Eur J Plant Pathol (2008) 122:3–18
DOI 10.1007/s10658-008-9341-y

Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology

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Received: 31 August 2007 / Accepted: 29 May 2008

Abstract Recent advances in the classification of downy mildews and white blister rusts are presented from ordinal to species level. Using molecular data (mainly LSU of nuclear ribosomal DNA and ITS rDNA data, but also cox2, beta-tubulin and NADH genes), ordinal, family and generic circumscriptions have been reconsidered and changed during the last years; species circumscription and concepts are also changing. These rearrangements also lead to a reevaluation of the traditional morphological characters used for classification. The recent changes have various implications for applied sciences (phytopathology, molecular biology) mainly at the species level; besides name changes for some taxa, revised species circumscriptions and improved species identification using genetic markers have important consequences on host ranges, source inocula and risk assessment of phytopathologically important taxa. However, there are also some substantial unresolved problems which need to be addressed in the future with new data and methods. These include the systematic position of some rarely sampled taxa, the phylogenetic relationships of the main downy mildew lineages to each other, more detailed molecular studies on speciation processes to develop appropriate sound species concepts and circumscriptions, and the development of a molecular bar coding system for downy mildews enabling reliable species identification. Applying molecular methods has the potential to greatly enhance our knowledge on the overall biodiversity of downy mildews.

Keywords *Albugo* · Molecular phylogeny · Peronosporales · Peronosporaceae · *Phytophthora* · Species concept · White blister rusts

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Introduction

Downy mildews and white blister rusts are members of the class Oomycetes (Peronosporomycetes), a comparatively small lineage with estimates of <1,000 species (Kirk et al. 2001). Due to their morphological, physiological and ecological similarities to fungi, the Oomycetes are traditionally treated within mycology; however, ultrastructural, biochemical and molecular phylogenetic data confirm that they are not related to true fungi (kingdom Fungi), but belong to the kingdom Chromista (Straminipila) which also contains the chromistan (heterokont) algae (Dick 2001, 2002; Kirk et al. 2001). Downy mildews are an important group of obligate biotrophic plant parasites, which have a great economic impact on numerous crops (e.g. *Plasmopara viticola* on *Vitis vinifera*, *Pseudoperonospora humuli* on *Humulus lupulus*, *Perono-*

spora tabacina on *Nicotiana* spp.). With the commercial large-scale production of some crop and ornamental plants, some downy mildew diseases have recently become of major concern, e.g. downy mildew disease of *Ocimum basilicum* (basil; e.g. Belbahri et al. 2005), *Eruca sativa* (rocket; e.g. Larran et al. 2006 and references cited therein), or *Rubus* spp. (arctic bramble and boysenberry; e.g. Hukkanen et al. 2006 and references cited therein). In several of these cases, classification of the causal agent is still unclear, which demonstrates our ignorance of biodiversity of downy mildews. With the rapid development of new research techniques and questions in applied and theoretical plant pathology, the interest in phylogeny of downy mildews has increased over the last years. The availability of a substantial number of additional new characters less prone to subjective interpretations in general led to a change of paradigms in classification, as phylogenetic hypotheses could be vigorously tested for the first time, which led to a shift from a phenetic to a phylogenetic classification. In addition, phylogenetic analyses of DNA sequence data also enabled a re-evaluation of morphological features in an evolutionary background and a re-investigation of species boundaries and host specificity. Significant progress has been made towards a phylogenetic classification, which is presented here. However, several important questions are still unresolved, and these will be briefly discussed.

General considerations on systematics and taxonomy

Before considering the changes, progress and challenges in downy mildews systematics in detail, some general considerations will be briefly outlined. There are often conflicts between taxonomists and applied biologists, originating from different and sometimes incongruent expectations on taxonomy and systematics. Although there have been different methodological approaches in taxonomy during its history, it is nowadays commonly accepted that phylogenetic relationships should be the primary basis of a taxonomic system (Lecointre and Le Guyader 2006). Therefore, the taxonomist seeks consistency of a taxonomic system with theories on phylogeny and evolution. Consequently, classification should be in line with well-supported phylogenetic hypotheses. With the increase of knowledge, reinterpretation of phylogenetic relationships leads necessarily to name changes. In addition, taxonomy and classification has to be consistent with the current rules of the International Code of Botanical Nomenclature. Application of the latter sometimes necessitates the change of well-established names, which are often felt unnecessary, cumbersome or complicated.

Non-taxonomists are primarily interested in using scientific names without the need for a deeper knowledge of taxonomy itself. Therefore, stability of names is highly desirable. Consequently, taxonomy should be easily applicable outside the taxonomic community, e.g. for purposes of identification. As another important requirement, taxonomy should be also appropriate for legal measures such as quarantine lists of species.

