Taylor and White 1989; White and Taylor 1989). The large, inner sporelike body is thought to represent the zygospore, while the surrounding structure would be equivalent to the hyphal envelope or mantle seen in certain modern Endogonales. The smaller internal spheres reported in some specimens are regarded as mycoparasites. Although there is an increasing body of circumstantial evidence to corroborate the hypothesis that at least some of the so-called sporocarps represent zygomycetous reproductive structures (Krings et al. 2010b, 2011b, c), structural features confirming the zygomycetous affinity of these interesting fossils have not been documented to date.

D. Glomeromycota

This monophyletic group of fungi represents one of the major drivers in modern ecosystems due to its role in global phosphorous and carbon cycles. This function is accomplished in approximately 80 % of land plants by means of a mutualistic symbiosis in the form of arbuscular mycorrhizae (Schüßler and Walker 2011). **The Glomeromycota can be traced back at least to the Early Devonian, where there is strong evidence of a mycorrhizal system in place in both the free-living gametophyte and sporophyte phases of several early land plants** (Remy et al. 1994b; Taylor et al. 1995, 2005b). There have been putative reports of glomeromycetes based on spores dating to the Precambrian (Pirozynski and Dalpé 1989) and Ordovician (Redecker et al. 2000, 2002); the latter report includes some hyphae. None of these reports, however, provides evidence of symbiotic associations, and there is concern that at least some of the alleged fossils may represent modern contaminants. Geologically younger spores showing a suite of features like those of modern Glomeromycota are common beginning in the Lower Devonian Rhynie chert (Kidston and Lang 1921b), slightly younger Devonian rocks (Stubblefield and Banks 1983), and into the Carboniferous (e.g., Wagner and Taylor 1981, 1982; Krings et al. 2011d), where they occur in both the matrix and within the tissues of various plants. Mesozoic records of Glomeromycota include specimens found in the fossilized dung of herbivorous dinosaurs (Kar et al. 2004; Sharma et al. 2005).

Some of the glomeromycotan spores from the Lower Devonian Rhynie chert provide details about certain features that are useful in identifying the mode of spore germination, but they also provide characters that are useful in determining their systematic affinities. Spores found in axes of *Asteroxylon mackiei* range up to 350 μm in diameter and possess a wall that consists of several distinct layers when examined in transmitted light. Associated with one spore wall layer is a round to oval, lobed structure that represents a germination shield, like those found in the modern genus *Scutellospora* (Fig. 10.9) (Dotzler et al. 2006). In modern forms, the germination shield produces one to several germ tubes at maturity. Other large spores from the Rhynie chert possess very complex walls and are comparable to those found in modern members of the Acaulosporaceae in which the spore develops laterally in the neck of a sporiferous saccule (Fig. 10.10) (Dotzler et al. 2008). These two studies demonstrate that even isolated spores can provide a wealth of information not only about functional aspects of the spores but also about the presence of structural and morphological details useful in more clearly defining the systematic affinities of the spores, which can be used as markers for minimal age dating in association with molecular data sets.

Although the presence of variously sized spores, some with structural features like those of modern Glomeromycota, confirms the presence of the group in the Paleozoic, the most convincing evidence of a biotrophic relationship with land plants is the presence of

arbuscules. The first unequivocal occurrence of these comes from both the sporophyte and gametophyte phases of the land plant *Aglaophyton major* (gametophyte = *Lyonophyton rhyniensis*) from the Lower Devonian Rhynie chert. The fungus occurs in a narrow circumferential zone of cortical cells 2–3 layers thick and consists of aseptate hyphae that give rise to intracellular branchlike structures that morphologically are identical to the modern physiological exchange structures in many extant plants. Arbuscule-like structures have also been reported in other Paleozoic and Mesozoic plant roots (Phipps and Taylor 1996; Strullu-Derrien et al. 2009; Schwendemann et al. 2011), as well as in structures that functioned as rooting organs (Fig. 10.11) (Krings et al. 2011d). While there are older land plants from the Silurian, to date none have been found that are sufficiently well preserved to test the hypothesis advanced by Pirozynski and Malloch (1975) that the earliest land plants were preadapted to living in a terrestrial ecosystem because of their biotrophic symbiotic relationship with a fungal partner.

E. Basidiomycota

Surprisingly, the fossil record of basidiomycetes is relatively poor, although today they are widespread and represent the primary decay agents of cellulose and lignin. Even the Lower Devonian Rhynie chert, which contains several of the major groups of fungi (i.e., Chytridiomycota, Blastocladiomycota, Glomeromycota, Ascomycota), lacks any evidence of fungi suggestive of being basidiomycetes. The lack of fossils of this group has led to the proposal of two possible reasons that basidiomycetous fungi were not present in this ecosystem: there was an absence of lignin in the Early Devonian or degradation was accomplished by other fungi. Like so many fossil groups, the failure to find features like those in extant groups provides an immediate obstacle to interpreting the affinities of organisms that may have initially evolved a different set of morphological or reproductive characters but nevertheless still possess the appropriate enzyme systems for degradation. It is also possible, as is often the case in paleomycology, that the group has simply not been discovered to date. There is some support for this latter suggestion by the Middle Devonian, where there are several examples of basidiomycete disease symptoms in the wood of the progymnosperm *Callixylon newberryi* (Stubblefield et al. 1985). Within the decaying wood are branched, septate hyphae, some of which contain both intercalary and terminal spores. No clamp connections were found, but the secondary xylem tracheids, decayed to varying degrees, show erosion troughs, cavities, and extensive lysis of the walls, similar to symptoms caused by modern-day white-rot fungi. Another indirect source of basidiomycete activity in the fossil record is patterns of secondary xylem decay in some Late Permian and Middle Triassic gymnosperm woods from Antarctica. The most conspicuous of these occurs in the form of circular areas (pockets) that range up to several millimeters in diameter and several centimeters long that are completely devoid of cells. In the adjacent wood are hyphae with clamp connections (Stubblefield and Taylor 1986). In addition, these woods show degradation of the cell walls in a sequential pattern, symptoms that are anatomically identical to white rot and white pocket rot in several extant woody plants.

To date, the oldest fossil evidence of a clamp connection comes from the Middle Mississippian (Visean) of central France (Krings et al. 2010c). The fungus occurs in the cortical tissues of a small fern and consists of clamp-bearing hyphae (Fig. 10.12) morphologically identical to those produced by many extant basidiomycetes, as well as intercalary and terminal swellings and structures resembling chlamydospores. The presence of callosities in some host cells suggests that the plant was alive at the time the fungus invaded the tissues. Other reports of late Paleozoic clamp-bearing hyphae, interestingly also associated with ferns, include *Palaeancistrus martinii* from the Upper Pennsylvanian of North America (Dennis 1970) and an unnamed form that thrives in the root mantle of *Psaronius* tree ferns from the Lower Permian of Germany (Barthel et al. 2010).

One basidiomycete group that has a more extensive fossil record are the polypores (surveyed in Fleischmann et al. 2007). Although there have been some reports of polypore basidiocarps from the Paleozoic and early Mesozoic, many of these reports have since been discounted or reinterpreted as other organisms (e.g., Pirozynski 1976; Hibbett et al. 1997b). In the Cretaceous and Cenozoic there are far more reports of polyporous fungi, and many of these can be closely related to modern genera. The oldest bona fide record of polypores comes from the Cretaceous of North America (Smith et al. 2004). Perhaps the most well-preserved fossil polyporous basidiocarp is *Ganodermites libycus* from the Neogene of North Africa (Fig. 10.13) (Fleischmann et al. 2007). This permineralized basidiocarp is stratified and shows pronounced growth increments. The hymenium has equidistantly arranged pores that contain clavate basidia and ellipsoidal basidiospores, each with a two-layered ganodermatoid wall. The extraordinary preservation of certain features in the fossil, especially the unique basidiospores, makes it possible to place the fossil within the modern Ganodermataceae.

F. Ascomycota

While the Ascomycota today constitute the largest group of fungi, the fossil record does not demonstrate this level of diversity. To some extent, there has been an inherent bias based on the long-held assumption that the group did not evolve until the Cretaceous (Pirozynski and Weresub 1979). Today, however, there are scattered reports of ascomycete fossils dating back to the middle Silurian of Sweden that have yielded hyphae and spores. The most often cited of these consists of chains of up to nine multiseptate spores as well as conidiogenous cells (phialides) (Fig. 10.14) (Sherwood-Pike and Gray 1985). Because these fossils were obtained in rock macerations, nothing is known about other stages of the life history or the relationship with other organisms. Slightly younger (Early Devonian) specimens from Siberia attributed to the Ascomycota include asci and structures interpreted as paraphyses that are regarded as evidence of the Microthyriales (Krassilov 1981).

To date, the oldest structurally preserved fossil attributed to the Ascomycota is *Paleopyrenomycites devonicus*, from the Lower Devonian Rhynie chert (Taylor et al. 2005c). This fossil is preserved in the flattened appendages of the land plant *Asteroxylon mackiei* and consists of perithecia approximately 400 μm in diameter. Each perithecium contains a short, ostiolate neck; within the perithecium are numerous asci intermixed with paraphyses. Each ascus contains up to 16 uniseriate or biseriate ascospores. What makes this fossil even more interesting is the presence of acervuli of thallic conidiophores producing cube-shaped arthrospores interspersed among the perithecia.

Mesozoic and Cenozoic rocks contain several excellent examples of epiphyllous fungi assigned to the Ascomycota. While most of these are associated with the leaves of angiosperms (e.g., Dilcher 1965; Smith 1980; Phipps and Rember 2004), a few have been reported on, or in association with, conifers and vascular cryptogams (e.g., Pons and Boureau 1977; Van der Ham and Dortangs 2005; Shi et al. 2010; García Massini et al. 2012). Most of these forms consist of isolated reproductive structures such as thyrothecia and pycnidia. Others consist of pseudoparenchymatous hyphae that radiate from ascomata. Several epiphyllous ascomycetes contain well-preserved hyphopodia that can be used to trace the life-history biology of the fungus. A wealth of information remains to be obtained about the nutritional mode of fossil epiphyllous ascomycetes and whether they represent generalist or specific types of parasites that can be correlated with distinct species of leaf fossils. Systematic data on fossil epiphyllous fungi may also be useful as another proxy record of various climate parameters through geologic time and space.

From Cenozoic rocks there are also several reports of excellent examples of fossil ascomycetes involved in various types of intricate interactions with other organisms. For example, from lower Eocene amber collected in India, Beimforde et al. (2011) reported on ectomycorrhizal fungi that compare closely to an extant member of the Dothidiomycetes. Other Eocene ascomycetes reported from permineralized specimens include mycoparasites (Currah et al. 1998) and a single loculoascomycetous ascoma (Mindell et al. 2007). An especially interesting case of fungal parasitism from the Eocene documents adaptive manipulation of

ants by a parasitic ascomycete in the form of stereotypical death grip scars preserved in angiosperm leaves from the famous Messel pit in northern Germany (Hughes et al. 2011).

VI. Lichens

It is surprising that the fossil record is not replete with reports of lichens, because the structure of the thallus in many forms would seem to have high preservational potential. Some reasons for the lack of fossil lichens may be the absence of a focus on these organisms, especially those that might have occurred early in the terrestrialization of the Earth, or the failure to recognize examples of these symbioses in the fossil record. The enigmatic Early Devonian fossil *Spongiophyton*, historically believed to represent a nonvascular plant form transitional between algae and land plants (Gensel et al. 1991), was interpreted as a lichen based on structural features seen in permineralized specimens (Stein et al. 1993). This interpretation has been supported by evidence from carbon-isotope ratios [Jahren et al. 2003; but see Fletcher et al. (2004) for arguments against] and from ultrastructural analyses (Taylor et al. 2004b). There have been a few reports of lichens from the Precambrian to the Ordovician (e.g., Hallbauer and van Warmelo 1974; Retallack 1994, 2009), but most of these have now been discounted (Cloud 1976; Waggoner 1995) or remain inconclusive. A structurally preserved Precambrian fossil that is morphologically similar to a lichen comes from the Ediacaran (Neoproterozoic) upper Doushantuo Formation of South China (Yuan et al. 2005). This structure, sometimes termed a biodictyon, consists of clusters of coccoid cells encased within a netlike arrangement of hyphae. It is not currently possible to demonstrate the physiological interactions of the bionts in this association, the fact that this Precambrian fossil shows a consistent, physical relationship of coccoid cells, and the enclosing netlike mycelium provides support for the hypothesis that this fossil is indeed a lichen or lichenlike association.

Another Proterozoic fossil with a netlike organization that has been interpreted to represent some level of fungal organization is *Tappania* (Butterfield 2005; Nagovitsin 2009), an organism previously described as an acritarch, a group of enigmatic fossil aquatic eukaryotes. In this fossil (Fig. 10.15), a series of filamentous processes with cross walls forms a series of anastomoses surrounding a central vesicle. This multicellular level of organization has been used to suggest that *Tappania* and a number of other acritarchs represent putative fungi that fall somewhere between Ascomycota and zygomycetous fungi. Establishing the biological affinities of these organisms, including whether they are in fact fungal, however, will require more definitive evidence.

The direct physical association between fungal hyphae and coccoid cells within a three-dimensional netlike organization is also present in the Early Devonian thallus *Winfrenatia reticulata* (Taylor et al. 1997; Karatygin et al. 2009). The thallus is constructed of two distinct zones, a lower one of superimposed layers of parallel hyphae and an upper zone of vertically oriented hyphae that are folded into loops so as to create a series of ridges and depressions. Extending into the depressions are aseptate hyphae arranged in a loosely organized netlike structure (Fig. 10.16). Within the lacunae of the net are unicells up to 16 μm in diameter, each surrounded by a thin sheath. Cells at the base of the depression are generally small and solitary, while those that are more distal show increasing divisions resulting in aggregations of up to 32 cells. The unicells of the photobiont in *W. reticulata* show many similarities to certain cyanobacteria, but the absence of diagnostic features of the hyphae means that the affinities of the mycobiont in *W. reticulata* remain unknown. Nevertheless, the structural organization displayed in this fossil, like the geologically earlier biodictyon in the Neoproterozoic, suggests that there was physiological interaction between the two components, as is seen in modern cyanobiont lichens.

There have been several reports of Cenozoic lichen thalli preserved as impressions and compressions in rocks (e.g., Peterson 2000) and amber (Rikkinen and Poinar 2008 and citations therein). An older specimen from the Middle Jurassic of northeastern China possesses

numerous features that suggest an affinity with the lichens (Wang et al. 2010). The thallus of *Daohugouthallus ciliiferus* consists of a series of elongate primary axes from which extend lateral and terminal branches that repeatedly dichotomize. Extending from all branches are elongate structures that appear similar to cilia in modern lichens.

Like the partners involved in mycorrhizae, the physiological relationships within a lichen symbiosis based on fossils is currently impossible to prove unequivocally. Nevertheless, the fact that lichens are repeatedly regarded as ancient organisms based on sequence data (e.g., Lutzoni et al. 2001), together with the scattered reports of fossils, indicates that the fossil record holds a potentially rich source of information not only about the character states present in ancient lichens, but also about the stages involved in the evolution of lichenization.

VI. Enigmatic Fossils

There are numerous reports of fossil organisms that possess many of the characters of fungi, and a number of these are known in great detail with regard to morphology or anatomy; however, for many of these, systematic assignment to a specific group remains problematic. The problem in deciphering the affinities or nutritional modes of these fossils has been due to either the presence of distinguishing features, for example, clamp connections and basidia, or, conversely, the absence of particular characteristics.

A. Uncertain Affinities

Most prominent among the enigmatic fossils that have variously been attributed to the fungi is the late Silurian–Early Devonian organism *Prototaxites*. Despite the fact that some *Prototaxites* specimens are large (some >1 m in diameter) and anatomically preserved, the affinities of this organism remain conjectural. The most comprehensive study involving multiple specimens suggests that this fossil may have had affinities with some basidiomycete group (Hueber 2001); its heterotrophic nutritional mode is also supported by isotopic analysis (Boyce et al. 2007; Hobbie and Boyce 2010). *Prototaxites* is constructed of three types of septate and nonseptate tubes or hyphae, and in certain sections of the axes, there are what are interpreted as growth increments of the sporophore. Doliporelike septal structures have been reported, but they have not been thoroughly investigated in multiple specimens or in different planes of a section (Schmid 1976). A few structures are interpreted as clamps and sterigmata, but these remain equivocal.

Another enigmatic organism that has been related to *Prototaxites* is *Nematasketum* (Burgess and Edwards 1988). It also consists of axially oriented tubes; the larger ones often branch and have irregular thickenings on the internal wall, while the internal surface of the narrower tube surface is sometimes smooth. On the outside of *Nematasketum* are tissues interpreted as a rind (Edwards and Axe 2012). In regions interpreted as sites of hyphal generation, *Nematasketum* exhibits differentially thickened branching tubes, whereas the hyphae are smooth in *Prototaxites*. Structures such as clamp connections, basidia, or septa have not been identified in any specimens to date. Although *Nematasketum* is well preserved as a charcoalification and has been examined by SEM, the affinities of this organism are still equivocal. *Prototaxites* and *Nematasketum*, as well as some other enigmatic thalloid organisms from the Silurian and Devonian known as nematophytes, may represent fungi, some type of symbiotic association such as lichens (Selosse 2002), or life forms that have no modern analogues. At present, these interesting fossils continue to defy attempts to place them within a modern systematic framework (Taylor et al. 2010).

Another fossil structure that possesses a number of fungal features is the Carboniferous taxon *Palaeosclerotium pusillum* (Rothwell 1972; Dennis 1976). This fossil appears to possess features of two major clades on modern fungi. There are hyphae with what appear to be doliporelike septa that have clamp connections like those in modern basidiomycetes, but *P. pusillum* also has cleistothecia, perhaps with asci, which are characteristics of the ascomycetes. It is not yet resolved whether this fossil

represents an example of mycoparasitism or whether it may be a fungus that shares multiple characters used to define modern groups (McLaughlin 1976; Singer 1977).

B. Plant–Fungal Associations with Uncertain Functions

There are a large number of fungi that colonize roots today whose particular functions remain unknown. A fungus that occurs in the roots of a Late Pennsylvanian calamite (*Astromyelon*) presents a set of interesting questions with regard to its affinities, function, and nutritional mode (Taylor et al. 2012). This fungus, *Cashhickia acuminata*, consists of aseptate hyphae that penetrate the outer periclinal and anticlinal (but not the inner periclinal) walls of the root cortical cells and extend approximately halfway through each cell lumen (Fig. 10.17). Some of the hyphae branch at right angles, and the tips are sharply pointed. While the presence of callosities associated with some of the infected cells suggests that the root was alive at the time of infection, the affinities of the fungus and its nutritional mode remain unknown. The presence of the pointed, aseptate hyphae that only penetrate from one side of the cell and extend only about halfway into the lumen may suggest that hyphal growth was perhaps influenced by some nutritional gradient. In general, these fossil hyphae appear similar to dark septate hyphae (DSE), modern root endophytic hyphae that represent some conidial forms whose function and affinities remain poorly understood (Mandyam and Jumpponen 2005).