Of course, ideally taxonomy should fit the needs of both taxonomists and non-taxonomists, and this is often possible if taxonomic decisions are made cautiously. In downy mildews, it has been possible up to now to avoid undesirable name changes for most phytopathologically important species. However, taxonomic changes are sometimes unavoidable to meet the standards of a consistent phylogenetic classification. This is no end in itself, but enables progress also in other research disciplines. Evidently, the non-taxonomist should be interested in reliable species concepts and boundaries, which are prerequisites for the development of reliable PCR-based identification systems, a record of the correct host ranges, and the application of pest control and quarantine measures.

Phylogenetic placement of Peronosporaceae (downy mildews) and Albuginaceae (white blister rusts)

Based on morphological and ultrastructural data, Oomycetes were subdivided into three subclasses, the Saprolegniomycetidae, Rhipidiomycetidae and Peronosporomycetidae, the latter including *Pythium*, *Phytophthora* and some other genera together with downy mildews and white blister rusts (Dick et al. 1984; Dick 1995). This subdivision was largely confirmed by subsequent molecular phylogenetic analyses (e.g. Dick et al. 1999; Riethmüller et al.

1999; Hudspeth et al. 2000; Petersen and Rosendahl 2000). However, the classification at lower ranks remained uncertain and changed quite substantially following molecular phylogenetic analyses (Table 1, Fig. 1). Whereas previously considered closely related to Peronosporaceae (Fig. 1a), the Albuginaceae were placed outside the Pythiaceae-Peronosporaceae lineage in cox2 (Fig. 1b), LSU/SSU (Fig. 1c) and ITS rDNA (Cooke et al. 2000) sequence analyses. Therefore, Albuginaceae should represent an ancient, evolutionarily–derived lineage of uncertain phylogenetic affinities (compare Fig. 1b,c). Early origin of Albuginaceae is in line with high sequence divergence (Riethmüller et al. 2002) and its unique conidial and oospore morphology (Riethmüller et al. 2002; Hudspeth et al. 2003; Thines and Spring 2005; Voglmayr and Riethmüller 2006). Consequently, Hudspeth et al. (2003) pleaded for exclusion of Albuginaceae from Peronosporales and for elevation to ordinal level, whereas Thines and Spring (2005) created even a new subclass, Albuginomycetidae.

As opposed to *Albugo*, DNA data confirmed a close phylogenetic relationship of downy mildews to the genus *Phytophthora* (e.g. Petersen and Rosendahl 2000; Cooke et al. 2000; Riethmüller et al. 1999, 2002; Hudspeth et al. 2003; Voglmayr 2003; Göker et al. 2007). There is strong molecular evidence that the genus *Peronophythora*, sometimes considered an

Table 1 Comparison of some ordinal, family and generic classifications of downy mildews, white blister rusts and relatives

Waterhouse (1973)	Kirk et al. (2001)	Riethmüller et al. (2002)	Göker et al. (2007)[a], Thines and Spring (2005)[b]
Peronosporales	Peronosporales	(no order name)	Peronosporales
Peronosporaceae	Peronosporaceae	Peronosporaceae	Peronosporaceae
Basidiophora	*Basidiophora*	*Basidiophora*	*Basidiophora*
Bremia	*Benua*	(*Benua*)	*Benua*
Bremiella	*Bremia*	*Bremia*	*Bremia*
Peronospora	*Bremiella*	*Paraperonospora*	*Graminivora*
Plasmopara	*Paraperonospora*	*Peronophythora*	*Hyaloperonospora*
Pseudoperonospora	*Peronospora*	(*Peronosclerospora*)	*Paraperonospora*
Sclerospora	*Plasmopara*	*Peronospora*	*Perofascia*
Albuginaceae	*Pseudoperonospora*	*Phytophthora*	*Peronosclerospora*
Albugo	Albuginaceae	*Peronospora*	*Peronospora*
Pythiaceae	*Albugo*	*Plasmopara*	*Peronospora*
Phytophthora	Pythiales	*Pseudoperonospora*	*Plasmopara*
Pythiogeton	Pythiaceae	*Sclerospora*	*Plasmoverna*
Pythium	*Halophytopthora*	Albuginaceae	*Protobremia*
Sclerophthora	*Peronophythora*	*Albugo*	*Pseudoperonospora*
Trachysphaera	*Phytophthora*	Pythiaceae	*Sclerospora*
	Pythium	*Lagenidium*	*Viennotia*
	Trachysphaera	*Pythium*	(family not formally classified)
	Pythiogetonaceae	(*Pythiogeton*)	*Phytophthora*
	Pythiogeton	(*Trachysphaera*)	Albuginales
	Sclerosporales	(*Sclerophthora*)	Albuginaceae
	Sclerosporaceae		*Albugo*
	Peronosclerospora		*Pustula*
	Sclerospora		*Wilsoniana*
	Verrucalvaceae		
	Sclerophthora		

For the Pythiaceae and Verrucalvaceae, only important genera (those with phytopathogenic and/or phylogenetic relevance for downy mildews) are listed (for complete genus lists, see the respective publications); from Peronosporaceae and Albuginaceae, all genera are considered. Taxa in parentheses: not included in the phylogenetic analyses. *Phytophthora* excluded from Peronosporaceae but placed in Peronosporales without formal family classification; other traditional Pythiaceae not classified.

[a] For downy mildews

[b] For white blister rusts

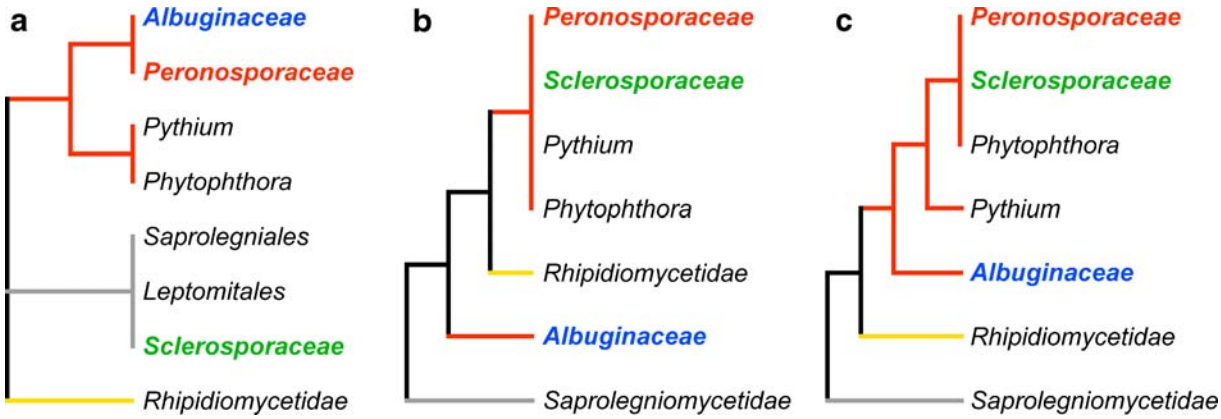

Fig. 1 Phylogenetic hypotheses among the Oomycetes, with special reference to obligate parasites and their closest relatives (*Pythium*, *Phytophthora*). **a** Topology reflecting the hierarchical classification of Dick (2001). **b** Topology obtained from cox2 sequence data (Hudspeth et al. 2003). **c** Topology obtained from SSU (Petersen and Rosendahl 2000) and LSU rDNA (Riethmüller et al. 2002) sequence data. *Red branches*: Peronosporomycetidae, *grey branches*: Saprolegniomycetidae, *yellow branches*: Rhipidiomycetidae. The three groups of obligate parasites (Albuginaceae = white blister rusts, Peronosporaceae = downy mildews, Sclerosporaceae = graminicolous downy mildews) are given in *colour* and *bold*; circumscription follows Kirk et al. (2001; see also Table 1). The molecular analyses are largely congruent except for the position of the Rhipidiomycetidae. Due to the isolated phylogenetic position of Albuginaceae, subclass Albuginomycetidae has been proposed (Thines and Spring 2005)

intermediate between *Phytophthora* and downy mildews, is not the closest relative to downy mildews and should rather be classified within *Phytophthora* (Riethmüller et al. 2002; Voglmayr 2003; Göker et al. 2007).

Monophyly versus polyphyly of downy mildews

The downy mildews (Peronosporaceae), in the traditional sense, are a morphologically diverse group, which is mainly united by obligate parasitism in combination with more or less complex conidio- or sporangiophores with determinate growth. In traditional morphological classifications it is generally assumed that obligate biotrophism of the downy mildews evolved only once.

In the first molecular phylogenetic analyses it was uncertain whether downy mildews are monophyletic or stem from different groups of *Phytophthora*, a situation which in fact has not yet been clarified with certainty (compare Riethmüller et al. 2002; Göker et al. 2003, 2007; Göker and Stamatakis 2006). In a recent multigene phylogeny involving five genes (Göker et al. 2007), monophyly of downy mildews was highly supported by various methods of phylogenetic reconstruction, which therefore seemed to be corroborated (Fig. 2a). However, in an analysis of the same dataset using different methods of phylogenetic reconstruction (Göker and Stamatakis 2006), downy mildews did not appear monophyletic, with the *Phytophthora infestans* group (*Phytophthora* 1) being embedded within the downy mildews clade (Fig. 2b). It is questionable whether this problem can be solved with comparably few DNA sequences alone; more detailed investigations on genome organisation and ultrastructure may provide better insights.

The paraphyly problem of *Phytophthora*

Phylogenetic classification using the monophyly criterion may raise severe problems for the genus *Phytophthora*, which is paraphyletic in most analyses in respect to downy mildews (e.g. Cooke et al. 2000, 2002; Göker et al. 2007; Fig. 2). Therefore, it remains open if a phylogenetic classification can be achieved without either merging downy mildews with *Phytophthora* or generic splitting of *Phytophthora* into several genera. It is foreseeable that these alternatives will not receive broad acceptance, representing a dilemma for classification. However, internal support for the tree backbone is usually low even in multigene analyses (e.g. Göker et al. 2007; Fig. 2), and additional data from other gene regions and taxa need to be collected before a robust phylogeny can be achieved.