Historically fossil roots have generally not been a major focus of terrestrial plant paleobotany, even in those instances in which they were permineralized and showed extraordinary cell and tissue detail. In recent years, however, they have been examined in greater detail because they can serve as host to mycorrhizal fungi and thus provide necessary information about patterns in mycorrhizal development through time (e.g., Strullu-Derrien et al. 2009; Krings et al. 2011d). Important work still to be done in such studies includes a comprehensive analysis of mycorrhizal relationships in extant plants based on structural (Brundrett 2009), molecular (Wang and Qiu 2006), and functional aspects (e.g., Bidartondo 2005). Examining the actual type of physiological exchange mechanism (e.g., Strullu-Derrien and Strullu 2007) will also be an important component of future studies involving the fossil record of mycorrhizae.

Despite the incredible amount of leaf biomass in the Carboniferous, **it is surprising that there are so few reports of fungi associated with leaves**. Since these leaves were produced by ferns, other vascular cryptogams, and gymnosperms (pteridosperms and cordaites), it has long been hypothesized that perhaps the production of secondary metabolites by these plants restricted endophyte colonization. However, as is apparently the case with other fossil fungi and the habitats they occupied, once recognized, fungi associated with leaves are rather common. For example, a small fern leaf from the Upper Pennsylvanian of France contains septate hyphae, some with prominent swellings, extending through the hypodermis of the leaf (Krings et al. 2009d). Because there is no observable host response, it is not possible to determine whether the host was alive at the time of infection or in some stage of decay, thus suggesting the fungus was some type of saprotroph.

A number of fungal remains that have recently been described from Carboniferous leaf tissue consist of diverse assemblages of propagules in multiple leaf specimens of the same plant (Fig. 10.18) (e.g., Dotzler et al. 2011; Krings et al. 2011c). These discoveries suggest that certain fungi were more widely distributed in these tropical Paleozoic habitats, while others appear to have had a rather limited host range. These differences inevitably raise the question of whether this situation reflects generalists and specialists among the fungi or is due merely to a current lack of resolution. Leaf endophytes today represent a significant percentage of overall fungal biodiversity, especially in tropical habitats. We believe that their current underrepresentation as fossils merely reflects a lack of attention to these fungi in the fossil record rather than some inherent biological control in the past. An important contribution to paleomycology in the future will be to look at early leaves and leaflike organs to determine when the phylloplane was first used as a fungal habitat.

VI. Conclusions

Paleomycology has been a relatively neglected area in mycology. The reasons for this lack of attention include an under appreciation of the diversity of forms in the fossil record, preservational and depositional biases, previous use of ineffective preparation techniques, and organism bias from multiple disciplines. Despite these limitations, the fossil record contains a wealth of information about fungi that cannot be obtained from any other source and that is an essential component in understanding the evolution of fungi and funguslike organisms in time and space. It has become increasingly evident that the fossil record of all major fungal lineages is, in fact, rather extensive and thus offers multiple opportunities to examine fungal biodiversity in deep time, interactions with other organisms in ancient ecosystems, and a range of nutritional modes and host reactions that are preserved in fossil plant tissues. Multiple approaches and avenues of investigation require an understanding of the fossil record of fungi. We believe that the solution to many important fungal evolutionary questions will be answered when the fossil record of these organisms is fully integrated with modern phylogenies and with the record of the organisms with which the fungi have interacted throughout geologic time. For a more in depth treatment of the field of paleomycology see *Fossil Fungi* (Taylor et al. 2015).

References

Andrews HN Jr (1980) The fossil hunters: in search of ancient plants. Cornell University Press, Ithaca, NY

Barthel M, Krings M, Rössler R (2010) Die schwarzen Psaronien von Manebach, ihre Epiphyten, Parasiten und Pilze. Semana 25:41–60

Batra LR, Segal H, Baxter RW (1964) A new Middle Pennsylvanian fossil fungus. Am J Bot 51:991–995

Baxter RW (1960) *Sporocarpon* and allied genera from the American Pennsylvanian. Phytomorph 10:19–25

Baxter RW (1975) Fossil fungi from American Pennsylvanian coal balls. Univ Kansas Paleontol Contrib 77:1–6

Belova MY, Akhmedov AM (2006) *Petsamomyces*, a new genus of organic-walled microfossils from the coal-bearing deposits of the Early Proterozoic, Kola Peninsula. Paleontol J 40:465–475

Beimforde C, Schäfer N, Dörfelt H, Nascimbene PC, Singh H, Heinrichs J, Reitner J, Rana RS, Schmidt AR (2011) Ectomycorrhizas from a lower Eocene angiosperm forest. New Phytol 192:988–996

Berbee LM, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota VIIB. Systematics and evolution. Springer, Berlin, pp 229–245

Bidartondo MI (2005) The evolutionary ecology of myco-heterotrophy. New Phytol 167:335–352

Blair JE (2009) Fungi. In: Hedges SB, Kumar S (eds) The timetree of life. Oxford University Press, New York, pp 215–219

Boullard B, Lemoigne Y (1971) Les champignons endophytes du *Rhynia gwynne-vaughanii* K. & L. Étude morphologique et deductions sur leur biologie. Botaniste 54:49–89

Boyce CK, Hotton CL, Fogel ML, Cody GD, Hazen RM, Knoll AH, Hueber FM (2007) Devonian landscape heterogeneity recorded by a giant fungus. Geology 35:399–402

Bradley WH (1967) Two aquatic fungi (Chytridiales) of Eocene age from the Green River Formation of Wyoming. Am J Bot 54:577–582

Bray PS, Anderson KB (2009) Identification of Carboniferous (320 million years old) class IC amber. Science 326:132–134

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Burgess ND, Edwards D (1988) A new Palaeozoic plant closely allied to *Prototaxites* Dawson. Bot J Linn Soc 97:189–303

Butterfield NJ (2005) Probable proterozoic fungi. Paleobiology 31:165–182

Cantrell SA, Dianese JC, Fell J, Gunde-Cimerman N, Zalar P (2011) Unusual fungal niches. Mycologia 103:1161–1174

Cash W, Hick T (1879) On fossil fungi from the lower coal-measures of Halifax. Proc Yorkshire Geol Polytech Soc 7:115–121

Caspary R, Klebs R (1907) Die Flora des Bernsteins und anderer fossiler Harze des ostpreußischen Tertiärs. Abh Königl Preuß Geol Landesanst 4:1–181

Cloud P (1976) Beginnings of biospheric evolution and their biogeochemical consequences. Paleobiology 2:351–387

Currah RS, Stockey RA, LePage BA (1998) An Eocene tar spot on a fossil palm and its fungal hyperparasite. Mycologia 90:667–673

Daghlian CP (1978) A new melioloid fungus from the Early Eocene of Texas. Palaeontology 21:171–176

Daugherty LH (1941) The Upper Triassic Flora of Arizona. Carnegie Inst Washington 526:1–108

Dennis RL (1970) A middle Pennsylvanian basidiomycete mycelium with clamp connections. Mycologia 62:578–584

Dennis RL (1976) *Palaeosclerotium*, a Pennsylvanian age fungus combining features of modern Ascomycetes and Basidiomycetes. Science 192:66–68

Dilcher DL (1965) Epiphyllous fungi from Eocene deposits in western Tennessee, USA. Palaeontographica 116B:1–54

Ding ST, Sun BN, Wu JY, Li XC (2011) Miocene *Smilax* leaves and associated epiphyllous fungi from Zhejiang, East China and their paleoecological implications. Rev Palaeobot Palynol 165:209–223

Dörfelt H, Schmidt AR (2005) A fossil *Aspergillus* from Baltic amber. Mycol Res 109:956–960

Dotzler N, Krings M, Taylor TN, Agerer R (2006) Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert. Mycol Progr 5:178–184

Dotzler N, Walker C, Krings M, Hass H, Kerp H, Taylor TN, Agerer R (2008) Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. Mycol Prog 8:9–18

Dotzler N, Taylor TN, Galtier J, Krings M (2011) *Sphenophyllum* (Sphenophyllales) leaves colonized by fungi from the Upper Pennsylvanian Grand-Croix cherts of central France. Zitteliana A 51:3–8

Dufrêne YF, Boonaert CJP, Gerin PA, Asther M, Rouxhet PG (1999) Direct probing of the surface ultrastructure and molecular interactions of dormant and germinating spores of *Phanerochaete chrysosporium*. J Bacteriol 181:5350–5354

Edwards D, Axe L (2012) Evidence for a fungal affinity for *Nematasketum*, a close ally of *Prototaxites*. Bot J Linn Soc 168:1–18

Elsik WC (1996) Fungi. In: Jansonius J, McGregor DC (eds) Palynology: principles and applications, volume 1 – principles. AASP Foundation, College Station, Texas, pp 293–305

Eshet Y, Rampino MR, Visscher H (1995) Fungal event and palynological record of ecological crisis and recovery across the Permian–Triassic boundary. Geology 23:967–970

Fleischmann A, Krings M, Mayr H, Agerer R (2007) Structurally preserved polypores from the Neogene of North Africa: *Ganodermites libycus* gen. et sp. nov. (Polyporales, Ganodermataceae). Rev Palaeobot Palynol 145:159–172

Fletcher BJ, Beerling DJ, Chaloner WG (2004) Stable carbon isotopes and the metabolism of the terrestrial Devonian organism *Spongiophyton*. Geobiology 2:107–119

Gadd GM (2008) Bacterial and fungal geomicrobiology: a problem with communities. Geobiology 6:278–284

Galtier J, Phillips TL (1999) The acetate peel technique. In: Jones TP, Rowe NP (eds) Fossil plants and spores: modern techniques. The Geological Society, London, pp 67–70

García Massini JL (2007) A possible endoparasitic chytridiomycete fungus from the Permian of Antarctica. Palaeontol Electron 103:16, 14 p, http://palaeo-electronica.org/2007_3/121/index.html

García Massini J, Channing A, Guido DM, Zamuner AB (2012) First report of fungi and fungus-like organisms from Mesozoic hot springs. Palaios 27:55–62

Gensel PG, Chaloner WG, Forbes WH (1991) *Spongiophyton* from the late lower Devonian of New Brunswick and Quebec, Canada. Palaeontology 34:149–168

Goeppert HR, Berendt GC (1845) Der Bernstein und die in ihm befindlichen Pflanzenreste der Vorwelt. Nicolaische Buchhandlung, Berlin, 125 pp

Graham A (1962) The role of fungal spores in palynology. J Paleontol 36:60–68

Hallbauer DK, van Warmelo KT (1974) Fossilized plants in thucholite from Precambrian rocks of the Witwatersrand, South Africa. Precambrian Res 1:199–212

Hass H, Taylor TN, Remy W (1994) Fungi from the Lower Devonian Rhynie chert: mycoparasitism. Am J Bot 81:29–37

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129–1133

Hermann TN, Podkovyrov VN (2006) Fungal remains from the late Riphean. Paleontol J 40:207–214

Hibbett DS, Grimaldi DA, Donoghue MJ (1995) Cretaceous mushrooms in amber. Nature 377:487

Hibbett DS, Grimaldi DA, Donoghue MJ (1997a) Fossil mushrooms from Miocene and Cretaceous ambers and the evolution of homobasidiomycetes. Am J Bot 84:981–991

Hibbett DS, Donoghue MJ, Tomlinson PB (1997b) Is *Phellinites digiustoi* the oldest homobasidiomycete? Am J Bot 84:1005–1011

Hibbett DS, Binder M, Wang Z, Goldman Y (2003) Another fossil agaric from Dominican amber. Mycologia 95:685–687

Hobbie EA, Boyce CK (2010) Carbon sources for the Palaeozoic giant fungus *Prototaxites* inferred from modern analogues. Proc R Soc B Biol Sci 277:2149–2156

Hower JC, O'Keefe JMK, Watt MA, Pratt TJ, Eble CF, Stucker JD, Richardson AR, Kostova IJ (2009) Notes on the origin of inertinite macerals in coals: observations on the importance of fungi in the origin of macrinite. Int J Coal Geol 80:135–143

Hueber FM (2001) Rotted wood-alga-fungus: the history and life of *Prototaxites* Dawson 1859. Rev Palaeobot Palynol 116:123–159

Hughes DP, Wappler T, Labandeira CC (2011) Ancient death-grip leaf scars reveal ant-fungal parasitism. Biol Lett 7:67–70

Hutchinson SA (1955) A review of the genus *Sporocarpon* Williamson. Ann Bot 19:425–435

Illman WI (1984) Zoosporic fungal bodies in the spores of the Devonian fossil vascular plant *Horneophyton*. Mycologia 76:545–547

Jahren AH, Porter S, Kuglitsch JJ (2003) Lichen metabolism identified in Early Devonian terrestrial organisms. Geology 31:99–102

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822

Joy WK, Willis AJ, Lacey WS (1956) A rapid cellulose peel technique in paleobotany. Ann Bot 20:635–637

Kalgutkar RM, Jansonius J (2000) Synopsis of fossil fungal spores, mycelia, and fructifications. Palynol Soc Contrib Ser 39:1–429

Kar RK, Sharma N, Kar R (2004) Occurrence of fossil fungi in dinosaur dung and its implication on food habit. Curr Sci 87:1053–1056

Karatygin V, Snigirevskaya NS, Vikulin SV (2009) The most ancient terrestrial lichen *Winfrenatia reticulata*: a new find and new interpretation. Paleontol J 43:107–114

Karling JS (1928) Studies in the Chytridiales III. A parasitic chytrid causing cell hypertrophy in *Chara*. Am J Bot 15:485–496

Karling JS (1973) A note on *Blastocladiella* (Blastocladiaceae). Mycopath Mycol Appl 49:169–172

Kidston R, Lang WH (1917) On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part I. *Rhynia gwynne-vaughani*, Kidston and Lang. Trans R Soc Edinb 51(Part 3(24)):763–784

Kidston R, Lang WH (1920a) On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part II. Additional notes on *Rhynia gwynne-vaughani*, Kidston and Lang; with descriptions of *Rhynia major*, n.sp., and *Hornea lignieri*, n.g. n.sp. Trans R Soc Edinb 52(Part 3 (24)):603–627

Kidston R, Lang WH (1920b) On Old Red Sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part III. *Asteroxylon mackiei*, Kidston and Lang. Trans R Soc Edinb 52(Part 3 (26)):643–680

Kidston R, Lang WH (1921a) On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part IV. Restorations of the vascular cryptogams, and discussion of their bearing on the general morphology of the Pteridophyta and the origin of the organisation of land-plants. Trans R Soc Edinb 52:831–854

Kidston R, Lang WH (1921b) On Old Red Sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part V. The Thallophyta occurring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit. Trans R Soc Edinb 52:855–902

Krassilov VA (1981) *Orestovia* and the origin of land plants. Lethaia 14:235–250

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007a) Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host response. New Phytol 174:648–657

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007b) An alternative mode of early land plant colonization by putative endomycorrhizal fungi. Plant Signal Behav 2:125–126

Krings M, Dotzler N, Taylor TN (2009a) *Globicultrix nugax* nov. gen. et spec. (Chytridiomycota), an intrusive microfungus in fungal spores from the Rhynie chert. Zitteliana A 48(49):165–170

Krings M, Dotzler N, Galtier J, Taylor TN (2009b) Microfungi from the upper Visean (Mississippian) of central France: Chytridiomycota and chytrid-like remains of uncertain affinity. Rev Palaeobot Palynol 156:319–328

Krings M, Galtier J, Taylor TN, Dotzler N (2009c) Chytrid-like microfungi in *Biscalitheca* cf. *musata* (Zygopteridales) from the Upper Pennsylvanian Grand-Croix cherts (Saint-Etienne Basin, France). Rev Palaeobot Palynol 157:309–316

Krings M, Dotzler N, Taylor TN, Galtier J (2009d) A Late Pennsylvanian fungal leaf endophyte from Grand-Croix, France. Rev Palaeobot Palynol 156:449–453

Krings M, Dotzler N, Longcore JE, Taylor TN (2010a) An unusual microfungus in a fungal spore from the Lower Devonian Rhynie chert. Palaeontol 53:753–759

Krings M, Dotzler N, Taylor TN, Galtier J (2010b) Microfungi from the upper Visean (Mississippian) of central France: structure and development of

the sporocarp *Mycocarpon cinctum* nov. sp. Zitteliana A 50:127–135

Krings M, Dotzler N, Galtier J, Taylor TN (2010c) Oldest fossil basidiomycete clamp connections. Mycoscience 52:18–23

Krings M, Taylor TN, White JF (2011a) Fungal sporocarps from the Carboniferous: an unusual specimen of *Traquairia*. Rev Palaeobot Palynol 168:1–6

Krings M, Dotzler N, Taylor TN (2011b) Mycoparasitism in *Dubiocarpon*, a fungal sporocarp from the Carboniferous. Neues Jahrb Geol Paläontol 262:241–245

Krings M, Taylor TN, Dotzler N, Galtier J (2011c) Fungal remains in cordaite (Cordaitales) leaves from the Upper Pennsylvanian of central France. Bull Geosci 86:777–784

Krings M, Taylor TN, Taylor EL, Dotzler N, Walker C (2011d) Arbuscular mycorrhizal-like fungi in Carboniferous arborescent lycopsids. New Phytol 191:311–314

Krings M, Taylor TN, Dotzler N, Persichini G (2012) Fossil fungi with suggested affinities to the Endogonaceae from the Middle Triassic of Antarctica. Mycologia 104:835–844

Langenheim JH (2003) Plant resins – chemistry, evolution, ecology, ethnobotany. Timber Press, Portland, OR

Lesquereux L (1877) A species of fungus recently discovered in the shales of the Darlington Coal Bed (Lower Productive Coal Measures, Alleghany River Series) at Cannelton, in Beaver County, Pennsylvania. Proc Am Philos Soc 17 (100):173–175

Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonizing dark septate endophytic fungi. Stud Mycol 53:173–189

McLaughlin DJ (1976) On *Palaeosclerotium* as a link between ascomycetes and basidiomycetes. Science 193:602

Meschinelli A (1898) Fungorum fossilium omnium, hucusque cognitorum, iconographia: Volumen unicum. Typis Aloysii Fabris and C, Venice

Millay MA, Taylor TN (1978) Chytrid-like fossils of Pennsylvanian age. Science 200:1147–1149

Mims CW, Richardson EA, Clay RP, Nicholson RL (1995) Ultrastructure of conidia and the conidium aging process in the plant pathogenic fungus *Colletotrichum gramnicola*. Int J Plant Sci 156:9–18

Mindell RA, Stockey RA, Beard G, Currah RS (2007) *Margaretbarromyces dictyosporus* gen. sp. nov.: a permineralized corticolous ascomycete from the Eocene of Vancouver Island, British Columbia. Mycol Res 111:680–684

Nagovitsin K (2009) *Tappania*-bearing association of the Siberian platform: biodiversity, stratigrahic position and geochronological constraints. Precambrian Res 173:137–145

Oliver FW (1903) Notes on fossil fungi. New Phytol 3:49–53

Peterson EB (2000) A overlooked fossil lichen (Lobariaceae). Lichenologist 32:298–300

Phillips TL, Avcin MJ, Berggren DE (1976) Fossil peat from the Illinois Basin. A guide to the study of coal-balls of Pennsylvanian age. Ill St Geol Educ Ser 11:1–39

Phipps CJ (2006) *Entopeltacites remberi* sp. nov. from the Miocene of Clarkia, Idaho, USA. Rev Palaeobot Palynol 145:193–200

Phipps CJ, Taylor TN (1996) Mixed arbuscular mycorrhizae from the Triassic of Antarctica. Mycologia 88:707–714