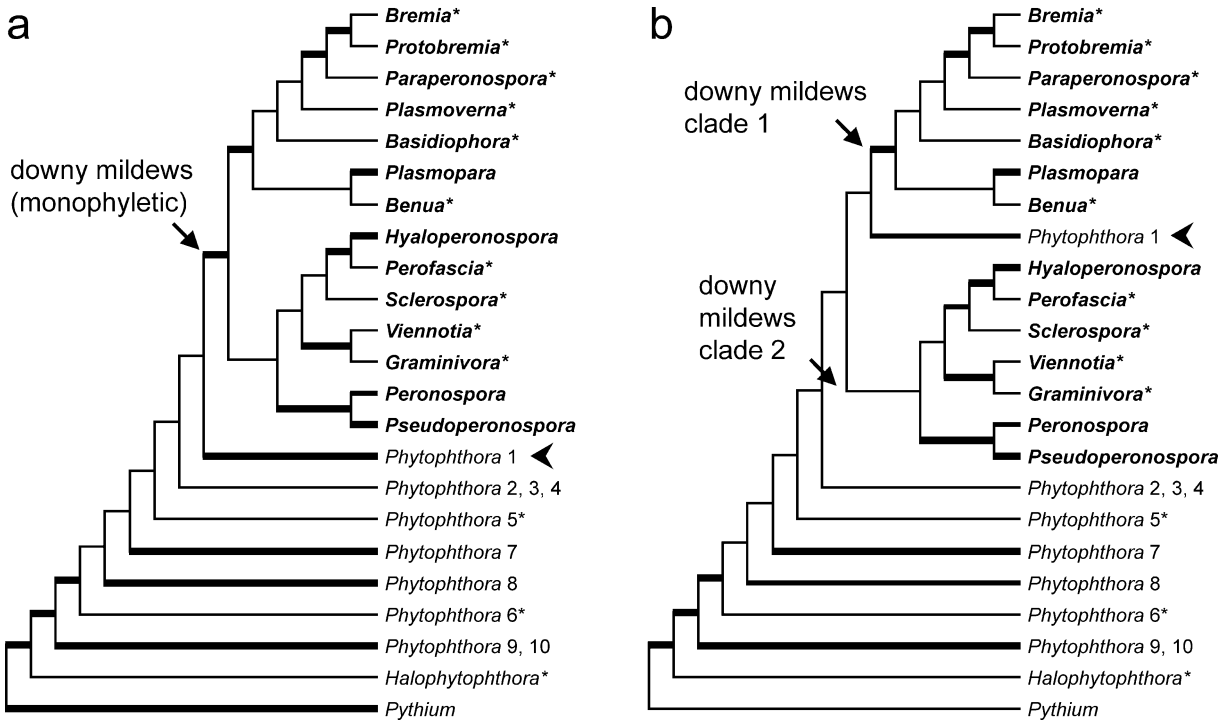

Fig. 2 Phylogenetic hypotheses among the downy mildews (genera in *bold*) inferred from DNA data. **a** Simplified tree illustrating the phylogenetic hypotheses of Göker et al. (2007) showing a single monophyletic downy mildews clade. **b** Simplified tree illustrating the phylogenetic hypotheses of Göker and Stamatakis (2006), showing polyphyly of two separate downy mildew clades; *Phytophthora* 1 (*P. infestans* group; arrowhead) is the closest relative of the downy mildews clade 1 containing, amongst others, *Plasmopara* and *Bremia*; the same tree topology was already largely revealed by Göker et al. (2003). *Bold* and medium branches indicate boostrap support equal or higher than 90% and 70%, respectively; genera marked with an *asterisk* are represented by a single taxon only. Note that in both trees the genus *Phytophthora* (closest relative of downy mildews) is paraphyletic. *Numbers* after *Phytophthora* correspond to the clade numbering in Cooke et al. (2000)

The status of the graminicolous downy mildews (Sclerosporaceae)

The controversial classification (Table 1, Fig. 1) of the graminicolous downy mildews (i.e. *Sclerospora* and *Peronosclerospora*) has been recently clarified with molecular phylogenetic data. Classification as a separate family and order (Dick et al. 1984), as well as the classification within the Saprolegniomycetidae (Dick et al. 1989), did not receive support from molecular data (Fig. 1). In all molecular phylogenetic investigations published so far (Riethmüller et al. 2002; Hudspeth et al. 2003; Göker et al. 2003, 2007; Thines et al. 2007, 2008), graminicolous downy mildews were unequivocally placed within Peronosporaceae. Therefore, separation of graminicolous downy mildews from downy mildews and classification within a separate family Sclerosporaceae has become obsolete (Figs. 1 and 2). The genus *Sclerophthora*, sometimes considered closely related to *Sclerospora* and *Peronosclerospora*, has been placed within *Phytophthora* in a recent molecular phylogenetic analysis (Thines et al. 2008), however, without significant internal support.

In addition to the graminicolous downy mildews in the strict sense (i.e. *Sclerospora*, *Peronosclerospora*), the species from the genera *Plasmopara* and *Bremia* parasitising Poaceae were also recently reclassified and transferred to newly described genera (Göker et al. 2003; Thines et al. 2006, 2007). Whether these are close relatives to *Sclerospora* and *Peronosclerospora* cannot be resolved with the current molecular data (Göker et al. 2007; Thines et al. 2008).

Phylogenetic relationship of the graminicolous downy mildews within the Peronosporaceae is much less clear. To date, only few accessions have been analysed. In some recent publications (Göker et al. 2007; Thines et al. 2007, 2008), graminicolous

downy mildews appear at varying basal positions within the downy mildew clade. However, this position lacks significant support.

Generic concepts in downy mildews

Generic concepts in downy mildews were (and still are) mainly based on conidio-/sporangiophore morphology in combination with conidial/sporangial morphology. Dichotomous versus monopodial branching of conidio-/sporangiophore, shape of the terminal branches and presence of conidia or sporangia were the primary features used for genus classification. However, interpretation of these morphological features was not always unequivocal and dependent on the observer, which sometimes resulted in conflicting generic concepts and delimitation (e.g. *Pseudoperonospora*: Skalický 1966 vs. Waterhouse 1973). Although the segregation of the genera *Paraperonospora* (Constantinescu 1989) and *Benua* (Constantinescu 1998) resolved some taxonomic problems of the morphological classification scheme of genera, generic classification remained problematic.

With the availability of molecular phylogenies, it soon became apparent that current generic classification and circumscription contained numerous problems and had to be adapted if standards of phylogenetic classification were applied (for a summary, see Table 2). Based on molecular and morphological features, the genera *Hyaloperonspora* and *Perofascia* were segregated from the large genus *Peronospora* (Constantinescu and Faheti 2002). The genus *Bremiella*, containing three species (Constantinescu 1991a), was shown to be polyphyletic, and all species were clearly embedded within *Plasmopara* (Riethmüller et al. 2002; Göker et al. 2007; Voglmayr and Thines

Table 2 Recent taxonomic changes at the generic level of white blister rusts and downy mildews based on molecular and morphological data

Pre-2000	Recent (post-2000)	References
Albugo	*Albugo* (sensu stricto)	Thines and Spring (2005)
	Pustula	Thines and Spring (2005)
	Wilsoniana	Thines and Spring (2005)
Basidiophora	*Basidiophora*	Riethmüller et al. (2002), Voglmayr et al. (2004), Göker et al. (2007)
Benua	*Benua*	Göker et al. (2007)
Bremia	*Bremia* (*B. lactucae*)	Voglmayr et al. (2004)
	Graminivora (*B. graminicola*)	Thines et al. (2006)
Bremiella	*Plasmopara*	Riethmüller et al. (2002), Voglmayr et al. (2004), Göker et al. (2007), Voglmayr and Thines (2007)
Paraperonospora	*Paraperonospora*	Riethmüller et al. (2002), Voglmayr et al. (2004), Göker et al. (2007)
Peronosclerospora	*Peronosclerospora*	Hudspeth et al. (2003), Thines et al. (2008)
Peronospora	*Peronospora*	Göker et al. (2007)
	Hyaloperonospora (*P. parasitica* s.l.)	Constantinescu and Faheti (2002), Göker et al. (2003, 2004)
	Perofascia (*P. lepidii*)	Constantinescu and Faheti (2002)
Plasmopara	Core *Plasmopara*	Voglmayr et al. (2004)
	Novotelnova (*Pl. savulescui*)	Voglmayr and Constantinescu (2008)
	Plasmoverna (*Pl. pygmaea* s.l.)	Constantinescu et al. (2005)
	Poakatesthia (*Pl. penniseti*)	Thines et al. (2007)
	Protobremia (*Pl. sphaerosperma*)	Voglmayr et al. (2004)
	Viennotia (*Pl. oplismeni*)	Göker et al. (2003)
Pseudoperonospora	*Pseudoperonospora*	Riethmüller et al. (2002), Göker et al. (2007)
Sclerospora	*Sclerospora*	Riethmüller et al. (2002), Göker et al. (2007)

The pre-2000 classification is according to the Dictionary of the Fungi (Kirk et al. 2001); the references refer to the post-2000 classifications

2007). On the other hand, the genus *Plasmopara* itself was shown to be a non-monophyletic genus, and several genera were segregated (see Table 2). Fortunately enough, it was nomenclaturally possible to maintain the use of the name *Plasmopara* for the bulk of species including the phytopathologically important ones (for details, see Constantinescu et al. 2005). The genus *Bremia*, consisting of two species (one on Asteraceae, one on Poaceae), was also shown to be polyphyletic, and the genus *Graminivora* was segregated for the grass parasite (Thines et al. 2006). Therefore, the number of accepted genera has gradually increased during the last years (one merged versus eight new genera). However, except for *Hyaloperonospora*, as none of these new genera is phytopathologically important, these changes have had little effect for phytopathologists.