Phipps CJ, Rember WC (2004) Epiphyllous fungi from the Miocene of Clarkia, Idaho: reproductive structures. Rev Palaeobot Palynol 129:67–79

Pirozynski KA (1976) Fossil fungi. Annu Rev Phytopathol 14:237–246

Pirozynski KA, Dalpé Y (1989) Geological history of the Glomaceae with particular reference to mycorrhizal symbiosis. Symbiosis 7:1–36

Pirozynski KA, Malloch D (1975) The origin of land plants: a matter of mycotrophism. BioSyst 6:153–164

Pirozynski KA, Weresub KL (1979) A biogeographic view of the history of ascomycetes and the development of their pleomorphism. In: Kendricks B (ed) The whole fungus, vol 1, Kananaskis Foundation. National Museum of the Natural Sciences, National Museums of Canada, and Kananaskis Foundation, Ottawa, ON, pp 93–123

Pons D, Boureau E (1977) Les champignons epiphylls d'un *Frenelopsis* du Cenomanian moyen de l'Anjou (France). Rev Mycol 41:349–358

Porter TM, Martin W, James TY, Longcore JE, Gleason FH, Adler PH, Letcher PM, Vilgalys R (2011) Molecular phylogeny of the Blastocladiomycota (Fungi) based on nuclear ribosomal DNA. Fungal Biol 115:381–392

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921

Redecker D, Kodner R, Graham LE (2002) *Palaeoglomus grayi* from the Ordovician. Mycotaxon 84:33–37

Remy W, Taylor TN, Hass H (1994a) Early Devonian fungi: a blastocladalean fungus with sexual reproduction. Am J Bot 81:690–702

Remy W, Taylor TN, Hass H, Kerp H (1994b) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci U S A 91:11841–11843

Renault B (1894) Sur quelques nouveaux parasites des *Lépidodendrons*. Soc Hist Nat d'Autun Procès Verbal Séances 1893:168–178

Renault B (1895a) Chytridinées fossiles du Dinantien (Culm). Rev Mycol 17:158–161

Renault B (1895b) Parasites des écorces de *Lépidodendrons*. Naturaliste 9:77–78
Renault B (1896a) Recherches sur les Bactériacées fossils. Ann Sci Nat Bot Sér 8(2):275–349
Renault B (1896b) Bassin Houiller et Permien d'Autun et d'Épinac. Fascicule IV: Flore fossile, deuxième partie (Études des Gîtes Minéraux de la France). Imprimerie Nationale, Paris
Renault B (1899/1900) Sur quelques microorganismes des combustibles fossils. Société de l'Imprimerie Théolier. J Thomas et Cie, Saint-Étienne (text 1900, atlas 1899)
Renault B (1903) Sur quelques nouveaux champignons et algues fossiles, de l'époque houillère. C R Acad Sci Paris 136:904–907
Renault B, Bertrand CE (1885) *Grilletia spherospermii*, Chytridiacée fossile du terrain houiller supérieur. C R Acad Sci Paris 100:1306–1308
Retallack GJ (1994) Were the Ediacaran fossils lichens? Paleobiology 20:523–544
Retallack GJ (2009) Cambrian–Ordovician nonmarine fossils from South Australia. Alcheringa 33:355–391
Rikkinen J, Poinar GO (2008) A new species of *Phyllopsora* (Lecanorales, lichen-forming Ascomycota) from the Dominican amber, with remarks on the fossil history of lichens. J Exp Bot 59:1007–1011
Rikkinen J, Dörfelt H, Schmidt AR, Wunderlich J (2003) Sooty moulds from European Tertiary amber, with notes on the systematic position of *Rosaria* (Cyanobacteria). Mycol Res 107:251–256
Rothwell GW (1972) *Palaeosclerotium pusillum* gen. et sp. nov., a fossil eumycete from the Pennsylvanian of Illinois. Can J Bot 50:2353–2356
Schmid R (1976) Septal pores in *Prototaxites*, an enigmatic Devonian plant. Science 191:287–288
Schmidt AR, Dörfelt H, Perrichot V (2007) Carnivorous fungi from Cretaceous amber. Science 318:1743
Schüßler A, Walker C (2011) Evolution of the 'plant-symbiotic' fungal phylum, Glomeromycota. In: Pöggeler S, Wöstemeyer J (eds) The Mycota XIV. Evolution of fungi and fungal-like organisms. Springer, Berlin Heidelberg New York, pp 163–185
Schwendemann AB, Decombeix A-L, Taylor TN, Taylor EL, Krings M (2011) Morphological and functional stasis in mycorrhizal root nodules as exhibited by a Triassic conifer. Proc Natl Acad Sci U S A 108:13630–13634
Selosse MA (2002) *Prototaxites*: a 400 myr old giant fossil, a saprophytic holobasidiomycete, or a lichen? Mycol Res 106:642–644
Sharma N, Kar RK, Agarwal A, Kar R (2005) Fungi in dinosaurian (*Isisaurus*) coprolites from the Lameta Formation (Maastrichtian) and its reflection on food habit and environment. Micropaleontology 51:73–82
Sheffy MV, Dilcher DL (1971) Morphology and taxonomy of fungal spores. Palaeontographica 133B:34–51
Sherwood-Pike MA, Gray J (1985) Silurian fungal remains: probable records of the class Ascomycetes. Lethaia 18:1–20
Shi G, Zhou Z, Xie Z (2010) A new *Cephalotaxus* and associated epiphyllous fungi from the Oligocene of Guangxi, South China. Rev Palaeobot Palynol 161:179–195
Singer R (1977) An interpretation of *Palaeosclerotium*. Mycologia 69:850–854
Singh SK, Chauhan MS (2008) Fungal remains from the Neogene sediments of Mahuadanr Valley, Latehar district, Jharkhand, India and their palaeoclimatic significance. J Palaeontol Soc India 53:73–81
Smith J (1898) On the discovery of fossil microscopic plants in the fossil amber of the Ayrshire coalfield. Trans Geol Soc Glasgow 10:318–322
Smith PH (1980) Trichothyriaceous fungi from the Early Tertiary of southern England. Palaeontol 23:205–212
Smith SY, Currah RS, Stockey RA (2004) Cretaceous and Eocene poroid hymenophores from Vancouver Island, British Columbia. Mycologia 96:180–186
Smith WG (1884) Diseases of field and garden crops chiefly such as are caused by fungi. MacMillan, London
Speranza M, Wierzchos J, Alonso J, Bettuchi L, Martín-Gonzlez A, Ascaso C (2010) Traditional and new microscopy techniques applied to the study of microscopic fungi included in amber. In: Méndez-Vilas A, Díaz J (eds) Microscopy: science, technology, application and education, vol 2, Formatex. Badajoz, Spain, pp 1135–1145
Stanevich AM, Maksimova EN, Kornilova TA, Mazukabzov AM, Gladkochub DP (2007) Microfossils of the late Proterozoic Debengdinskaya Formation of the Olenekskiy uplift. Bull Tomsk Polytech Univ 311:9–14
Stein WE, Harmon GD, Hueber FM (1993) *Spongiophyton* from the Lower Devonian of North America reinterpreted as a lichen. Am J Bot 80(6 Suppl Abstr):93
Strullu-Derrien C, Strullu D-G (2007) Mycorrhization of fossil and living plants. C R Palevol 6:483–494
Strullu-Derrien C, Rioult JP, Strullu DG (2009) Mycorrhizas in Upper Carboniferous *Radiculites*-type cordaitalean rootlets. New Phytol 182:561–564
Stubblefield SP, Banks HP (1983) Fungal remains in the Devonian trimerophyte *Psilophyton dawsonii*. Am J Bot 70:1258–1261
Stubblefield SP, Taylor TN (1983) Studies of Paleozoic fungi. I. The structure and organization of *Traquairia* (Ascomycota). Am J Bot 70:387–399
Stubblefield SP, Taylor TN (1986) Wood decay in silicified gymnosperms from Antarctica. Bot Gaz 147:116–125
Stubblefield SP, Taylor TN (1988) Recent advances in paleomycology. New Phytol 108:3–25
Stubblefield SP, Taylor TN, Miller CE, Cole GT (1983) Studies in Carboniferous fungi. II. The structure and organization of *Mycocarpon*, *Sporocarpon*, *Dubiocarpon*, and *Coleocarpon* (Ascomycotina). Am J Bot 70:1482–1498
Stubblefield SP, Taylor TN, Beck CB (1985) Studies of Paleozoic fungi. V. Wood-decaying fungi in *Cal-*

lixylon newberryi from the Upper Devonian. Am J Bot 72:1765–1774

Sung GH, Poinar GO Jr, Spatafora JW (2008) The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses. Mol Phylogenet Evol 49:495–502

Taylor TN (1993) Fungi. In: Benton MJ (ed) The fossil record 2. Chapman & Hall, London, pp 9–13

Taylor TN, White JF Jr (1989) Fossil fungi (Endogonaceae) from the Triassic of Antarctica. Am J Bot 76:389–396

Taylor TN, Hass H, Remy W (1992a) Devonian fungi: interactions with the green alga *Palaeonitella*. Mycologia 84:901–910

Taylor TN, Remy W, Hass H (1992b) Fungi from the Lower Devonian Rhynie chert: Chytridiomycetes. Am J Bot 79:1233–1241

Taylor TN, Galtier J, Axsmith BJ (1994) Fungi from the Lower Carboniferous of central France. Rev Palaeobot Palynol 83:253–260

Taylor TN, Remy W, Hass H, Kerp H (1995) Fossil arbuscular mycorrhizae from the Early Devonian. Mycologia 87:560–573

Taylor TN, Hass H, Kerp H (1997) A cyanolichen from the lower Devonian Rhynie chert. Am J Bot 84:992–1004

Taylor TN, Klavins SD, Krings M, Taylor EL, Kerp H, Hass H (2004a) Fungi from the Rhynie chert: a view from the dark side. Trans R Soc Edinb Earth Sci 94:457–473

Taylor TN, Krings M, Klavins SD, Taylor EL (2005a) *Protoascon missouriensis*, a complex fossil microfungus revisited. Mycologia 97:725–729

Taylor TN, Kerp H, Hass H (2005b) Life history biology of early land plants: deciphering the gametophyte phase. Proc Natl Acad Sci U S A 102:5892–5897

Taylor TN, Hass H, Kerp H, Krings M, Hanlin RT (2005c) Perithecial ascomycetes from the 400 million year old Rhynie chert: an example of ancestral polymorphism. Mycologia 97:269–285

Taylor TN, Taylor EL, Krings M (2009) Paleobotany: the biology and evolution of fossil plants, 2nd edn. Academic, Burlington, MA

Taylor TN, Taylor EL, Decombeix AL, Schwendemann A, Serbet R, Escapa I, Krings M (2010) The enigmatic Devonian fossil *Prototaxites* is not a rolled-up liverwort mat: comment on the paper by Graham et al. (AJB 97: 268–275). Am J Bot 97:1074–1078

Taylor TN, Krings M, Dotzler N, Galtier J (2011) The advantage of thin section preparations over acetate peels in the study of late Paleozoic fungi and other microorganisms. Palaios 26:239–244

Taylor TN, Krings M, Galtier J, Dotzler N (2012) Fungal endophytes in *Astromyelon*-type (Sphenophyta, Equisetales, Calamitaceae) roots from the Upper Pennsylvanian of France. Rev Palaeobot Palynol 171:9–18

Taylor TN, Krings M, Taylor EL (2015) Fossil fungi. Academic, Burlington, MA

Taylor WA, Free C, Boyce CK, Helgemo R, Ochoads J (2004b) SEM analysis of *Spongiophyton* interpreted as a fossil lichen. Int J Plant Sci 165:875–881

Tiffney BH, Barghoorn ES (1974) The fossil record of the fungi. Occas Papers Farlow Herb 7:1–42

Tripathi SKM (2009) Fungi from palaeoenvironments: their role in environmental interpretations. In: Misra JK, Deshmukh SK (eds) Fungi from different environments. Science Publishers, Lucknow, India, pp 1–27

Unger F (1850) Genera et species plantarum fossilium. Wilhelm Baumüller, Vienna

Vajda V, McLoughlin S (2004) Fungal proliferation at the Cretaceous–Tertiary boundary. Science 303:1489

Van der Ham RWJM, Dortangs RW (2005) Structurally preserved ascomycetous fungi from the Maastrichtian type area (NE Belgium). Rev Palaeobot Palynol 136:48–62

Visscher H, Sephton MA, Looy CV (2011) Fungal virulence at the time of the end-Permian biosphere crisis. Geology 39:883–886

Waggoner BM (1995) Ediacaran lichens: a critique. Paleobiology 21:393–397

Wagner CA, Taylor TN (1981) Evidence for endomycorrhizae in Pennsylvanian age plant fossils. Science 212:562–563

Wagner CA, Taylor TN (1982) Fungal chlamydospores from the Pennsylvanian of North America. Rev Palaeobot Palynol 37:317–328

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang X, Krings M, Taylor TN (2010) A thalloid organism with possible lichen affinity from the Jurassic of northeastern China. Rev Palaeobot Palynol 162:591–598

Weiss FE (1904) A mycorrhiza from the Lower Coal Measures. Ann Bot 18:255–265

Wells AK, Hill RS (1993) Epiphyllous microorganisms as palaeoclimate estimators: the developmental sequence of fungal 'germlings' on their living host. Aust Syst Bot 6:377–386

White JF, Taylor TN (1989) Triassic fungi with suggested affinities to the Endogonales (Zygomycotina). Rev Palaeobot Palynol 61:53–61

White JF, Taylor TN (1991) Fungal sporocarps from Triassic peat deposits in Antarctica. Rev Palaeobot Palynol 67:229–236

Williamson WC (1878) On the organization of the fossil plants of the Coal-Measures. Part IX. Philos Trans R Soc Lond 169:319–364

Yuan X, Xiao S, Taylor TN (2005) Lichen-like symbiosis 600 million years ago. Science 308:1017–1020

11 Phylogenomics Enabling Genome-Based Mycology

Jason E. Stajich[1]

CONTENTS

I. **Application of Genomics to Molecular Phylogenetics** 279
A. Phylogenetic Reconstruction from Targeted Genes 279
B. Genome-Based Phylogenetic Reconstruction 281
C. Sequencing 1000 Fungal Genomes....... 283
II. **Genomics Enabling Comparative Biology** . 284
A. Evolution of Gene Structures in the Genome 284
B. Synteny and Gene Order Evolution...... 285
C. Evolution of Gene Families 286
D. From Molecules to Morphology 287
III. **Conclusions**................................ 288
References.................................. 288

I. Application of Genomics to Molecular Phylogenetics

The application of molecular phylogenetics to studies of fungal evolution ushered in an era of more accurate methods for reconstructing the history and relationships of fungi. The advent of high-throughput genome sequencing has even further improved the rate at which systematics can resolve species relationships. The application of genome sequences to phylogenetics has also improved the accuracy of species tree reconstructions and focused efforts on obtaining samples and downstream data analyses rather than one-by-one efforts to obtain amplification from each sample.

[1]Department of Plant Pathology & Microbiology and Institute for Integrative Genome Biology, University of California-Riverside, Riverside, CA 92521, USA; e-mail: jason.stajich@ucr.edu

A. Phylogenetic Reconstruction from Targeted Genes

Amplification and sequencing of ribosomal DNA (rDNA) has been a widely successful tool for molecular phylogenetic identification of fungi (Bruns et al. 1992; White et al. 1990). **The advent of polymerase chain reaction (PCR) and the development of universal primers for amplification of fungi opened avenues for phylogenetic reconstruction** from rDNA molecules, providing methods less prone to homoplasy and often with greater resolution. Molecular-based phylogenetic trees were shown to be more robust and avoided the homoplasy that characterized many morphological characters as a result of convergent evolution (e.g., gills of mushrooms, truffles) (Hibbett et al. 1997; McLaughlin et al. 1995; Moncalvo et al. 2002). The individual loci within the rDNA provide needed variation in phylogenetic resolution: on average, the 18S (small subunit—SSU) and 28S (large subunit—LSU) genes provided support for deep branches in the fungal tree while the internal transcribed spacers (ITS1 and ITS2) were useful for species- and genus-level resolution. However, as further fungal phylogenetic research was pursued, it emerged that rDNA genes had limitations in the resolution of phylogenetic relationships, and additional markers were sought (Seifert et al. 1995). **Multilocus sequencing and combined analyses of markers have mostly overcome problems in connection with the limited phylogenetic resolution of rDNA loci** (James et al. 2006; Schoch et al. 2009). The development of additional loci organized by phylogenetic informativeness has also shown improvements in species tree

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)
© Springer-Verlag Berlin Heidelberg 2015

resolution from gene phylogenies (Townsend 2007) so that loci can be chosen that are tuned for the evolutionary distance to be resolved. The culmination of this work was a series of revisions of the entire fungal phylogenetic tree, with 200 species based on multiple markers (Hibbett et al. 2007; James et al. 2006).

However, locus-by-locus amplification has challenges and disadvantages and is a low-throughput operation. Optimization of PCR conditions for some species is required due to variation in sequence bias and the length of the target loci and, when performed by either sample or locus, can be time consuming. In addition, locus-by-locus PCRs will work best if one is starting with a pure isolate of a species. This can be difficult to obtain for some species that are reticent to grow under laboratory conditions or often impossible for obligate symbionts. Finally, the resulting PCR product must be cloned and sequenced, which incurs reagent and sequencing costs for each sample attempted and requires potential needs for optimization of PCR primers for some clades of fungi.

An alternative to locus-by-locus targeted amplification is a random shotgun sequencing of whole-genome DNA to generate a low-coverage (5–10×) of the genome. **With current next-generation sequencing techniques it is possible to obtain draft-level genome coverage of a 40 Mb genome in 1/20 of an Illumina Hi-Seq lane.** Using the paired-end approach would generate roughly 200 million reads that are 100 bp long with current technology, or 40 gigabases (4×10^9). Splitting this among 20 samples provides 2 gigabases (20 million bases) per genome, or 50× coverage of a 40 Mb haploid genome. This level of sequencing is not sufficient to create a high-quality de novo assembly of genomes but should be sufficient for extracting a predefined set of gene markers (Lemmon and Lemmon 2012). Improvements to the next-generation sequencing technologies, including upgrades to Illumina sequencing at 250 bp long reads and the development of other sequencing platforms (e.g., Ion Torrent, PacBio, Nanopore), will continue to lower the cost of these approaches to data collection for phylogenomics (Quail et al. 2012).

Genome sequencing involves a random sample of DNA and so provides roughly even coverage of the genome. However, much of the genome evolves too rapidly or lacks correspondence across species for use in many phylogenetic analyses. Intergenic regions, introns, and transposable elements will be part of the sampled genome but are unlikely to be useful for phylogenetic reconstruction for more than the closest of species or for population genetic analyses. A more targeted sample of only the gene regions can be obtained by sequencing the expressed transcripts of mRNA. This is achieved with protocols for so-called RNA-Seq that reverse-transcribe mRNA into cDNA using either random primers or at least one primer that is complementary to the poly-A tail found on eukaryotic mRNAs. The mRNA library must also be normalized, or the majority of the sequences obtained will originate from the most highly expressed genes, including mostly ribosomal RNA, which will be only a limited and biased sample of the genes. To convert the short reads of RNA-Seq into usable long sequences for analyses, a tool such as Trinity (Grabherr et al. 2011) is well suited to construct a de novo assembly of the reads. These assembled transcripts can then be compared to a preselected set of target markers and extraction and alignment of the sequences performed for phylogenetic analyses (Hittinger et al. 2010; Rokas and Abbot 2009).