Morphology revisited: shortcomings, new features and interpretations

The results of the molecular phylogenetic investigations also stimulated reevaluation of the morphological features traditionally used for classification and the search for new, previously neglected characters (Spring and Thines 2004; Voglmayr et al. 2004; Constantinescu et al. 2005; Thines 2006; Voglmayr and Riethmüller 2006). Already Hall (1996) stressed the necessity of thorough morphological investigations, besides other methods, to obtain a more satisfactory classification. Downy mildews and white blister rusts show a low morphological complexity, and comparatively few characters are available for classification. However, taking the whole spectrum of these few available characters into account, it is astonishing that even fewer selected characters had been used in traditional morphological classifications (i.e. some features of conidio-/sporangiophore morphology and conidial/sporangial morphology; for a synopsis, see Waterhouse 1973). In addition, these few characters often had not been critically studied and evaluated for more than a few species, and often data were just taken non-critically from the original descriptions. The morphology of the few common and phytopathologically important downy mildew species was comparatively well known, but the vast majority of species were much less studied. Therefore, morphological classification and interpretation was mainly based on the few phytopathologically important species, neglecting much of the morphological diversity.

With the advent of molecular systematics, the morphological features used for classification were reinterpreted in a phylogenetic context, making previous interpretations of conidio-/sporangiophore morphology too simple, partly incorrect or unsuitable for generic classification (e.g., Riethmüller et al. 2002; Voglmayr et al. 2004; Thines et al. 2006). Nevertheless, importance of known but previously neglected features became apparent, as is the case for haustorial morphology. Presence of different haustorial types in downy mildews was recorded by Fraymouth (1956), but these were never used for classification. Recently, several haustorial types could be shown to be diagnostic for several lineages (e.g. *Hyaloperonospora*: Constantinescu and Faheti 2002; *Plasmopara*, *Bremia* and their close relatives: Riethmüller et al. 2002; Göker et al. 2003; Voglmayr et al. 2004; Thines et al. 2006; *Pseudoperonospora*: Voglmayr et al. 2004).

In addition, the importance of searching for additional features to be used in classification was emphasised by Spring (2004) and Spring and Thines (2004). Ideally, these should be preserved in herbarium specimens to enable investigation of as many representatives as possible, which would be difficult if living specimens were necessary. As herbarium specimens can be used, Spring (2004) and Spring and Thines (2004) argued for investigations of ultrastructural features by scanning electron microscopy (SEM) to reveal additional features. Thines (2006, 2007b) presented the results of detailed morphological investigations of downy mildews including SEM studies of conidio-/sporangiophores and sporangia. He produced a morphological character matrix and analysed it within the constraint of recently published molecular phylogenetic investigations to reveal and evaluate morphological synapomorphies. He concluded that the classical features used for classification like conidio-/sporangiophore branching or sporangial germination are not diagnostic for phylogenetic lineages above the generic level. However, the fine structure of the ultimate branchlets (revealed by SEM) and haustorial shape were considered to be phylogenetically informative.

The genus *Albugo* sensu lato has also recently been extensively re-investigated using light microscopy

and SEM. Thines and Spring (2005) emphasised that different sporangial ornamentation types revealed by SEM were diagnostic for the *Albugo* clades revealed by molecular data, and segregated the two genera *Wilsoniana* (for species on Caryophyllidae) and *Pustula* (for species on Asteraceae). Voglmayr and Riethmüller (2006) confirmed the SEM data of Thines and Spring (2005) and added detailed light microscopical and SEM data of the oospores, which were also shown to be diagnostic for the different molecular phylogenetic lineages of *Albugo* sensu lato. Constantinescu and Thines (2006) investigated and clarified sporangiogenesis in Albuginaceae; they confirmed the presence of sporangial dimorphism (primary and secondary sporangia) for all species investigated.

Biochemical characters

Apart from DNA sequence data, few characters at the biochemical level have been used for classification of downy mildews. Recently, fatty acids were recorded as potentially promising for the characterisation of downy mildew species (e.g., Spring et al. 2003; Spring, 2004; Spring and Thines 2004; Spring et al. 2005). In the case of *Albugo* sensu lato, Spring et al. (2005) recorded significant differences between three species and considered fatty acid pattern characteristic for the species. Investigations on closely related species are still missing, and therefore additional data are necessary to evaluate applicability and resolution limits of fatty acid composition for taxonomic differentiation.

Species concepts in downy mildews

The species concept is probably the most controversial issue in downy mildew systematics, partly due to experimental difficulties to test it and partly due to its profound implications for researchers outside the systematics research community. A well-written account of the history and implications of the different species concepts is given in Hall (1996). In downy mildews, several species concepts were applied, which resulted in highly different numbers of accepted species depending on the criteria used. The main problem in species delimitation in downy mildews is that there are numerous indications that, due to their obligate parasitism, they often have narrow host ranges and therefore represent genetically distinct species. On the other hand, host specificity is not always paralleled by morphological distinctness. Therefore, if morphology is used as a primary criterion for species definition, only a few species can be defined and accepted in many lineages, resulting in genetically heterogeneous species.

Historically, two approaches were commonly followed, which were both mainly based on host ranges: the splitting approach of Gäumann (1918, 1923) versus the lumping approach of Yerkes and Shaw (1959). Gäumann (1918, 1923) advocated a narrow species concept in *Peronospora*, based on his results of cross-inoculation studies and minute morphological differences. Each species was usually assumed to be confined to one host genus or even a few host species (one host-one species concept; see Hall 1996). Conversely, Yerkes and Shaw (1959) argued that host specificity is neither sufficient nor suitable for the recognition of a species without clear-cut morphological differences. As a consequence, they lumped the numerous *Peronospora* species on Brassicaceae and Chenopodiaceae each into a single species (*Peronospora parasitica* and *P. farinosa*, respectively), resulting in a wide one host family-one species concept.

Both the splitting as well as the lumping approach have sincere shortcomings. Using the narrow species concept, identification of morphologically similar species is often difficult or impossible without correct identification of the host. In addition, high host specificity has rarely been conclusively demonstrated, weakening the primary underlying assumption of the narrow species concept.

In a wide species concept, there is the problem that genetically distinct or even unrelated entities may be classified in the same species, raising incorrect assumptions on biology and host ranges. This is especially problematic if host jumps are common and parasitism on the same host family has evolved multiple times, resulting in polyphyletic species. However, due to its easier applicability, the approach to classify all accessions of a given host family within a single species was widely followed by phytopathologists and molecular biologists.

Impact of molecular data on downy mildew species concept and circumscription

Recently, molecular phylogenetic investigations have enabled the evaluation of the species problem using new perspectives and have led to the shift from a morphological to a phylogenetic species concept. A biological species concept directly addressing mating barriers has never been applied to downy mildews due to sincere methodological difficulties, and it is unlikely that these can be overcome. Therefore, reproductive isolation can only be indirectly assessed, e. g. by genetic distance of sequence data. The impact of molecular data is manifold: (1) numerous additional characters are available for recognition and distinction; (2) presence and amount of reproductive isolation can be assessed; (3) presence and amount of genetic distances provide indirect but strong evidence for host specificity and host ranges; (4) molecular data are less variable and prone to subjective interpretation than morphological data; (5) molecular data provide a sound basis for species identification even if morphological data are missing or incomplete; (6) pathotypes or races, the basic entities for experiments in applied sciences, can be properly attributed to a species and their phylogenetic relationships can be assessed. Therefore, in the absence of sound morphological characters, the species concept is increasingly based on molecular evidence of reproductive isolation, which is a general tendency within mycology. Consequently, morphologically similar cryptic species are often recognised as distinct species if reproductive isolation and genetic distinctness can be demonstrated. However, evaluation of species boundaries by molecular data require thorough sampling throughout the distribution area to assess genetic variability as well as reproductive isolation, and at best several molecular markers should be used for corroboration of species boundaries.

Due to easy amplification and variability, the ITS rDNA region has been used in most investigations addressing the species concept in downy mildews and white blister rusts (e.g. Choi et al. 2003, 2005, 2006, 2007a, b, c, d; Voglmayr 2003; Göker et al. 2004; Scott et al. 2004; Cunnington 2006; Spring et al. 2006; Voglmayr et al. 2006; Landa et al. 2007; García Blázquez et al. 2008). However, the mitochondrial cox2 region may also be a promising candidate to resolve species boundaries and for species identification (e.g. Choi et al. 2006, 2007d). Interestingly, the current evidence from molecular phylogenetic investigations often supports a narrow species concept as advocated by Gäumann (1918, 1923), although there are sometimes marked differences in detail. In the following, the results of recent molecular investigations will be briefly summarised for the different genera.

Hyaloperonospora

According to Constantinescu and Faheti (2002), about 140 species names were published attributable to this genus. In their separation of *Hyaloperonospora* from *Peronospora*, Constantinescu and Faheti (2002) only recognised six morphologically distinct species, and accessions from most hosts of Brassicaceae were placed in *Hyaloperonospora parasitica*. However, subsequent molecular phylogenetic investigations demonstrated that the latter was a paraphyletic assemblage with respect to the other five *Hyaloperonospora* species, and that many more species should be accepted based on the high genetic distances observed (Choi et al. 2003; Göker et al. 2003, 2004; Voglmayr 2003). Usually, these genetically distinct entities deserving species rank have a narrow host range and are confined to host genera or even species; however, in some cases accessions from the same host do not form a monophylum (e.g. from *Armoracia rusticana*; see Göker et al. 2004). Therefore, it is problematic when species are determined solely on host association, as this is often but not always conclusive. The case study of *Hyaloperonospora* is also relevant for investigations at the molecular level of plant–pathogen interactions, as numerous studies are performed with the plant model organism *Arabidopsis thaliana* and its *Hyaloperonospora* parasite. The parasite is usually named *H. parasitica*, but it is genetically quite distinct from *H. parasitica* sensu stricto which is confined to *Capsella bursa-pastoris* (Göker et al. 2004); therefore, the name *H. arabidopsidis* should be used for the *Arabidopsis* parasite.