To best achieve uniformity from these sampling approaches, a set of conserved loci must be developed to insure targeting of most of the same loci across studies (Hittinger et al. 2010; Rokas and Abbot 2009). These loci should be single-copy or at least have paralogs that can be easily distinguished from each other. They can be generated based on a reference set of fungal genomes representing the phylogenetic diversity of the fungal kingdom. Comparative analysis of these genomes using methods to cluster orthologous genes would generate a compact set of loci that can be targeted. One method to accomplish this is to use the HAL pipeline to extract gene sets from the genomes (Robbertse et al. 2006, 2011). This approach was used to develop a target set for the Fungal Tree of Life II project and works by first

building a matrix of sequence similarities of all genes found in all the genomes and identifying the clusters that have only one copy per species. Often some flexibility is allowed in the criteria, which permits some species to be missing a gene to accommodate annotation errors or gene-loss events. The assumption is that these losses or missing data are random. Another phylogenomic approach, implemented in the PhylomeDB project, constructs phylogenetic gene trees from the ortholog clusters (Gabaldon 2008; Huerta-Cepas et al. 2008; Huerta-Cepas et al. 2011). These gene trees can be tested against known phylogenies or, for consistent species relationships, resolved in the different clusters in order to select only a subset of markers that perform best for species tree construction of the targeted organisms.

The dependence on PCR dictates the choice of markers for phylogenetic studies in which genes can be amplified with common primers. The identification of conserved genes, where a single set of primers amplifies a very broad taxonomic sample, was crucial for expansion beyond rDNA loci. The protein-coding genes Translation Elongation Factor 1 alpha (EF1α, also called TEF1 in *Saccharomyces cerevisiae*) and RNA Polymerase II subunits 1 and 2 (RPB1 and RPB2) have been developed as useful phylogenetic markers (Baldauf and Palmer 1993; Liu et al. 1999). These three protein-coding genes were included in addition to rDNA as the sampled loci for the Assembling the Fungal Tree of Life (AFTOL) project. They demonstrated improved resolution of the fungal phylogenetic tree as markers with a variety of evolutionary rates (James et al. 2006). Markers with slower evolutionary rates resolve older nodes in the phylogenetic tree, while those with faster rates are more useful for younger nodes in the tree**. The multilocus approach provided strong support for many previously unresolved relationships in the fungal tree of life.** The evolutionary range of a marker to resolve nodes of a particular age in a tree is its phylogenetic informativeness. In computing phylogenetic informativeness one calculates the probability of a marker possessing the most appropriate evolutionary rate for a set of nodes in a phylogenetic tree within a given epoch (Townsend 2007). This approach enables a principled selection of markers for the species in a study that allows one to find an optimal set to resolve both deep and shallow nodes of the phylogeny (Lopez-Giraldez and Townsend 2011; Townsend 2007; Townsend et al. 2012). While the AFTOL project collected these markers by PCR and sequencing of individual loci, the availability of whole-genome sequencing enables identification of the groups of orthologs and their evaluation to identify a marker set that is most phylogenetically informative for the taxa selected.

B. Genome-Based Phylogenetic Reconstruction

To fully utilize genome sequences in phylogenomics requires the construction of species trees from genome or transcriptome sequences of a group of species. To produce these trees, a set of orthologous genes must be identified from the set of genomes to be used in the phylogenetic analysis. Genes are clustered into homologous groups on the basis of their protein similarity. These clusters are further processed using tools to evaluate the consistency of the similarity, and in some cases the consistency of the phylogenetic signal, in order to best identify the subset of clusters of genes that are truly orthologous. To identify genes useful for phylogenetic analysis, simple criteria are applied such as selecting only orthologous clusters with one gene present per species in a cluster. Other approaches accommodate clusters with paralogous genes by choosing only one from a set of “in-paralogs,” genes duplicated within a single species. Each set of putatively orthologous sequences is aligned as a multiple alignment, and the resulting alignment is filtered for ambiguously aligned positions. This trimmed alignment is then analyzed using phylogenetic tools that first choose an appropriate model of sequence evolution. Finally, the trimmed alignment is subjected to a variety of phylogenetic reconstruction methods that include parsimony, distance-based methods such as neighbor joining, maximum likelihood, and Bayesian phylogenetic software.

This operation can be performed in an automated fashion following a series of logical steps in a defined workflow. **Several general workflow systems have been developed for bioinformatics,** including Galaxy (Goecks et al. 2010) and Taverna (Hull et al. 2006; Oinn et al. 2004; Tan et al. 2010) or combinations of both (Abouelhoda et al. 2012). These systems are typically Web-based tools designed for users to interact with Web browser software. Analyses can also be run within UNIX environments of Linux or Mac OS X with a series of steps executed as scripts that are run by the user. The improved standardization of these pipelines has reduced the amount of bioinformatics training required to adopt these approaches to new study systems. Further development of these workflow systems to support phylogenetic analyses and enable evolutionary model selection, hypothesis testing, and bootstrap analysis will simplify accessto phylogenomics for inferring species relationships.

To identify orthologous genes, groups of similar sequences must be identified (Fang et al. 2010). One approach uses clustering to build a network of genes based on sequence similarity computed from pairwise alignments using tools such as BLASTP and then clusters the network using Markov clustering (MCL) (van Dongen 2000; van Dongen and Abreu-Goodger 2012), implemented first in Tribe-MCL (Enright et al. 2002). Additional refinements to the MCL approach **for orthologous genes was developed as OrthoMCL, which corrects for paralogous gene families and has been shown to be one of the more accurate and automated approaches** (Chen et al. 2006, 2007). A variation on this approach that simply tries to find single-copy genes suitable for phylogenetic analyses was developed in the pipeline HAL and has been successfully applied to many fungal genome projects (Robbertse et al. 2006, 2011). Other fungal databases of gene families include FUNYBASE, which provides a presentation of multigene families as well as single-copy gene families across the fungi (Marthey et al. 2008), and the OrthoMCL-DB database, which houses a collection of eukaryotic and bacterial orthologous gene clusters, all based primarily on sequence similarity (Chen et al. 2006; Fischer et al. 2011).

Fungal species trees generated from fungal genomes give a similar perspective on the relationships inferred from only a few loci (Capella-Gutierrez et al. 2012; Fitzpatrick et al. 2006; Marcet-Houben and Gabaldon 2009; Robbertse et al. 2006). The degree of resolution of the tree can depend on whether there are conflicting signals in the loci. **Evaluation of supertrees from gene trees of multiple orthologs can be used to identify incongruences, since there will be multiple topologies supported among the gene trees** (Creevey and McInerney 2005; Fitzpatrick et al. 2006; Haggerty et al. 2009). These incongruences can be caused by horizontal gene transfer, differential rates of evolution, and incomplete lineage sorting. It has also been shown that in some cases nearly all individual gene trees of universally found orthologs can have phylogenetic patterns that are different from those of trees derived from concatenated alignments, indicating that testing the congruence of the phylogenetic signal is an important part of constructing species trees from gene trees (Salichos and Rokas 2013). Work from the genomic data of *S. cerevisiae* and related species showed that for more than 1,070 orthologous families from genomes of 23 species, each had different topologies from that estimated from a concatenated alignment.

Phylogenomic approaches also use gene families from similarity clusters to build candidate orthologous families but then use phylogenetic trees to assess orthologous and paralogous groups to provide detail on the history of gene families. One approach, PhylomeDB, provides the historical perspective for each gene in a target genome by presenting a tree with the copies of orthologs and paralogs for a selected set of genomes (Gabaldon 2008; Huerta-Cepas et al. 2008, 2011). The relationship of the genes within a family can also be assessed as to their relationship as paralogs (by duplication) or as true orthologs (by speciation). This reconciliation of gene relationships can be made through gene tree–species tree reconciliation using software such as NOTUNG (Stolzer et al. 2012) or TreeBEST (Vilella et al. 2009).

In some cases, **genes' history are not simply the same as the species history**. This can be **the result of events such as horizontal gene transfer (HGT),** where genes from one species are exchanged directly with another. There are many documented examples of fungi accepting bacterial genes (Hall and Dietrich 2007; Hall et al. 2005; Marcet-Houben and Gabaldon 2010) as well as oomycete and fungal exchanges (Richards et al. 2011). In many cases, the acquisition of these genes can be interpreted as a gain of function for a new biological process for the species in its ecological niche. One example is the URA1 gene in *Saccharomyces cerevisiae*, which is required for uracil biosynthesis and is a HGT of a dihydroorotate dehydrogenase (DHOD) enzyme from *Lactobacillus* bacteria (Gojkovic et al. 2004; Hall et al. 2005). The confirmation of the gene's history was revealed through the gene tree, which had a pattern of fungal gene copies embedded within a large bacterial phylogeny, indicating the fungal copy was derived from a bacterial donor. The eukaryotic version of the DHOD gene is found in most fungi, but not in *S. cerevisiae* and *Saccharomyces* sensu stricto. Within the *Saccharomyces* sensu stricto clade, species contain the bacterial version of this gene, which can function in anaerobic conditions. Acquisition of the DHOD gene by HGT was an important aspect of the evolution of a lifestyle that allows these yeasts to ferment alcohol. The comparison of gene trees of the copies of DHOD indicated that *S. cerevisiae* and relatives' copies were embedded within the bacterial phylogeny, while the eukaryotic version is found in all species outside the clade. Examination of genomes of *Kluveryomyces* species also revealed copies of both eukaryotic and eubacterial origins, confirming that some present-day species have maintained both copies.

Another scenario that can cause incongruence between a gene tree and the expected species tree (or the consensus among many but not all genes) is incomplete lineage sorting. This can occur when multiple alleles of a gene exist in a population and persist in the formation of new species, but eventually the polymorphism sorts out as different fixed alleles in the subsequent lineages where, for example, one group of species inherits allele A while others inherit allele B. This will make the divergence between species seem older than it actually is. This incomplete sorting can occur with multiple alleles of a gene or sometimes multiple duplicate copies of a gene, so that two lineages may each only have one copy of a gene family, but they are different by paralogy as ancient duplications that predate the species divergence. This can be a complicating factor when these duplicates have acquired different functions and display different rates of evolution, or when there are multiple species in a lineage that could have fixed alternative versions of a duplicate gene, causing them to group by which allele was fixed, not by the speciation.

C. Sequencing 1000 Fungal Genomes

Questions on how to resolve the fungal tree of life, on a better understanding of the origins of novelty in enzyme families or morphology, and on the evolution of genome structure can be addressed by comparing genome sequences from fungi across the kingdom. Several genome-sequencing projects have focused on a comprehensive sampling and genome sequencing of the Fungi, including projects housed at the U.S. Department of Energy's Joint Genome Institute (Martin et al. 2011) or the Broad Institute's Fungal Genome Initiative (Cuomo and Birren 2010). **One approach, the 1000 Fungal Genomes project (**http://1000.fungalgenomes.org**) or 1KFG, seeks to produce two genomes from each of the more than 500 families of fungi**. A deep sampling of genomes from across the fungal phylogeny will provide an important resource for evolutionary studies on how gene content changes as morphology or lifestyle changes and for phylogenetic resolution of the tree of life.

Within kingdom Fungi there are diverse families of enzymes, secondary metabolite production, and novel proteins to be discovered by expanding the inventory of fungal genes. Other large-scale projects have used genome sequencing to explore phylogenomic diversity. The Genomic Encyclopedia of Bacteria and Archaea

sequenced and analyzed 1,000 bacterial and archaeal genomes (Wu et al. 2009). The researchers' analysis revealed that the rate of discovery of new protein families continued to increase as new genomes were considered, and the curve representing family discovery did not indicate a saturation of the number of new families, even when all 1,000 were included. As hundreds of fungal genomes are sequenced and gene families compared, it will be important to understand whether the same diversity is exposed in Fungi.

Phylogenetic studies will be able to take advantage of the complete genome sequences produced through 1KFG and other efforts. **The availability of this deep sampling of genomes from Fungi should assist in providing better support for the backbone of the fungal tree.** For future projects that will apply low-coverage genome sequencing for phylogenetic studies, having a reference genome from a nearby clade will enable better assembly of the data. Furthermore, the extensive genome sampling will assist in building data sets of genes and their conservation profile through the fungi, allowing for identification of the core fungal genes found in all species, and those specific to the phyla or classes. The origins and functions of these gene groupings may provide additional insight into the biology and mechanisms of morphological differences among groups of fungi.

II. Genomics Enabling Comparative Biology

Comparative biology in Fungi comprises the study of the evolution of characters, particularly with a focus on morphology, lifestyles, and sexual reproduction (Lutzoni et al. 2001; Schoch et al. 2009). Many fungal characteristics, such as spore color (Hopple and Vilgalys 1999) and morphology (Hibbett 2004; Hibbett 2007; Hibbett and Binder 2002), have been shown to be evolutionarily labile and change rapidly, making them homoplastic characters and uninformative for phylogenetic reconstruction. Other phylogenetic analyses have shown that the shape and ultrastructure of organelles in zoosporic fungi are consistent with molecular phylogenetic trees, indicating that some morphological characters are robust enough to use as phylogenetic characters (Barr 1980; Letcher et al. 2008). However, DNA sequences and the proteins they encode can be some of the most useful data for phylogenetics because they tend to change in a predictable fashion.

With the availability of genome sequencing, an inventory of genes in a species and comparison of gene content among species can comprise an alternative approach to studying changes between species. The genes identified in a genome sequence can predict some aspects of lifestyle, such as the specializations of rusts (Duplessis et al. 2011) or the potential to process only some carbohydrates, and thus explain a specialized ecology (Eastwood et al. 2011) or predict whether a species is sexual (Galagan et al. 2005), which can subsequently be tested (O'Gorman et al. 2009).

Studies of how genomes have evolved can provide more insight into an organism's past through what can be considered molecular archaeology. These characters can include the evolution of how genes are encoded in the genome, chromosome structure and number, and the copy number of gene family members. The specific gene content of clades of organisms can also provide some timing for when specific processes may have originated, back to the most recent common ancestor of the group. Dating approximately when genes or groups of genes were gained or lost can help establish when shifts in function and ecology may have occurred in fungal history.

A. Evolution of Gene Structures in the Genome

The exon and intron structure of genes can inform the relationships between organisms because gene structures tend to be more similar among orthologous genes (Irimia and Roy 2008). A **striking pattern in gene structure was observed among the evolutionary clades of Fungi**. The Saccharomycotina yeasts (*Candida albicans*, *S. cerevisiae*) and the Taphrinomycotina fission yeast *Schizosaccharomyces*

pombe had on average one or no introns per gene, while the filamentous ascomycetes and basidiomycetes displayed a much broader range of intron distribution. This finding suggested that an ongoing process of intron change had occurred during Fungal evolution and could have been driven either by widespread gains or series of losses in these yeasts. Several studies examined the positions of the introns in orthologous genes and found evidence that introns were common in the ancestor of the Fungi but that subsequent loss of introns had occurred, most extremely in the branches leading to the Saccharomycotina and the branch leading to *S. pombe* (Carmel et al. 2007, 2009; Nielsen et al. 2004; Stajich et al. 2007). This ongoing process of loss and gain, but predominantly of loss, can be linked to ecology and lifestyle changes of these fungi and the effective population sizes of the species (Lynch 2006; Lynch and Conery 2003). The transition from a filamentous to yeast form may have altered the effective population size, allowing for selection to be more effective at purging the mildly deleterious introns. Further studies of mechanisms of intron changes suggest loss could occur through recombination or integration of spliced mRNA into the genome (Fink 1987; Stajich and Dietrich 2006). Intron gain has also been posited to occur through several mechanisms, reviewed in Roy and Irimia (2009, 2012), that include recruitment of the existing exonic sequence, tandem duplication, invasion during double-stranded break repair, organellar DNA insertion, and transposition of an intron by locus-specific targeting. An example of duplication leading to intron gain was observed in the *Cryptococcus* species (Sharpton et al. 2008), while locus-specific targeting was found in the accumulation of introns in *Magnaporthe oryzae* (Nielsen et al. 2004). The intronization of coding sequence and insertions were also observed to be major drivers in intron changes in *Fusarium* and *Cryptococcus* species (Croll and McDonald 2012). Recent discoveries of specific elements have shed additional light on intron gains. A proliferating element called an introner, which resembles an intron with canonical splice sites and efficient removal from transcript, was found in the genome of the picoeukaryote *Micromonas* in thousands of nearly identical copies within gene sequences. The further discovery of intronerlike elements in the Dothideomycete *Cladosporium fulvum* and five other fungi (van der Burgt et al. 2012) indicates that this mechanism might also contribute to intron creation in fungi.

B. Synteny and Gene Order Evolution

Beyond the conservation of gene structure among species, understanding how the arrangement of genes along a chromosome evolves can be important to picking out the orthologous genes and understanding the mechanisms of chromosome evolution. **A significant event in the history of the Saccharomycotina yeasts was the ancestral whole-genome duplication (WGD).** These fungi also have high rates of duplication, providing a rich environment for studies of these contributing factors to genome evolution (Dujon 2010; Koszul et al. 2006). A careful curation of the gene order in a collection of *Saccharomyces* species (Byrne and Wolfe 2005) provided a map to determine the evolutionary mechanisms by which the large number of duplicated genes in *S. cerevisiae* were created. The fate of these duplicate genes can be examined in multiple "experiments" by examining the different patterns of gene loss among the duplicates (Gordon et al. 2009) that ultimately lead to the formation of new species (Scannell et al. 2006, 2007). Large-scale duplication also occurs in other lineages of the Fungi, including the early diverging Mucoromycotina, where at least one WGD occurred (Ma et al. 2009).

Additional **studies of synteny have been useful in discovering clusters of genes that have moved**. This includes the identification of secondary metabolite clusters (Palmer and Keller 2010; Sanchez et al. 2012), for example, the aflatoxinlike gene cluster (Bradshaw et al. 2013), the cyclosporin gene cluster (Bushley et al. 2013), fumonisin (Khaldi and Wolfe 2011), or the sterigmatocystin gene cluster (Slot and Rokas 2011). Gene clusters of related function have also been demonstrated to be transferred, and breaks in synteny make it

easy to pick out the inserted regions. One example is the transfer of nitrate assimilation genes that were transferred as a unit from a basidiomycete into the ancestor of the ascomycete *Trichoderma reesei* (Slot and Hibbett 2007).

The DAL (Wong and Wolfe 2005) and GAL clusters (Slot and Rokas 2010) present other mechanisms of genome evolution where clusters of genes migrated to a common location from scattered locations throughout the *Saccharomyces* genomes. Syntenic comparisons of these genomes permitted the conclusion that the clusters were created, not transferred, because of the lack of shared gene order among the gene homologs in the related species.

Synteny is also often used to identify the mating type (MAT) region of fungi due to the shared gene order that can be found throughout the Dikarya. For instance, **the mating type loci of *Cryptococcus neoformans* was first inferred through the identification of a lack of shared gene order around the locus** (Lengeler et al. 2002) in a comparison of two strains of opposite mating types. Similarly for previously putatively asexual *Aspergillus fumigatus*, a pattern of gene order was used to find MAT genes between species (Galagan et al. 2005) and later enabled confirmation of the capability for a sexual cycle (O'Gorman et al. 2009).

An interesting pattern of gene order was discovered among the Dothideomycetes (Ascomycota). Strict synteny is not maintained, and so genes on homologous chromosomes were not found in the same order when species were compared. However, the gene content of chromosomes, that is, the complement of genes found, was preserved, but the order was scrambled. This **property of gene content conservation but not gene order was dubbed mesosynteny** (Hane et al. 2011) and presents a curious but currently unexplained evolutionary pattern that has only been observed in the Dothideomycetes.

C. Evolution of Gene Families

Phylogenomic approaches can also shed light on gene family evolution. **Examining the size of gene families and how these sizes change among species can provide correlative evidence for adaptations.** One approach is to count the number of copies of a gene family in two species and see whether they are significantly different. However, a more phylogenetic approach would take into account the evolutionary distance and the copy numbers across a clade of species (Hahn et al. 2005). One implementation, CAFE, estimates the rates of gene gain and loss among families for each branch of a phylogenetic tree (De Bie et al. 2006; Han et al. 2013). This allows for tests of shifts in rates of duplication or loss that can be examined in the context of the evolution of other traits such as a shift from host-associated to saprotrophic lifestyles or domestication. By identifying gene families with unusual patterns of gene duplication or loss, inferences can be made about the relative importance and suggest the changes that may be important to a lifestyle. In Fungi, it appears that many of the changes involve gene families related to metabolism.

Many studies have taken this approach, identifying changes in carbohydrate-active enzymes (CAZymes). CAZymes include carbohydrate synthesis and cleaving enzymes, and their curation in **the CAZy database has provided a resource for studying the evolution of these families across Fungi** (Cantarel et al. 2009; Levasseur et al. 2013). Changes in family composition have been interpreted in switches in ecological association or in the preferred nutritional substrate. For example, a study among three species of *Aspergillus* was able to relate some of the differences in enzyme potential to their biological niche (Coutinho et al. 2009). Perhaps one of the most identifiable associations of fungi is with wood-rotting basidiomycetes. Brown rotters are unable to degrade lignin and leave brown remains of a decomposed wood, while white rotters are able to break down lignin, leaving white remains. Comparing the genome sequences of a broad sample of 31 genomes, including 7 brown rotters and 9 white rotters, made it possible to reconstruct the history and timing of the evolution of rotting mechanisms among basidiomycetes (Floudas et al. 2012). This work revealed that expansion of lignin-degrading enzymes occurred early in the evolution of the

Agaricomycotina and correlated with the significant decrease in the deposition of organic carbon (coal) toward the end of the Permian. Furthermore, **gene losses were found to accompany the transition to brown rot status, and brown rot evolved several times independently** in these fungi. Work that investigated the dry rot fungus *Serpula lacrymans* along with the brown rot fungus *Postia placenta* also showed that smaller gene families of oxidoreductases in these two separate lineages were likely caused by expansions in the white rot fungi, but that CAZY families were reduced in size in the brown rotters (Eastwood et al. 2011).

Approaches to studying pathogenic fungi have also used **gene family comparisons to understand what genomic changes supported the adaptations that led to virulence.** A comparison of the amphibian pathogen *Batrachochytrium dendrobatidis* with a relative that is nonpathogenic identified pathogen-specific expansions of metallo-, serine, and aspartyl proteases (Joneson et al. 2011). The CAZy family CBM18 was also found to be greatly expanded only in the *B. dendrobatidis* pathogen, predicting some role for changes in how the cells sense or interact with chitin (Abramyan and Stajich 2012). Gene family contraction can occur as well. In the mammalian pathogenic fungi *Coccidioides immitis* and *C. posadasii*, several gene families related to the degradation of plant material such as cellulases and pectinases are absent. These families are missing in all the examined genomes in the Onygenales clade but are present in the more distant phyto-saprotrophic Aspergilli and Eurotiales (Sharpton et al. 2009), while expansions of protease families likely used for keratin degradation were identified that may be linked to the keratinophilic nature of these fungi. Similar findings of an expanded ratio of proteases to CAZy families were observed in the genomes of the Onygenales pathogens *Paracoccidioides lutzii* and *P. brasiliensis* (Desjardins et al. 2011).

Differences between hemibiotrophic and necrotrophic plant pathogens could be related to their repertoire of CAZy enzymes. The necrotrophic fungi do not need to avoid inducing cell death since they rely on dead tissue for nutrition, while hemibiotrophs avoid host detection in the early stages of infection and obtain nutrients from a living host. Comparison of the genomes of species with these differing lifestyles reveals in part a pattern of carbohydrate enzymes consistent with these predictions (de Wit et al. 2012). An analysis to compare genomes among the Dothediomycetes (Ohm et al. 2012) also showed **expansions and contractions of CAZy families that tracked with lifestyles.** A comparison of two *Colletotrichum* species found that *C. higginsianum* had an increased repertoire of enzymes for pectin degradation, peptidases, and effectors, along with secondary metabolites, in comparison to the maize-infecting *C. graminicola* (O'Connell et al. 2012). Overall, this work and others suggest that changes in copy number of some metabolic genes, especially those involved in carbohydrate metabolism, are correlated with lifestyle changes and pathogenesis mode.

However, large-scale changes in gene families are not strictly related to metabolism. For example, the expansions of kinase and RAS small GTPase families in the basidiomycete *Laccaria bicolor* suggest a major change in signaling pathways that may be related to changes from saprotroph to symbiont (Rajashekar et al. 2009). Families of genes including P450, hydrophobins, laccases, and kinases are dramatically increased in the basidiomycetes (Hoegger et al. 2004; Ide et al. 2012; Plett et al. 2012; Stajich et al. 2010). As more genomes are sequenced from fungi, it will be important to see whether the patterns of family changes continue to be good predictors of ecological niche and host-associated lifestyles.

D. From Molecules to Morphology

Much of the comparative biology of Fungi has focused on how shapes change, such as the ultrastructure of organelles and fruiting body form (Barr 1980; Hibbett 2007; Hibbett and Binder 2002; Letcher et al. 2008; Velez et al. 2011). Changes in phenotype are caused by changes in the DNA, but we do not yet have the ability to predict which changes will impact morphology. However, inferences and

correlations in gene interactions can be drawn as to the presence or absence of genes and the observed morphological changes in a group of organisms (Date and Marcotte 2003). For example, a life stage with flagellated zoospores is found in the Chytridiomycota and Blastocladiomyocta but is missing in the Dikarya. This difference predicts the presence of flagellar genes only in the zoosporic fungi. **Sequence similarity searches confirm** this pattern, and **flagellar genes are restricted to the early diverging lineages** but missing in species lacking a flagellated life stage based on the analysis of several available zygomycete genomes from the Mucoromycotina and Entomophthoromycotina.

A second example of changes in structure is **the evolution of the septal pore plugging proteins**, which are necessary to plug septal pores that link adjacent cells in hyphae upon hyphal wounding. Without these proteins, hyphae would leak cytoplasm after wounding, while intact septal plugs can protect the remainder of the colony. Two different types of protein perform these tasks in the filamentous basidiomycetes and ascomycetes. In the Pezizomycotina (Ascomycota), the Woronin body is the septal plug (Buller 1933; Voronin 1865) and is encoded by the *hex* gene (Jedd 2011; Jedd and Chua 2000). Isolation and sequencing of proteins responsible for the septal pore cap in Agaricomycotina (Basidiomycota), an analogous but nonhomologous structure to the Woronin body, identified a protein the authors later named SPC18 (van Driel et al. 2008). However, the sequences for these proteins are not similar to each other, indicating that these identified components of septal plugs have independent origins.

Further studies are needed to link observed changes in ultrastructure and morphology in the Fungi to the underlying molecular changes. One effort to collect the descriptions of the structure of fungal cellular components is being undertaken by the AFTOL project in the development of the Structural and Biochemical Database (AFTOL 2013) (see McLaughlin et al., Chap. 9, this volume). The project has curated six categories of processes or structures in Fungi: nuclear division, motile cell types, septum and septal pore cap, sterol composition, apical organization, and meiosporangium where meiotic spore products or apparatus for their delivery are formed. This collection of characters linked to the inventory of genes from genome sequences will provide even more insight into the evolution of these characters. Research on the evolution of these and other aspects of cell biology changes in Fungi will be a rich area of future research.

III. Conclusions

New technologies have improved the access to genomic data for all biological systems. Molecular phylogenetics has greatly benefited from the reduced barrier to access to sequence data, and mycology has reaped these benefits to improve the ability to describe species independent of morphology or cultivability. The future of mycology with these genomics-enabled tools may look different. One possibility is that genome sequencing will become routine enough that sample identification will proceed with a whole-genome-sequencing approach and bioinformatics analysis of these data. A necessary development will be streamlined tools for analysis so that sequence data can be processed in a standardized fashion. Studies in genome evolution and phylogenetics will certainly benefit from these approaches. However, cell biology and pathogenesis will be able to regularly use transcriptome sequencing to examine gene expression in a routine manner, and sequencing to diagnose plant or animal infections can also be expected to be a primary means of classifying unknown infections. The application of genomics methods will not remove the need to study and understand fungi from the microscopic to the ecosystem level, but it should reduce the time it takes to collect sequence data and improve the power and resolution of studies of fungal evolution.

References

Abouelhoda M, Issa SA, Ghanem M (2012) Tavaxy: integrating Taverna and Galaxy workflows with

cloud computing support. BMC Bioinformatics 13:77
Abramyan J, Stajich JE (2012) Species-specific chitin-binding module 18 expansion in the amphibian pathogen *Batrachochytrium dendrobatidis*. mBio 3:e00150-12
AFTOL Structural and Biochemical Database (2013) http://aftol.umn.edu/. Cited 1 Dec 2013
Baldauf SL, Palmer JD (1993) Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. Proc Natl Acad Sci U S A 90:11558–11562
Barr DJS (1980) An outline for the reclassification of the Chytridiales, and for a new order, the *Spizellomycetales*. Can J Bot 58:2380–2394
Bradshaw RE, Slot JC, Moore GG, Chettri P, de Wit PJ, Ehrlich KC, Ganley AR, Olson MA, Rokas A, Carbone I, Cox MP (2013) Fragmentation of an aflatoxin-like gene cluster in a forest pathogen. New Phytol 198:525–535
Bruns TD, Vilgalys R, Barns SM, Gonzalez D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisburg WG, Sogin ML (1992) Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. Mol Phylogenet Evol 1:231–241
Buller AHR (1933) Researches on fungi, vol V. Hafner, New York
Bushley KE, Raja R, Jaiswal P, Cumbie JS, Nonogaki M, Boyd AE, Owensby CA, Knaus BJ, Elser J, Miller D, Di Y, McPhail KL, Spatafora JW (2013) The genome of *Tolypocladium inflatum*: evolution, organization, and expression of the cyclosporin biosynthetic gene cluster. PLoS Genet 9:e1003496
Byrne KP, Wolfe KH (2005) The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species. Genome Res 15:1456–1461
Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37: D233–D238
Capella-Gutierrez S, Marcet-Houben M, Gabaldon T (2012) Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. BMC Biol 10:47
Carmel L, Rogozin IB, Wolf YI, Koonin EV (2007) Patterns of intron gain and conservation in eukaryotic genes. BMC Evol Biol 7:192
Carmel L, Rogozin IB, Wolf YI, Koonin EV (2009) A maximum likelihood method for reconstruction of the evolution of eukaryotic gene structure. Methods Mol Biol 541:357–371
Chen F, Mackey AJ, Stoeckert CJ Jr, Roos DS (2006) OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. Nucleic Acids Res 34:D363–D368
Chen F, Mackey AJ, Vermunt JK, Roos DS (2007) Assessing performance of orthology detection strategies applied to eukaryotic genomes. PLoS One 2:e383
Coutinho PM, Andersen MR, Kolenova K, vanKuyk PA, Benoit I, Gruben BS, Trejo-Aguilar B, Visser H, van Solingen P, Pakula T, Seiboth B, Battaglia E, Aguilar-Osorio G, de Jong JF, Ohm RA, Aguilar M, Henrissat B, Nielsen J, Stalbrand H, de Vries RP (2009) Post-genomic insights into the plant polysaccharide degradation potential of *Aspergillus nidulans* and comparison to *Aspergillus niger* and *Aspergillus oryzae*. Fungal Genet Biol 46 (Suppl 1):S161–S169
Creevey CJ, McInerney JO (2005) Clann: investigating phylogenetic information through supertree analyses. Bioinformatics 21:390–392
Croll D, McDonald BA (2012) Intron gains and losses in the evolution of *Fusarium* and *Cryptococcus* fungi. Genome Biol Evol 4:1148–1161
Cuomo CA, Birren BW (2010) The fungal genome initiative and lessons learned from genome sequencing. Methods Enzymol 470:833–855
Date SV, Marcotte EM (2003) Discovery of uncharacterized cellular systems by genome-wide analysis of functional linkages. Nat Biotechnol 21:1055–1062
De Bie T, Cristianini N, Demuth JP, Hahn MW (2006) CAFE: a computational tool for the study of gene family evolution. Bioinformatics 22:1269–1271
de Wit PJ, van der Burgt A, Okmen B, Stergiopoulos I, Abd-Elsalam KA, Aerts AL, Bahkali AH, Beenen HG, Chettri P, Cox MP, Datema E, de Vries RP, Dhillon B, Ganley AR, Griffiths SA, Guo Y, Hamelin RC, Henrissat B, Kabir MS, Jashni MK, Kema G, Klaubauf S, Lapidus A, Levasseur A, Lindquist E, Mehrabi R, Ohm RA, Owen TJ, Salamov A, Schwelm A, Schijlen E, Sun H, van den Burg HA, van Ham RC, Zhang S, Goodwin SB, Grigoriev IV, Collemare J, Bradshaw RE (2012) The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry. PLoS Genet 8:e1003088
Desjardins CA, Champion MD, Holder JW, Muszewska A, Goldberg J, Bailao AM, Brigido MM, Ferreira ME, Garcia AM, Grynberg M, Gujja S, Heiman DI, Henn MR, Kodira CD, Leon-Narvaez H, Longo LV, Ma LJ, Malavazi I, Matsuo AL, Morais FV, Pereira M, Rodriguez-Brito S, Sakthikumar S, Salem-Izacc SM, Sykes SM, Teixeira MM, Vallejo MC, Walter ME, Yandava C, Young S, Zeng Q, Zucker J, Felipe MS, Goldman GH, Haas BJ, McEwen JG, Nino-Vega G, Puccia R, San-Blas G, Soares CM, Birren BW, Cuomo CA (2011) Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. PLoS Genet 7:e1002345
Dujon B (2010) Yeast evolutionary genomics. Nat Rev Genet 11:512–524

Duplessis S, Cuomo CA, Lin YC, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL, Chiu R, Coutinho PM, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grabherr MG, Kodira CD, Kohler A, Kues U, Lindquist EA, Lucas SM, Mago R, Mauceli E, Morin E, Murat C, Pangilinan JL, Park R, Pearson M, Quesneville H, Rouhier N, Sakthikumar S, Salamov AA, Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan GA, Henrissat B, Van de Peer Y, Rouze P, Ellis JG, Dodds PN, Schein JE, Zhong S, Hamelin RC, Grigoriev IV, Szabo LJ, Martin F (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc Natl Acad Sci U S A 108:9166–9171

Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, Asiegbu FO, Baker SE, Barry K, Bendiksby M, Blumentritt M, Coutinho PM, Cullen D, de Vries RP, Gathman A, Goodell B, Henrissat B, Ihrmark K, Kauserud H, Kohler A, LaButti K, Lapidus A, Lavin JL, Lee YH, Lindquist E, Lilly W, Lucas S, Morin E, Murat C, Oguiza JA, Park J, Pisabarro AG, Riley R, Rosling A, Salamov A, Schmidt O, Schmutz J, Skrede I, Stenlid J, Wiebenga A, Xie X, Kues U, Hibbett DS, Hoffmeister D, Hogberg N, Martin F, Grigoriev IV, Watkinson SC (2011) The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science 333:762–765

Enright AJ, Van Dongen S, Ouzounis CA (2002) An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30:1575–1584

Fang G, Bhardwaj N, Robilotto R, Gerstein MB (2010) Getting started in gene orthology and functional analysis. PLoS Comput Biol 6:e1000703

Fink GR (1987) Pseudogenes in yeast? Cell 49:5–6

Fischer S, Brunk BP, Chen F, Gao X, Harb OS, Iodice JB, Shanmugam D, Roos DS, Stoeckert CJ Jr (2011) Using OrthoMCL to assign proteins to OrthoMCL-DB groups or to cluster proteomes into new ortholog groups. Curr Protoc Bioinformatics 35:6.12.1–6.12.19. doi:10.1002/0471250953.bi0612s35

Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol Biol 6:99

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martinez AT, Otillar R, Spatafora JW, Yadav JS, Aerts A, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho PM, de Vries RP, Ferreira P, Findley K, Foster B, Gaskell J, Glotzer D, Gorecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens JA, Kallen N, Kersten P, Kohler A, Kues U, Kumar TK, Kuo A, LaButti K, Larrondo LF, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, McLaughlin DJ, Morgenstern I, Morin E, Murat C, Nagy LG, Nolan M, Ohm RA, Patyshakuliyeva A, Rokas A, Ruiz-Duenas FJ, Sabat G, Salamov A, Samejima M, Schmutz J, Slot JC, St John F, Stenlid J, Sun H, Sun S, Syed K, Tsang A, Wiebenga A, Young D, Pisabarro A, Eastwood DC, Martin F, Cullen D, Grigoriev IV, Hibbett DS (2012) The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719

Gabaldon T (2008) Large-scale assignment of orthology: back to phylogenetics? Genome Biol 9:235

Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S, Lee SI, Basturkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D'Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Penalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW (2005) Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438:1105–1115

Goecks J, Nekrutenko A, Taylor J (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol 11:R86

Gojkovic Z, Knecht W, Zameitat E, Warneboldt J, Coutelis JB, Pynyaha Y, Neuveglise C, Moller K, Loffler M, Piskur J (2004) Horizontal gene transfer promoted evolution of the ability to propagate under anaerobic conditions in yeasts. Mol Genet Genomics 271:387–393

Gordon JL, Byrne KP, Wolfe KH (2009) Additions, losses, and rearrangements on the evolutionary route from a reconstructed ancestor to the modern *Saccharomyces cerevisiae* genome. PLoS Genet 5: e1000485

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29:644–652

Haggerty LS, Martin FJ, Fitzpatrick DA, McInerney JO (2009) Gene and genome trees conflict at many levels. Philos Trans R Soc Lond B Biol Sci 364:2209–2219

Hahn MW, De Bie T, Stajich JE, Nguyen C, Cristianini N (2005) Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res 15:1153–1160

Hall C, Dietrich FS (2007) The reacquisition of biotin prototrophy in *Saccharomyces cerevisiae* involved horizontal gene transfer, gene duplication and gene clustering. Genetics 177:2293–2307

Hall C, Brachat S, Dietrich FS (2005) Contribution of horizontal gene transfer to the evolution of *Saccharomyces cerevisiae*. Eukaryot Cell 4:1102–1115

Han MV, Thomas GW, Lugo-Martinez J, Hahn MW (2013) Estimating gene gain and loss rates in the presence of error in genome assembly and annotation using CAFE 3. Mol Biol Evol 30:1987–1997

Hane JK, Rouxel T, Howlett BJ, Kema GH, Goodwin SB, Oliver RP (2011) A novel mode of chromosomal evolution peculiar to filamentous Ascomycete fungi. Genome Biol 12:R45

Hibbett DS (2004) Trends in morphological evolution in Homobasidiomycetes inferred using maximum likelihood: a comparison of binary and multistate approaches. Syst Biol 53:889–903

Hibbett DS (2007) After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (Agaricomycetes) in the early 21st century. Mycol Res 111:1001–1018

Hibbett DS, Binder M (2002) Evolution of complex fruiting-body morphologies in homobasidiomycetes. Proc Biol Sci 269:1963–1969

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc Natl Acad Sci U S A 94:12002–12006

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Hittinger CT, Johnston M, Tossberg JT, Rokas A (2010) Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life. Proc Natl Acad Sci U S A 107:1476–1481

Hoegger PJ, Navarro-Gonzalez M, Kilaru S, Hoffmann M, Westbrook ED, Kues U (2004) The laccase gene family in *Coprinopsis cinerea* (*Coprinus cinereus*). Curr Genet 45:9–18

Hopple JS Jr, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Mol Phylogenet Evol 13:1–19

Huerta-Cepas J, Bueno A, Dopazo J, Gabaldon T (2008) PhylomeDB: a database for genome-wide collections of gene phylogenies. Nucleic Acids Res 36: D491–D496

Huerta-Cepas J, Capella-Gutierrez S, Pryszcz LP, Denisov I, Kormes D, Marcet-Houben M, Gabaldon T (2011) PhylomeDB v3.0: an expanding repository of genome-wide collections of trees, alignments and phylogeny-based orthology and paralogy predictions. Nucleic Acids Res 39:D556–D560

Hull D, Wolstencroft K, Stevens R, Goble C, Pocock MR, Li P, Oinn T (2006) Taverna: a tool for building and running workflows of services. Nucleic Acids Res 34:W729–W732

Ide M, Ichinose H, Wariishi H (2012) Molecular identification and functional characterization of cytochrome P450 monooxygenases from the brown-rot basidiomycete *Postia placenta*. Arch Microbiol 194:243–253

Irimia M, Roy SW (2008) Spliceosomal introns as tools for genomic and evolutionary analysis. Nucleic Acids Res 36:1703–1712

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822

Jedd G (2011) Fungal evo-devo: organelles and multicellular complexity. Trends Cell Biol 21:12–19

Jedd G, Chua NH (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2:226–231

Joneson S, Stajich JE, Shiu SH, Rosenblum EB (2011) Genomic transition to pathogenicity in chytrid fungi. PLoS Pathog 7:e1002338

Khaldi N, Wolfe KH (2011) Evolutionary origins of the fumonisin secondary metabolite gene cluster in *Fusarium verticillioides* and *Aspergillus niger*. Int J Evol Biol 2011:423821

Koszul R, Dujon B, Fischer G (2006) Stability of large segmental duplications in the yeast genome. Genetics 172:2211–2222

Lemmon AR, Lemmon EM (2012) High-throughput identification of informative nuclear loci for

shallow-scale phylogenetics and phylogeography. Syst Biol 61:745–761

Lengeler KB, Fox DS, Fraser JA, Allen A, Forrester K, Dietrich FS, Heitman J (2002) Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. Eukaryot Cell 1:704–718

Letcher PM, Velez CG, Barrantes ME, Powell MJ, Churchill PF, Wakefield WS (2008) Ultrastructural and molecular analyses of *Rhizophydiales* (*Chytridiomycota*) isolates from North America and Argentina. Mycol Res 112:759–782

Levasseur A, Drula E, Lombard V, Coutinho PM, Henrissat B (2013) Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnol Biofuels 6:41

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808

Lopez-Giraldez F, Townsend JP (2011) PhyDesign: an online application for profiling phylogenetic informativeness. BMC Evol Biol 11:152

Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940

Lynch M (2006) The origins of eukaryotic gene structure. Mol Biol Evol 23:450–468

Lynch M, Conery JS (2003) The origins of genome complexity. Science 302:1401–1404

Ma LJ, Ibrahim AS, Skory C, Grabherr MG, Burger G, Butler M, Elias M, Idnurm A, Lang BF, Sone T, Abe A, Calvo SE, Corrochano LM, Engels R, Fu J, Hansberg W, Kim JM, Kodira CD, Koehrsen MJ, Liu B, Miranda-Saavedra D, O'Leary S, Ortiz-Castellanos L, Poulter R, Rodriguez-Romero J, Ruiz-Herrera J, Shen YQ, Zeng Q, Galagan J, Birren BW, Cuomo CA, Wickes BL (2009) Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication. PLoS Genet 5:e1000549

Marcet-Houben M, Gabaldon T (2009) The tree versus the forest: the fungal tree of life and the topological diversity within the yeast phylome. PLoS One 4: e4357

Marcet-Houben M, Gabaldon T (2010) Acquisition of prokaryotic genes by fungal genomes. Trends Genet 26:5–8

Marthey S, Aguileta G, Rodolphe F, Gendrault A, Giraud T, Fournier E, Lopez-Villavicencio M, Gautier A, Lebrun MH, Chiapello H (2008) FUNYBASE: a FUNgal phYlogenomic dataBASE. BMC Bioinformatics 9:456

Martin F, Cullen D, Hibbett D, Pisabarro A, Spatafora JW, Baker SE, Grigoriev IV (2011) Sequencing the fungal tree of life. New Phytol 190:818–821

McLaughlin DJ, Berres ME, Szabo LJ (1995) Molecules and morphology in basidiomycete phylogeny. Can J Bot 73(Suppl 1):S684–S692

Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Catherine Aime M, Hofstetter V, Verduin SJ, Larsson E, Baroni TJ, Greg Thorn R, Jacobsson S, Clemencon H, Miller OK Jr (2002) One hundred and seventeen clades of euagarics. Mol Phylogenet Evol 23:357–400

Nielsen CB, Friedman B, Birren B, Burge CB, Galagan JE (2004) Patterns of intron gain and loss in fungi. PLoS Biol 2:e422

O'Connell RJ, Thon MR, Hacquard S, Amyotte SG, Kleemann J, Torres MF, Damm U, Buiate EA, Epstein L, Alkan N, Altmuller J, Alvarado-Balderrama L, Bauser CA, Becker C, Birren BW, Chen Z, Choi J, Crouch JA, Duvick JP, Farman MA, Gan P, Heiman D, Henrissat B, Howard RJ, Kabbage M, Koch C, Kracher B, Kubo Y, Law AD, Lebrun MH, Lee YH, Miyara I, Moore N, Neumann U, Nordstrom K, Panaccione DG, Panstruga R, Place M, Proctor RH, Prusky D, Rech G, Reinhardt R, Rollins JA, Rounsley S, Schardl CL, Schwartz DC, Shenoy N, Shirasu K, Sikhakolli UR, Stuber K, Sukno SA, Sweigard JA, Takano Y, Takahara H, Trail F, van der Does HC, Voll LM, Will I, Young S, Zeng Q, Zhang J, Zhou S, Dickman MB, Schulze-Lefert P, Loren V, van Themaat E, Ma LJ, Vaillancourt LJ (2012) Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nat Genet 44:1060–1065

O'Gorman CM, Fuller H, Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457:471–474

Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, Barry KW, Condon BJ, Copeland AC, Dhillon B, Glaser F, Hesse CN, Kosti I, LaButti K, Lindquist EA, Lucas S, Salamov AA, Bradshaw RE, Ciuffetti L, Hamelin RC, Kema GH, Lawrence C, Scott JA, Spatafora JW, Turgeon BG, de Wit PJ, Zhong S, Goodwin SB, Grigoriev IV (2012) Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. PLoS Pathog 8:e1003037

Oinn T, Addis M, Ferris J, Marvin D, Senger M, Greenwood M, Carver T, Glover K, Pocock MR, Wipat A, Li P (2004) Taverna: a tool for the composition and enactment of bioinformatics workflows. Bioinformatics 20:3045–3054

Palmer JM, Keller NP (2010) Secondary metabolism in fungi: does chromosomal location matter? Curr Opin Microbiol 13:431–436

Plett JM, Gibon J, Kohler A, Duffy K, Hoegger PJ, Velagapudi R, Han J, Kues U, Grigoriev IV, Martin F (2012) Phylogenetic, genomic organization and expression analysis of hydrophobin genes in the ectomycorrhizal basidiomycete *Laccaria bicolor*. Fungal Genet Biol 49:199–209

Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y (2012) A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Bios-

ciences and Illumina MiSeq sequencers. BMC Genomics 13:341
Rajashekar B, Kohler A, Johansson T, Martin F, Tunlid A, Ahren D (2009) Expansion of signal pathways in the ectomycorrhizal fungus *Laccaria bicolor*—evolution of nucleotide sequences and expression patterns in families of protein kinases and RAS small GTPases. New Phytol 183:365–379
Richards TA, Soanes DM, Jones MD, Vasieva O, Leonard G, Paszkiewicz K, Foster PG, Hall N, Talbot NJ (2011) Horizontal gene transfer facilitated the evolution of plant parasitic mechanisms in the oomycetes. Proc Natl Acad Sci U S A 108:15258–15263
Robbertse B, Reeves JB, Schoch CL, Spatafora JW (2006) A phylogenomic analysis of the Ascomycota. Fungal Genet Biol 43:715–725
Robbertse B, Yoder RJ, Boyd A, Reeves J, Spatafora JW (2011) Hal: an automated pipeline for phylogenetic analyses of genomic data. PLoS Curr 3, RRN1213
Rokas A, Abbot P (2009) Harnessing genomics for evolutionary insights. Trends Ecol Evol 24:192–200
Roy SW, Irimia M (2009) Mystery of intron gain: new data and new models. Trends Genet 25:67–73
Roy SW, Irimia M (2012) Genome evolution: where do new introns come from? Curr Biol 22:R529–R531
Salichos L, Rokas A (2013) Inferring ancient divergences requires genes with strong phylogenetic signals. Nature 497:327–331
Sanchez JF, Somoza AD, Keller NP, Wang CC (2012) Advances in *Aspergillus* secondary metabolite research in the post-genomic era. Nat Prod Rep 29:351–371
Scannell DR, Byrne KP, Gordon JL, Wong S, Wolfe KH (2006) Multiple rounds of speciation associated with reciprocal gene loss in polyploid yeasts. Nature 440:341–345
Scannell DR, Frank AC, Conant GC, Byrne KP, Woolfit M, Wolfe KH (2007) Independent sorting-out of thousands of duplicated gene pairs in two yeast species descended from a whole-genome duplication. Proc Natl Acad Sci U S A 104:8397–8402
Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239
Seifert KA, Wingfield BD, Wingfield MJ (1995) A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. Can J Bot 73:760–767
Sharpton TJ, Neafsey DE, Galagan JE, Taylor JW (2008) Mechanisms of intron gain and loss in *Cryptococcus*. Genome Biol 9:R24
Sharpton TJ, Stajich JE, Rounsley SD, Gardner MJ, Wortman JR, Jordar VS, Maiti R, Kodira CD, Neafsey DE, Zeng Q, Hung CY, McMahan C, Muszewska A, Grynberg M, Mandel MA, Kellner EM, Barker BM, Galgiani JN, Orbach MJ, Kirkland TN, Cole GT, Henn MR, Birren BW, Taylor JW (2009) Comparative genomic analyses of the human fungal pathogens *Coccidioides* and their relatives. Genome Res 19:1722–1731
Slot JC, Hibbett DS (2007) Horizontal transfer of a nitrate assimilation gene cluster and ecological transitions in fungi: a phylogenetic study. PLoS One 2:e1097
Slot JC, Rokas A (2010) Multiple GAL pathway gene clusters evolved independently and by different mechanisms in fungi. Proc Natl Acad Sci U S A 107:10136–10141
Slot JC, Rokas A (2011) Horizontal transfer of a large and highly toxic secondary metabolic gene cluster between fungi. Curr Biol 21:134–139
Stajich JE, Dietrich FS (2006) Evidence of mRNA-mediated intron loss in the human-pathogenic fungus *Cryptococcus neoformans*. Eukaryot Cell 5:789–793
Stajich JE, Dietrich FS, Roy SW (2007) Comparative genomic analysis of fungal genomes reveals intron-rich ancestors. Genome Biol 8:R223
Stajich JE, Wilke SK, Ahren D, Au CH, Birren BW, Borodovsky M, Burns C, Canback B, Casselton LA, Cheng CK, Deng J, Dietrich FS, Fargo DC, Farman ML, Gathman AC, Goldberg J, Guigo R, Hoegger PJ, Hooker JB, Huggins A, James TY, Kamada T, Kilaru S, Kodira C, Kues U, Kupfer D, Kwan HS, Lomsadze A, Li W, Lilly WW, Ma LJ, Mackey AJ, Manning G, Martin F, Muraguchi H, Natvig DO, Palmerini H, Ramesh MA, Rehmeyer CJ, Roe BA, Shenoy N, Stanke M, Ter-Hovhannisyan V, Tunlid A, Velagapudi R, Vision TJ, Zeng Q, Zolan ME, Pukkila PJ (2010) Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). Proc Natl Acad Sci U S A 107:11889–11894
Stolzer M, Lai H, Xu M, Sathaye D, Vernot B, Durand D (2012) Inferring duplications, losses, transfers and incomplete lineage sorting with nonbinary species trees. Bioinformatics 28:i409–i415

Tan W, Missier P, Foster I, Madduri R, Goble C (2010) A comparison of using Taverna and BPEL in building scientific workflows: the case of caGrid. Concurr Comput 22:1098–1117

Townsend JP (2007) Profiling phylogenetic informativeness. Syst Biol 56:222–231

Townsend JP, Su Z, Tekle YI (2012) Phylogenetic signal and noise: predicting the power of a data set to resolve phylogeny. Syst Biol 61:835–849

van der Burgt A, Severing E, de Wit PJ, Collemare J (2012) Birth of new spliceosomal introns in fungi by multiplication of introner-like elements. Curr Biol 22:1260–1265

Van Dongen S (2000). Graph clustering by flow simulation. PhD thesis, University of Utrecht, Utrecht

Van Dongen S, Abreu-Goodger C (2012) Using MCL to extract clusters from networks. Methods Mol Biol 804:281–295

Van Driel KG, van Peer AF, Grijpstra J, Wosten HA, Verkleij AJ, Muller WH, Boekhout T (2008) Septal pore cap protein SPC18, isolated from the basidiomycetous fungus *Rhizoctonia solani*, also resides in pore plugs. Eukaryot Cell 7:1865–1873

Velez CG, Letcher PM, Schultz S, Powell MJ, Churchill PF (2011) Molecular phylogenetic and zoospore ultrastructural analyses of *Chytridium olla* establish the limits of a monophyletic Chytridiales. Mycologia 103:118–130

Vilella AJ, Severin J, Ureta-Vidal A, Heng L, Durbin R, Birney E (2009) EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. Genome Res 19:327–335

Voronin MS (1865) Zur Entwicklungsgeschichte des *Ascobolus pulcherrimus* Cr. und einiger Pezizen. Senckenbergische Naturforschende Gesellschaft, Frankfurt

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322

Wong S, Wolfe KH (2005) Birth of a metabolic gene cluster in yeast by adaptive gene relocation. Nat Genet 37:777–782

Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, Hooper SD, Pati A, Lykidis A, Spring S, Anderson IJ, D'Haeseleer P, Zemla A, Singer M, Lapidus A, Nolan M, Copeland A, Han C, Chen F, Cheng JF, Lucas S, Kerfeld C, Lang E, Gronow S, Chain P, Bruce D, Rubin EM, Kyrpides NC, Klenk HP, Eisen JA (2009) A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature 462:1056–1060

Biosystematic Index

A

Acarosporaceae, 100
Aciculoconidium, 15
Acremonium, 189
Acrospermales, 160
Adelococcaceae, 131
Agaricomycetes, 181
Agaricomycotina, 235, 287, 288
Agaricus, 181
  A. campestris, 181
Aglaophyton major, 268
Agyriaceae, 108
Agyriales, 108
Agyrium, 107, 109
Aigialaceae, 151, 156, 166
Ajellomycetaceae, 128, 129, 133
Albugo, 213
Aleuriaceae, 48
Aliquandostipitaceae, 151, 158
Aliquandostipite, 157–158
  A. khaoyaiensis, 157
Allomyces, 266
Alternaria, 150, 156
Alyxoria, 165
Amanita, 216
Ambrosiozyma, 8–9, 15
Amniculicolaceae, 151
Amphisphaeriaceae, 67
Anamylopsoraceae, 103
Angulospora, 40
Anisogramma anomala, 81
Anisomeridium, 153
Anteaglonium, 157
Antennulariaceae, 159
Apiosporina, 162
Arachnomyces, 129
Arachnomycetaceae, 128, 129
Arachnomycetales, 123, 128, 129
Archaeorhizomyces, 14
Arthonia, 145, 147, 152, 164–166
  A. endlicheri, 165
  A. intexta, 147
  A. muscigena, 152
Arthoniaceae, 152, 164–166
Arthoniales, 143, 148, 149, 152, 154, 163–166
Arthophacopsis, 166
Arthopyrenia, 156
Arthopyreniaceae, 152, 155
Arthothelium, 164
Arthrobotrys, 37, 40
Arthrodermataceae, 128, 133
Ascobolaceae, 42, 43, 46, 48
Ascobolus, 44, 46
  A. carbonarius, 46
Ascobotryozyma, 16, 19
Ascocorticiaceae, 72
Ascocoryne, 77
Ascodesmidiaceae, 46
Ascodesmis, 42, 46, 48
  A. nigricans, 244
Ascoidea, 14
Ascomycetes, 233, 235, 242, 249, 288
Ascomycota, 3, 232, 235, 237, 240, 247, 286
Ascosphaera, 128, 129, 133
Ascosphaeraceae, 128
Ascosphaerales, 121, 129
Aspergillaceae, 124–126
Aspergillus, 123–126, 132, 134–136, 264, 287
  A. alliaceus, 126
  A. collembolorum, 262
  A. fischeri, 126
  A. flavus, 125, 126, 134, 135
  A. fumigatus, 122, 126, 132, 134, 286
  A. lentulus, 126

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)

© Springer-Verlag Berlin Heidelberg 2015

A. nidulans, 126, 134
A. niger, 125, 134, 135
A. ochraceus, 125, 134
A. oryzae, 126, 135, 136
A. parasiticus, 126, 134, 135
A. sojae, 135, 136
A. sydowii, 134
A. tamarii, 135
A. terreus, 125, 126, 134
Aspergillus section *Aspergillus*, 122
Aspergillus section *Fumigati*, 125
Asterinaceae, 147, 148, 161, 162, 166
Asterinales, 161
Asteroxylon mackiei, 267, 269
Astromyelon, 272
Aulographina pinorum, 161
Aureobasidium pullulans, 160
Auriscalpium vulgare, 244

B
Babjeviella, 16
Baeomycetaceae, 102
Balansia, 60
Barnettozyma, 15
Barriopsis, 160
Basidiomycetes, 233, 249
Basidiomycota, 3, 231, 232, 235, 237, 247, 288
Batrachochytrium dendrobatidis, 287
Baudoinia compniacensis, 160
Beauveria, 64
Bionectriaceae, 63
Bipolaris, 149, 188
Blastobotrys, 16
Blastocladiomycota, 233, 240, 251, 288
Blastomyces, 129
B. dermatitidis, 129, 133
Boletus, 216
Boliniales, 66
Botryosphaeria, 156, 160
Botryosphaeriaceae, 151, 155, 160
Botryosphaeriales, 156, 160, 161
Botryozyma, 16
Botrytis, 79
B. cinerea, 79
Brachyphoris, 40
Brefeldiellaceae, 161
Brettanomyces, 15
Briancoppinsia, 149, 164
Brigantiaeaceae, 109
Bulgariaceae, 72
Burenia, 14
Byssochlamys, 126, 127

C
Cainiaceae, 67
Caliciaceae, 103
Caliciales, 131
Callixylon newberryi, 268
Caloscyphaceae, 46
Caloscypha fulgens, 42, 46
Calosphaeriales, 65
Candelariaceae, 103
Candida, 16
C. albicans, 6, 284
C. cylindracea, 4
C. jeffriesii, 4–5
C. oleophila, 6
C. shehatae, 4
Capnodiaceae, 158, 159, 166
Capnodiales, 152, 158, 159, 161, 167
Capronia pilosella, 121
Cashhickia acuminata, 272
Catillariaceae, 108
Catinella olivacea, 149
Celotheliaceae, 131
Celotheliales, 131
Celothelium, 131
C. longisporum, 123
Cenococcum geophilum, 149, 150, 157
Cephaliophora, 44
Cephaloascus, 14
Ceratocystis, 64
Chaetomium, 60
Chaetosartorya, 125
Chaetosphaeriales, 66
Chaetothyriaceae, 130, 159
Chaetothyriales, 121, 123, 130–132, 153, 159
Chaetothyriomycetidae, 123, 130–132, 134, 136, 158
Chaetothyrium, 123
Chlorociboria, 75
Chorioactidaceae, 42, 44, 46–47
Chrysosporium, 125, 128
Chrysothrix, 165
Chrysotricacaceae, 164
Chrysotrichaceae, 152, 164, 165
Chytridiales, 250
Chytridiomycetes, 218, 250
Chytridiomycota, 233, 240, 249, 251, 288
Cirrosporium, 137
C. novae-zelandiae, 123, 132

Citeromyces, 16
Cladochytriales, 250
Cladoniaceae, 105
Cladosporium, 158
  C. fulvum, 158
Clathrospora heterospora var. *simmonsii*, 145
Clavicipitaceae, 63
Clavispora, 15
Clypeosphaeriaceae, 67
Coccidiascus, 16
Coccidioides, 122
  C. immitis, 129, 133, 287
  C. posadasii, 133, 287
Coccocarpiaceae, 107
Coccodiniaceae, 159
Coccotremataceae, 108
Cochliobolus, 149, 156, 188
Coelomycetes, 149
Coenogoniaceae, 105
Collemataceae, 107
Colletotrichum, 60, 287
  C. graminicola, 287
  C. higginsianum, 287
Coniochaetales, 66
Conocybe, 216
Conotrema, 106
Coprinopsis, 216
Coprinus, 216
Cordyceps, 60
Cordycipitaceae, 63
Coronophorales, 63
Cortinarius, 216
Corynasca, 155
Coryneliales, 62, 123, 129, 130, 134
Corynelia uberata, 122
Corynespora cassiicola, 155
Cryphonectria parasitica, 59
Cryptococcus, 285
Cryptothecia, 147, 164
Cucurbitariaceae, 156
Curvularia, 156
Cyberlindnera, 15
Cyniclomyces, 15
Cyphellophoraceae, 130
Cystocoleus, 152, 158
Cyttaria, 70, 75
Cyttariales, 69–70

D
Dactylaria, 40
Dactylella, 40
Dactylellina, 40
Dactylomyces, 126
Dactylospora, 131, 132
Daohugouthallus ciliiferus, 271
Davidiella, 158
Davidiellaceae, 159
Dawsophila, 152, 164
Debaryomyces, 14
  D. hansenii, 4
Dekkera, 8, 15
Delitschiaceae, 156
Dendrographa, 152, 165
Dermateaceae, 72
Diaporthales, 65
Diaporthe, 65
Diatrypaceae, 67
Dibotryon, 162
Dichlaenoideae, 127
Dichotomomyces, 125
Dicranidion, 40
Diddensiella, 16
Didymella, 151, 167
Didymellaceae, 156
Dimidiographa, 165
Diplodia, 160
Dipodascopsis, 15
Dipodascus, 14
Discinaceae, 42, 47, 50
Disciotus, 47
Discula destructiva, 65
Dolabra, 131
Dothidea, 144, 145, 150
Dothideaceae, 150, 160
Dothideales, 144, 145, 153, 154, 158, 160, 163
Dothideomyceta, 143–145, 152–154, 157,
    163, 167
Dothideomycetes, 126, 129, 131, 286
Dothideomycetidae, 144, 145, 151, 154, 158, 160
Dothidotthia, 156
Dothidotthiaceae, 156
Dothiorella, 160
Drechslerella, 40
Dubiocarpon, 267
Dwayaangam, 40

E
Elaphomyces, 122, 127, 128, 134
Elaphomycetaceae, 123, 127–128
Eleutherascus, 42, 46
Elixiaceae, 110
Elsinoaceae, 151, 160

Elsinoe
E. australis, 160
E. fawcettii, 160
Emericella, 124–126
E. nidulans, 125
Endogone, 266
Endomyces, 14
Engerulaceae, 161
Enterographa, 152, 165
Epibryaceae, 130
Epibryon, 134
Epichloë, 60
Eremascaceae, 128
Eremascus, 128, 129
Eremothecium, 5, 15
Eremothecium gossypii, 4
Erysiphales, 62, 69–70, 235
Erythrodecton, 165
Euallomyces, 266
Eucladiella, 266
Eupenicillium, 124–126
Eurotiales, 121, 123, 127–129, 131, 132, 135, 287
Eurotiomycetes, 122, 131, 134, 137, 158, 159, 167
Eurotiomycetidae, 123, 130, 131, 136
Eurotium, 122, 124–126, 132

F
Farlowiella, 147, 157
Fennellia, 126
Fulvophyton, 165
Fusarium, 59
Fuscideaceae, 110
Fusicladium, 162

G
Gaeumannomyces graminis, 65
Galactomyces, 14
G. candidus, 5
Gamsylella, 40
Ganodermataceae, 269
Ganodermites libycus, 263, 269
Geneaceae, 48
Geoglossaceae, 72
Geoglossomycetes, 77–78
Geoglossum, 77
Geosmithia, 127
Geotrichum, 14
Glaziella aurantiaca, 47
Glaziellaceae, 47
Glomerella, 63, 69
Glomerellales, 63
Glomeromycota, 235, 240, 247
Gloniopsis, 157
Glonium, 157
Gonapodya, 266
Graphidaceae, 97, 105
Guignardia, 161
Gyalectaceae, 105
Gymnoascaceae, 128, 129

H
Halosphaeriaceae, 64
Hanseniaspora, 15
Helicogonium, 14
Helicoma, 162
Helicomyces, 162
Helicoon, 40
Helicosporium, 162
Helotiaceae, 72
Helotiales, 70
Helvellaceae, 41–42, 47, 48, 50
Hemiphacidiaceae, 72
Herpotrichiellaceae, 130
Heydenia, 43
Histoplasma, 129
H. capsulatum, 122, 129, 133
H. capsulatum var. *duboisii*, 133
H. duboisii, 133
Humariaceae, 48
Hyalina, 40
Hyalorbilia, 40, 41
Hyaloscyphaceae, 72
Hymenoscyphus, 76
Hyphomycetes, 149, 161
Hyphopichia, 9, 14
Hypocrea, 63, 188
Hypocreaceae, 63
Hypocreales, 63–64
Hypocreomycetidae, 62
Hypomyces, 60
Hyponectriaceae, 68
Hysteriaceae, 147, 155, 157
Hysteriales, 145, 147, 155, 157, 160
Hysterium, 157
Hysterographium, 157

I
Icmadophilaceae, 108
Ingaderia, 152

J

Jahnula, 157
Jahnulales, 151, 155, 157, 158
Jimwhitea circumtecta, 263, 266
Julella avicenniae, 155

K

Kalapuya, 47
Kallistoskypha, 46
Karstenellaceae, 47
Karstenella vernalis, 47
Kazachstania, 15
Kellermania, 188
Kickxellomycotina, 232, 241
Kloeckera, 15
Kluyveromyces, 15, 283
  K. marxianus, 4
Kodamaea, 15
Koerberiaceae, 107
Komagataella, 16
Koralionastetales, 62
Kregervanrija, 15
Kryphiomyces catenulatus, 266
Kuraishia, 16
Kurtzmaniella, 14

L

Laboulbeniales, 62, 78
Laboulbeniomycetes, 78
Laccaria bicolor, 287
Lachancea, 15
  L. kluyveri, 9
Lachnocladiaceae, 233
Lactarius, 216
Lalaria, 14
Lamprospora, 42
Lecanactis latebrarum, 165
Lecanographa, 165
Lecanographaceae, 164, 165
Lecanoraceae, 104
Lecanorales, 90, 91
Lecanoromycetes, 131, 143, 144, 152, 157, 166
Lecideaceae, 105
Lecophagus, 40
Leiothecium, 125, 127
Lentitheciaceae, 151, 156
Leotia, 75
Leotiaceae, 72
Leotiales, 69
Leotioaceae, 72
Leotiomycetes, 57, 128
Leptopeltidaceae, 161
Leptosphaeria, 151, 156
  L. maculans, 151
Leptosphaeriaceae, 156
Leptosphaerulina, 154
Letrouitiaceae, 109
Leucoangium, 47
Lichenoconium, 153
Lindgomycetaceae, 151, 156
Lipomyces, 4, 15
Lobariaceae, 107
Lobulomycetales, 250
Loculoascomycetes, 144, 149, 150, 154, 158
Lodderomyces, 14
Lophiaceae, 157
Lophiostomataceae, 156
Lophium mytilinum, 155
Loramycetaceae, 72
Loxospora, 108
Lulworthiales, 62
Lyonophyton rhyniensis, 268

M

Macrorhabdus, 16
Magnaporthales, 65
Magnaporthe, 187
  M. oryzae, 59, 285
  M. poae, 65
Magnusiomyces, 14
Malbranchea, 128
Manglicolaceae, 158
Manglicola guatemalensis, 158
Massalongiaceae, 107
Massariaceae, 156, 157
Massarinaceae, 156
Massarineae, 156
Mazosia, 152
Medeolaria, 75
Medeolariales, 74
Megalosporaceae, 109
Megasporaceae, 108
Melanconis, 65
Melanommatales, 155, 157
Melanospora, 63
Melanosporales, 64
Meliolales, 62, 68–69
Metacoleroa, 162
Metacordyceps, 64

Metarhizium, 64
Metschnikowia, 15
  M. fructicola, 6
Meyerozyma, 14
  M. guilliermondii, 4
Microascales, 64–65
Micropeltidaceae, 161
Micropeltis, 161
Microsporidia, 192, 241
Microsporum, 128, 133
Microthyriaceae, 145, 147, 161, 162, 166
Microthyriales, 161
Microthyrium microscopicum, 145, 147
Millerozyma, 14
Miltideaceae, 108
Monacrosporium, 40
Monascaceae, 123, 127–128
Monascella, 42
Monascus, 125, 127
  M. purpureus, 135, 136
Monoblastiaceae, 152, 153, 162, 163
Montagnulaceae, 156
Morchellaceae, 41–42, 47–48, 50
Morosphaeriaceae, 151, 156, 166
Mucoromycotina, 241
Muyocopron, 161
Mycena, 216
Mycenella, 216
Mycocaliciales, 123, 131
Mycocaliciomycetidae, 123, 131, 134, 136
Mycocalicium, 123
Mycocarpon, 267
Mycosphaerella, 159
Mycosphaerellaceae, 151, 159, 161
Mycrothyriaceae, 161
Mycrothyrium microscopicum, 161
Myeloconidaceae, 105
Myriangiaceae, 160
Myriangiales, 144, 145, 158, 160
Mytilinidiaceae, 157
Mytilinidiales, 145, 155, 157, 160
Myxotrichaceae, 70, 128
Myxozyma, 15

N
Nadsonia, 16
Nakaseomyces, 15
Nakazawaea, 16
Nannizziopsiaceae, 128
Naumovozyma, 15
Nectria, 63
Nectriaceae, 63
Nematasketum, 271
Neocallimastigomycota, 240–241, 251
Neolecta, 14, 20
Neomicrothyrium, 161
Neopetromyces, 126
Neosartorya, 124–126
  N. fumigata, 125
Nephromataceae, 107
Neurospora crassa, 60
Niessliaceae, 63
Nizziopsaceae, 129
Nothia aphylla, 263, 266
Nothojafnea thaxteri, 49

O
Ochrolechiaceae, 108
Octospora, 42
Odontotremataceae, 105
Ogataea, 4, 15
  O. polymorpha, 4
Olpidium, 251, 265
Onygena, 122, 128
Onygenaceae, 128
Onygenales, 121, 123, 128–129, 131, 132, 287
Oomycota, 216, 217, 219, 234
Opegrapha, 147, 152, 165
Opegraphaceae, 147, 152, 154, 164, 165
Ophiocordycipitaceae, 63
Ophioparmaceae, 110
Ophiostomatales, 65–66
Ophiostoma ulmi, 59
Orbicula parietina, 43
Orbilia, 37, 39, 40
  O. xanthostigma, 39
Orbiliaceae, 35–36, 40
Orbiliales, 35–36
Orbiliomycetes, 35–38, 40, 50
Ostreichnon, 157
Ostropales, 90
Otideaceae, 48

P
Pachysolen, 16
  P. tannophilus, 4
Paecilomyces, 125–127
Palaeancistrus martinii, 268
Palaeoblastocladia milleri, 266

Palaeonitella cranii, 265
Palaeosclerotium pusillum, 271
Paleopyrenomycites devonicus, 269
Pannariaceae, 107
Paracoccidioides, 133
  P. brasiliensis, 129, 133, 287
  P. lutzii, 287
Paradoxomyces, 166
Paralecanographa, 165
Paramicrothyrium, 161
Paraschismatomma, 165
Parmeliaceae, 104
Parmulariaceae, 161, 166
Passalora fulva, 158
Patellariaceae, 157
Patellariales, 157, 160
Peltigeraceae, 107
Peltigerales, 91
Penicillium, 123–127, 134–136, 212
  P. camemberti, 135
  P. chrysogenum, 122, 126, 136
  P. roquefortii, 135
  subgenus *Biverticillium*, 124, 127
Pentagenella, 152
Perigrapha, 166
Pertusariaceae, 108
Pestalotia, 67
Peterjamesia circumscripta, 165
Peterozyma, 16
Petromyces, 126
Peziza, 48, 233
Pezizaceae, 41–44, 48
Pezizales, 37, 41
Pezizomycetes, 35–37, 41–43, 46–50
Pezizomycotina, 35, 126, 130, 232, 235, 237, 288
Phacidiaceae, 72
Phacographa, 165
Phacothecium, 166
Phaeomoniella, 131
Phaeosphaeriaceae, 153, 156
Phaeotrichaceae, 146, 162
Phaeotrichum, 148
Phaffomyces, 15
Phakopsora pachyrhizi, 212
Phaneromycetaceae, 105
Phialoascus, 14
Phialocephala, 75
Phialophora, 65
Phialosimplex, 125
Phlyctidaceae, 105
Phoebus, 166
Phoma, 153, 156, 165
Phomopsis, 65
Phycopeltis, 152
Phyllachorales, 62
Phyllosticta, 161
Phymatotrichopsis, 42, 44
  P. ominivora, 42, 44
Physciaceae, 103
Phytophthora, 216
  P. infestans, 206
Pichia, 15
  P. kluyveri, 4
Piedraiaceae, 158
Piedraia hortae, 158
Pinus, 162
Placopyrenium canellum, 123
Placynthiaceae, 107
Planistromellaceae, 188
Plectocarpon, 166
Plectomycetes, 121, 127, 129, 136
Pleomassariaceae, 155
Pleosporaceae, 156
Pleosporales, 144, 145, 147, 151, 154–160, 162, 167
Pleosporineae, 150, 156
Pleosporomycetidae, 144, 145, 151, 154, 155, 157, 160, 162
Pneumocystis, 14, 21
  P. carinii, 235
Podocarpaceae, 134
Podospora, 67
Polystomellaceae, 161
Porinaceae, 105
Porpidiaceae, 105
Postia placenta, 287
Priceomyces, 14
Prosthemium, 155
Protoascon missouriensis, 266
Protomyces, 5, 14
Protomycopsis, 14
Protoventuria, 162
Psaronius, 268
Pseudallescheria, 64
Pseudocercospora, 159
Pseudorbilia, 40
Pseudotripoconidium, 40
Pseudotulostoma, 127, 128, 134
  P. volvata, 122, 127
Psilopezia, 49
Pucciniomycotina, 232, 235, 237
Pyrenidium, 153

Pyrenomycetes, 59, 129, 130
Pyrenophora, 151, 156
Pyrenula, 123, 154
  P. aspistea, 154
  P. concatervans, 123
  P. pseudobufonia, 154
Pyrenulaceae, 131
Pyrenulales, 123, 131
Pyricularia, 65, 187
Pyronema, 44
Pyronemataceae, 41–43, 46, 48–50
Pythium, 216
Pyxidiophora, 78
Pyxidiophorales, 78

R
Racodium, 152, 158
Ramalinaceae, 104
Ramularia, 159
Rasamsonia, 127
Reichlingia, 149, 165
Requiellaceae, 131
Rhizaria, 231
Rhizinaceae, 41–42, 44, 49
Rhizina undulata, 42, 49
Rhizocarpaceae, 108
Rhizophydiales, 250
Rhytidhysteron rufulum, 157
Rhytisma, 79
Rhytismatales, 69–70
Roccella, 152, 163, 233
  R. galapagoensis, 166
Roccellaceae, 152, 164–166
Roccellographa, 165
Roccellographaceae, 165
Roussoella, 156
Roussoellopsis, 156
Rutstroemiaceae, 72

S
Saccharomyces, 4, 15
  S. cerevisiae, 283
Saccharomycodes, 15
Saccharomycopsis, 16
Saccharomycotina, 3–27
Saccobolus, 46
Sagenomella, 125, 127
Sagiolechiaceae, 105
Saitoella, 14, 17
Saprochaete, 14
Sarcoscyphaceae, 42–44, 49
Sarcosomataceae, 42, 44, 49
Sarrameana, 108
Saturnispora, 15
Scheffersomyces, 14
  S. stipitis, 4
Schismatomma, 152, 165
Schizoblastosporion, 16
Schizosaccharomyces, 13, 14
  S. pombe, 284
Schizothyriaceae, 29, 32, 159, 161
Schwanniomyces, 14
Sclerococcum sphaerale, 131
Sclerophyton, 165
  S. cerebriforme, 165
  S. muriforme, 165
Sclerospora graminicola, 213
Sclerotinia, 79
  S. sclerotiorum, 79
Sclerotiniaceae, 72
Scutellospora, 267
Scutellosporites devonicus, 263
Septoria, 156, 167
Serpula lacrymans, 287
Sordariales, 66–67
Sordariomycetes, 57, 126
Sordariomycetidae, 62
Sparria, 165
Spathaspora, 14
  S. passalidarum, 4–5
Spathulospora, 68
Spencermartinsia, 160
Spencermartinsiella, 16
Sphenophyllum, 263
Sphinctrinaceae, 131
Spilocaea, 162
Spongiophyton, 270
Sporocarpon, 263, 267
Sporopachydermia, 16
Sporormiaceae, 147, 156
Sporostigma, 149
Stachybotrys chartarum, 64
Starmera, 15
Starmerella, 4, 16
Stereocaulaceae, 105
Stictidaceae, 105
Stictis, 106
Stigmina, 162
Stirtonia, 147
Stomiopeltis, 161
  S. versicolor, 161
Stramenopiles, 231
Straminipila, 217

Strigulaceae, 162, 163
Sugiyamaella, 16
Sympoventuria, 162
Sympoventuriaceae, 162

T
Talaromyces, 124, 127
  T. marneffei, 134
Taphridium, 14
Taphrina, 5, 14
Taphrinales, 235
Taphrinomycotina, 3–27
Tappania, 263, 270
Teloschistaceae, 109
Teratosphaeriaceae, 158–160, 166
Terfezia, 235
Tetrapisispora, 15
Tetraplosphaericeae, 156
Thecotheus, 46
Thelebolaceae, 70
Thelebolales, 70
Thelebolus, 70
Thelotremataceae, 97
Thermoascaceae, 123
Thermoascus, 126, 127
Thermomyces, 127
Torula compniacensis, 160
Torulaspora, 4, 15
Tothia, 161
Trapeliaceae, 109
Trapeliales, 108
Traquairia, 267
Trematosphaeriaceae, 156
Trentepohlia, 131, 152
Trentepohliales, 152
Trichocoma, 127, 128
  T. paradoxa, 122, 127
Trichocomaceae, 123, 124, 126–127
Trichoderma, 60, 188
  T. reesei, 286
Trichodermataceae, 188
Trichomeriaceae, 130
Trichomonascus, 9, 16
Trichophyton, 128, 133
Trichosphaeriales, 62, 69
Tridentaria, 40
Trigonopsis, 16
Trinacrium, 40
Trypetheliaceae, 152, 154
Trypetheliales, 162
Tuber, 235
  T. magnatum, 49
  T. melanosporum, 49
Tuberaceae, 44, 47, 49–50
Tuberales, 44, 127
Tubeufiaceae, 155, 161–162
Tylophorella, 149
Tylophoron, 148, 164
Tyrannosorus pinicola, 162

U
Umbilicariaceae, 110
Uncinocarpus reesii, 129
Uredo, 189
  U. betae, 189
Urnula craterium, 42
Ustilaginomycotina, 235

V
Vahliellaceae, 107
Valsa, 65
Vanderwaltozyma, 15
Vararia, 233
Varicosporina, 64
Venturia, 162
  V. inaequalis, 162
Venturiaceae, 151, 155, 161, 162
Venturiales, 162
Verrucariaceae, 131
Verrucariales, 123, 130–131
Verticillium, 63
Vibrisseaceae, 72
Vizellaceae, 161
Volkartia, 14

W
Wegea, 149
Wickerhamia, 14
Wickerhamiella, 16
Wickerhamomyces, 15
  W. anomalus, 6
  W. canadensis, 9
Winfrenatia reticulata, 263, 270

X
Xeromyces, 125, 127
Xylaria, 60
Xylariaceae, 67
Xylariales, 67
Xylariomycetidae, 62

Y
Yamadazyma, 14
Yarrowia, 16
Y. lipolytica, 4

Z
Zoopagomycotina, 241
Zopfia, 145, 148, 156
Zopfiaceae, 147, 148, 156
Zwackhia, 165
Zwackhiomyces, 153
Zygoascus, 16
Zygosaccharomyces, 4, 15
Zygotorulaspora, 15
Zymoseptoria, 159

Subject Index

A
Access and benefit sharing (ABS), 215
Acetate peels, 264
Acronym, 207
Agar, 215
Agglutination, 9
Amsterdam Declaration on Fungal Nomenclature, 183
Anamorphs, 8, 21, 27–29, 31, 33, 37, 61, 183
  states, 44
Ancient DNA (aDNA), 211
  length of, 214
Annotations, 211
Antibiotics, 24
Apical organization, 230, 232, 237, 248–249
Apical ring, 61
Apothecia
  lirellate, 94, 164
  perithecioid, 94
Apothecioid, 144, 145, 149, 159, 160
Appressoria, 92
Ascogenous hyphae, 145, 148
Ascohymenial, 95, 144
Ascolocular, 95, 144
  development, 144
Ascoma, 38–39, 42
  apothecial ascoma, 57
  exothecia, 43
  hypogeous, 41
  hysteriaceous, 157
  maculiform, 164
  mazaediate, 164
  perithecial ascoma, 57
  ptycothecia, 43
  stereothecia, 43
  truffle, 43
Ascomata, 93
Ascospores, 39, 43, 61, 97, 269
  muriform, 163–165
Ascostroma, 147
Ascus, asci, 39, 43, 61, 232, 233, 239
  bitunicate, 96, 147, 148, 150, 157
  dehiscence, 97
  fissitunicate, 148, 150, 154
  inoperculate, 37
  operculum, 43
  prototunicate, 96
  pseudoprototunicate, 148
  rostrate, 148
  semifissitunicate, 148
  suboperculate, 43
  unitunicate, 96
Aspergillaceae
  asexual morphology, 125
  self-fertility, 125–126
  sexual morphology, 125–126
Atractosome, 232
Author citations, 198

B
Baking process, 4
Basidiospore, 232, 233
Basidium, 239
Basionym, 195
Beverage, 4–5
  spoilage, 4–6
Biochemical characters, 229, 233–237, 239, 251
Biocontrol, 5–6, 60
Biosafety, 215
Biosecurity, 215
Biotrophy, 68, 151

Systematics and Evolution, 2nd Edition
The Mycota VII Part B
D.J. McLaughlin and J.W. Spatafora (Eds.)

© Springer-Verlag Berlin Heidelberg 2015

Brewing process, 4
Bryophyte parasites, 42

C
Candidiasis, 6
Canopy fungi, 40
Carboniferous, 153, 260, 263–267, 272
  Mississippian, 271
  Pennsylvanian, 271, 272
Carboniferous coal-ball, 264
Cell lysis, 213
Cellular membranes, 220
Cellular proteins, 220
Cell wall, 234
Cenozoic rocks, 261, 264, 269
Centriole, 230, 232
Centrum, 60, 144, 149, 155, 157, 162
Character evolution, 237, 239
Character state, 230, 237–240, 250
Chemical fixation, 232, 242, 244, 245, 247, 248, 250
Cherts, 260, 263, 265, 266
Chestnut blight, 59
Chlamydospores, 8
Clamp connections (fossil), 263, 268, 271
Cleistothecia, 148
Cleistothecioid, 144
Coal-balls, 263, 264, 266
Coal geology, 260
Coccidioidomycosis, 133
Coelomycete, 61
Collecting
  fungal specimen, 207
Collection management, 215
Commensals, 152
Conidia, 98
  aquatic, 40
Conservation, 182
Contamination
  DNA, 212
  of herbarium DNA samples, 212
Convention on Biological Diversity (CBD), 215
Coprophilic fungi, 156, 162
Cretaceous, 264, 269
Crozier, 61
Cryofixation, 229, 232, 244, 248, 249
Cryogenic storage, 217–219
  dynamic vapor phase, 218
Cryopreservation, 217–219
Cryoprotectants, 218, 220
Cryovials, 218
Culture, 193
  availability, 23–26
  axenic, 214
  collections, 26
  ex-epitype, 195
  ex-holotype, 195
  ex-neotype, 195
  ex-type, 193, 195
  preservation, 216, 217, 220, 222
  purification, 24
Culture specimens, dried, 208
  preparing, 209
  storage of, 209–210
Cystidia, 232, 237

D
Devonian, 153, 167, 266–271
Devonian Rhynie chert, 263, 265, 267, 268
Diazonium Blue B (DBB), 233
Dictyosomes, 232
Differential scanning calorimetry (DSC), 220
Dimethyl sulfoxide (DMSO), 218
Direct reference in new combination, 197
Divergence time, 99
DNA
  barcoding, 215
  degradation of, 214
  quality
    from herbarium specimens, 214
DNA extraction, 207, 212–214
  bead beating, 214
  cell lysis, 213
  clean area, 213
Dogwood anthracnose, 65
Dolipore, 231, 235
Drought tolerance, 220
Dutch elm disease, 59

E
Ectomycorrhizae, 134
Effectors, 151
Embedding, 243, 245–247, 251
Endophytes, 60, 150, 151, 153, 155, 160, 272
Endospores, 7–8
Environmental sequences, 199
Environmental study, 79–80
Enzymatic degradation, 214

Eocene, 261, 263, 269
Epiphyllous fungi (fossil), 261, 269
Epiphytes, 150, 155, 158
Epitype, 209
Ethylene glycol, 218
Eurotiomycetes, 122, 131, 134, 137
  animal pathogens, 132
  Coccidioidomycosis, 133
  dermatophytes, 133
  ectomycorrhizae, 134
  extremophilism, 132
  food contamination, 135
  human pathogens, 134
  industrial uses, 136
  lichens, 134
  metabolites, 135
  myrmecophytes, 135
  onygenalean pathogens, 132–134
  plant pathogens, 134
Exciples, 164, 165
Ex-type, 193, 195

F
Flagellar apparatus, 230, 251
Flagellum, 230
Food contamination, 135
Food spoilage, 4–6
Fossils, 100, 167
  record, 259, 260, 265, 268–273
Freeze-dried material
  reactivation, 221–222
Freeze drying, 220
  dehydration, 220
  primary drying, 220
  secondary drying, 220
  sublimation of ice, 220
Freeze substitution, 244, 245
Frozen storage, 25
Fungal fossils, 261, 262
Fungal Subcellular Ontology (FSO), 230, 240–242
Fungicolous fungi, 158

G
Genome, 78–79, 156, 160, 167
Genome based phylogenetic reconstruction, 281–283
Genomics enabling comparative biology, 284–288
Geobiochemistry, 260
Geobiology, 260
Germ slit, 67
Glass ampoules, 220, 221
Glass transition temperature, 220
Glomeromycotan spores (fossil), 267
Glycerol, 218
Golgi, 230
  equivalents, 232
Growth in culture, Orbiliomycetes, 41

H
Hamathecium, 144, 147, 162
Hartig net, 42
Haustoria, 92
Hemiangiocarpous development, 147
Hemibiotrophic, 151, 156
Herbarium cabinets, 209
Herbarium sheets, 208
Herbarium specimens
  annotations, 211
  cross contamination, 212
  destructive sampling, 211
  DNA extraction, 212–214
  labeling, 209
  mounting sheets, 208
  paper packets, 208
Holomorph, 183
Holotype, 209, 215
Homology, 237
Homonym, 181, 194
Host response, 263, 265, 272
Hyperparasites, 155, 161
Hyphal apex, 232, 248, 249
Hyphal branching, 232
Hyphomycete, 61
Hyphopodia, 161
Hysterothecia, 144
Hysterothecium, 157

I
Ice-crystal dynamics, 217
Ice-crystals
  intracellular, 218
Identifier, 191, 194
Index Fungorum, 190
Index Herbariorum, 207, 209, 210
Inoperculate discomycetes, 69
Insect infestations, 209

Insect pests, 212
Interascal pseudoparenchyma, 144
International Botanical Congress (IBC), 180
International Code, 149
International Code of Nomenclature (ICN) for algae, fungi and plants, 180–198
International Commission on the Taxonomy of Fungi (ICTF), 187
International Mycological Congress (IMC), 183
Isidia, 98

J
Jurassic, 270–271

K
Karyogamy, 9

L
Labeling fungal specimens, 209
Lactose, 220
L-drying, 25
Lichenicolous fungi, 144, 147, 152, 153, 164, 166
Lichenization, 100
Lichens, 91, 134, 153, 233
  combined classification, 150
  crustose, 152, 162–165
  fruticose, 152, 163, 165
  subfruticose, 165
Lifestyles, Lecanoromycetes, 91
Liquid nitrogen, 217
Liquid paraffin, 216
Long-term culture preservation, 216, 217, 220, 222
Lyophilization, 25, 220
  cooling rates, 220
  glass transition temperature, 220
Lyoprotectants, 220
Lysine synthesis, 235

M
Marine species, 131–132
Material transfer agreement (MTA), 215
Meiosis, 9, 232, 247, 248
Melzer's reagent, 233
Meristematic growth, 153
Mesosynteny, 167
Metabolically active preservation, 215
Metabolism, 216
Metabolites, 99
Methanol assimilating yeasts, 4
Microbodies, 232, 237
Microscala, 232
Microwave fixation, 246
Mineral oil, 216–217
Mites, 212, 216
  infestations, 216
Mitochondrial cristae, 230
Mitosis, 232, 247, 250, 251
Motile cell, 230–233, 237, 239, 240, 251
Mounting sheets, 208
Multigene analyses, 13
MycoBank, 191
Mycoparasitism, 153
Mycorrhiza, 42

N
Name
  illegitimate, 181
  invalid, 181
  legitimate, 181
  new combination, 197
  registration, 190–191
  replacement, 197
  sanctioned, 192–193, 195
  superfluous, 181
Necrotrophy, 151
Nematophyta, 271
Nematophytes, 271
Nomenclature, 179
  dual, 182
  original material, 195
Nomen Novum, 197
Nuclear division, 40, 230, 232, 233, 237, 239, 240, 247–248

O
One Name Per Fungus, 182–189
Ontology, 240–242
Operculate, 45
Original material, 195
Ostiole, 144, 145, 155

P
Paleodiversity, 260
Paleoecology, 260

Paleomycology, 268, 272, 273
Paper packets, 208
Paraphyses, 39–40, 43, 98, 232
Paraphysoids, 144
Parasites, 49, 144, 150, 152, 155, 162
  species, 42, 150, 151, 156
Pathogens, 6, 132–134, 143–144, 150, 156, 159, 162
Peptone, 220
Periodic subculturing, 215
Periphyses, 144, 155, 158
Periphysoids, 144, 158
Perithecia, 94, 147, 150, 162
Permanent slide mounts, 208
Permian, 262, 263, 268
Photobionts, 91, 152, 165
Phylogeny, 61–62, 230–237, 249, 281–283
  analysis, 238–240
  and classification, 13–23
  programs, 239
Phytosanitary regulations, 215
Plant diseases, 5
Plant parasites, 49
Plasmogamy, 9
Plectenchyma, 144, 147
Plectomycetes
  characteristics, 121
Polymerase chain reaction (PCR), 211
Polypore basidiocarps (fossil), 269
Polyspory, 100
Postmortem degradation, 214
Post-preservation survival, 218
Powdery mildew, 76
Precambrian, 261, 266, 270
Predation
  nematode, 38
Preservation
  amber, 263, 264, 269, 270
  Mississippian, 263
  Pennsylvanian, 263, 268
  permineralizations, 262, 263
  petrifaction, 263
Preservation methods, 215
Priority, 181, 188
Proterozoic (fossil), 263, 270
Protologue, 192
Pseudohyphae, 7
Pseudoparaphyses, 144, 145, 151, 154, 156–158, 160, 162, 163
Publication
  effective, 181, 189–190
  electronic, 189–190
  ranks, 181
  valid, 181
Pullulan, 160
Pyrenocarpous lichens, 162

Q
Quality standards, 215
Quellkörper, 63

R
Recombinant proteins, 4
Red bread mold, 78
Registration of fungal names, 190–191
Rejection of fungal names, 182
Replaced synonym, 195–197
Requesting a specimen, 210
Residual moisture, 220
Rhynie chert, 260, 265–267, 269
Riboflavin, 4
Rice blast, 59
Rock-inhabiting fungi (RIF), 146, 153, 158, 159
Roots, 268, 272

S
Saccharides, 220
Safety cabinet, 218, 219, 222
Sanctioning, 182, 192, 195
Saprobes, 38, 42, 144, 150, 151, 156, 159, 161, 163, 166
  dung, 42
Saprotrophic nutritional mode, 81
Sclerotia, 149, 150
Scutate wall of thyriothecia, 144
Section staining, 246–247
Sedimentology, 260
Septal pore, 230–232, 235, 237, 240, 245
  apparatus, 232, 237, 239
  cap, 232, 237
Septal structures, 40, 44, 46–49
Septate (true) hyphae, 7
Septum, 230, 231, 239, 240
Sequencing 1000 fungal genomes, 283–284
Shipment of cultures, 26
Silurian, 263, 268, 269, 271
Skim milk, 220
Sodium glutamate, 220

Soil Clone Group I (SCGI), 23
Sooty mold, 158, 159, 167
Soredia, 98
Special Committee on Electronic Publication, 189
Special Committee on Nomenclature of Fungi with a Pleomorphic Life Cycle, 183
Spindle pole body (SPB), 230, 232, 239, 240, 247
Spitzenkörper, 232, 249
Spore body, 39
Sporocarps (fossil), 263, 266–267
Sporodochia, 164
Sterol, 235, 239
Structural and Biocemical Database (SBD), 230, 237–240, 252
Subcellular characters, 230, 232, 249
Substrate, Lecanoromycetes, 91
Summer patch pathogen, 65
Symplechosome, 232
Synanamorphs, 61
Synonym
  facultative, 193
  heterotypic, 193
  homotypic, 193
  nomenclatural, 193
  obligate, 193
  replaced, 195–197
  taxonomic, 193

T
Taxonomy, 179
Teleomorph, 183
Teliospores, 243, 248
Thalli, Lecanoromycetes, 93
Thermoascaceae
  asexual morphology, 126
  sexual morphology, 126
Thin-sections, 263, 264
Thyriothecia, 144, 147, 161
Thyriothecioid, 161
Transport regulations, 215
Trapping mechanisms, 38
Trehalose, 220
Triassic, 266–268
Trichocomaceae
  asexual morphology, 127
  sexual morphology, 127
  taxonomy, 126–127
Truffles, 49
Type, 181, 192
  epitype, 196–197
  fungal cultures, 193
  holotype, 193, 194
  isosyntype, 195
  isotype, 195
  lectotype, 193, 195
  neotype, 196
  paratype, 195
  specimen, 209
  syntype, 195

U
Ultrastructure, 61, 229, 248, 250
Unique acronym, 209

V
Vapor phase, 217
Voucher specimens, 206

W
Wine making process, 4
Woronin bodies, 237

X
Xylose fermentation, 236

Y
Yeast, 233, 235, 236, 247
  asexual reproduction, 7
  D1/D2 domains, rRNA gene, 12
  distribution, 3–4
  ecology, 3–4
  fission, 7
  genotypic classification, 12–13
  industrial uses, 4–5
  methanol assimilating, 4
  occurrence, 3–4
  phenotypic characterization, 11–12
  purification of cultures, 24
  septate (true) hyphae, 7
  sexual reproduction, 9–11
Yeast budding
  bipolar, 7

enteroblastic, 7
holoblastic, 7
multilateral, 7
Yeast culture
acidified media, 23
isolation, 23–26
maintenance, 23–26
malt-peptone, 222
membrane filters, 24
osmotic media, 24
temperature, 23

Z

Zoospore, 230, 249, 250
Zwischengruppe, 147
Zygomycete, 232, 247, 251
Zygosporic fungi, 232