Peronospora

The molecular phylogenetic analyses dealing with the genus *Peronospora* (e. g. Voglmayr 2003; Choi et al.

2007c; García Blázquez et al. 2008) also provide evidence for a narrow species circumscription. Species parasitising e.g. Chenopodiaceae do not form a monophyletic lineage (Voglmayr 2003; Choi et al. 2007c), but are interspersed with species from other host families and can often also be separated morphologically (Choi et al. 2007c). Interestingly, the type species of *Peronospora*, *P. rumicis*, a parasite of *Rumex* spp. (Polygonaceae), is embedded within a group of species infecting Chenopodiaceae (Choi et al. 2007c), which may indicate a recent host jump. Choi et al. (2007c) demonstrated that *Peronospora effusa* (spinach downy mildew) is genetically homogeneous world-wide, but distinct from *P. farinosa* sensu stricto. Reasons for this genetic homogeneity may include the recent introduction to most of its present growth area as well as pathogen transmission by seeds, enabling rapid dispersal from a small geographic source area via international seed trade. Choi et al. (2008a), investigating five *Peronospora* species from different species of *Chenopodium*, recorded significant molecular and morphological differences, and they concluded that these are well-distinct species and should not be merged with *P. farinosa*. Therefore, the approach of Yerkes and Shaw (1959) to merge all species on Chenopodiaceae under *P. farinosa* appears to be inappropriate, as *P. farinosa* according to Yerkes and Shaw (1959) is evidently a polyphyletic assemblage. However, further studies using additional variable genes are required to reveal and evaluate the phylogenetic species, as the ITS region does not give significant support or resolution for many nodes of the backbone (Voglmayr 2003; Choi et al. 2007c).

Traditionally, from de Bary (1863) onwards, only two *Peronospora* species have been recognised on Fabaceae, *P. trifoliorum* and *P. viciae*, which were observed to differ in their oospore ornamentation. Gäumann (1923) and subsequent authors again described numerous new species from different host species, resulting in >100 *Peronospora* binomials described from 25 host genera of Fabaceae (Constantinescu 1991b). The results of Voglmayr (2003) indicated that *Peronospora* on Fabaceae does not form a monophyletic lineage, although most accessions including those from *Vicia* and *Trifolium* were united in a single monophyletic clade. However, the clades did not correspond to the classical two species recognised from Fabaceae and showed that more than two species are involved. Cunnington (2006) confirmed high genetic distances between accessions from different hosts, which were traditionally included in *P. viciae*. In the most extensive study on *Peronospora* parasites of Fabaceae, García-Blázquez et al. (2008) showed that numerous host-specific lineages are present on Fabaceae. Although their study did not include *Peronospora* species from other host families, there is evidence that they do not even form a monophyletic lineage (Voglmayr 2003), a situation comparable to the *Peronospora* species on Chenopodiaceae. Although there are numerous nomenclatural problems left in the Peronosporas from Fabaceae which need additional studies (García-Blázquez et al. 2008), the results are roughly concordant with the classification of Gäumann (1923).

Molecular phylogenies using ITS data not only often confirmed a rather narrow species concept in *Peronospora*, but also helped to clarify species attribution. Scott et al. (2004) identified the parasite of oilseed poppy (*Papaver somniferum*) from Tasmania, which was previously listed as *Peronospora arborescens*, as *P. cristata*, and both species were shown to be genetically distinct. Conversely, Landa et al. (2007) reported *Peronospora arborescens* as the causal agent of downy mildew of *P. somniferum* from Spain. Therefore, apparently both *P. arborescens* and *P. cristata* can infect *P. somniferum* and are of different importance in various regions of the world.

Pseudoperonospora

Conversely to the other examples listed above, in *Pseudoperonospora* molecular data provided evidence for conspecificity of species from different host families. Choi et al. (2005) performed a study including *Pseudoperonospora humuli* (from *Humulus* spp., Cannabinaceae) and *P. cubensis* (from *Citrullus vulgaris*, *Cucumis* spp. and *Cucurbita* spp., Cucurbitaceae). Distinction as separate species was mainly based on their occurrence on two non-related host families, but almost identical ITS sequences and absence of significant morphological differences indicate conspecificity. Therefore, Choi et al. (2005) synonymised *Pseudoperonospora humuli* with *P. cubensis*. These data confirm recent and possibly multiple host shift from Cannabinaceae to Cucurbitaceae. However, for detailed insights into the evolu-

tionary processes involved, the ITS data offer too little resolution, and molecular markers with higher resolution should be applied.

ITS length differences as potential markers for species

In addition to phylogenetic analyses of ITS data, the structure of the ITS itself has also recently received increasing attention. This appears to be especially promising for the *Plasmopara-Bremia* clade, which was shown to have an ITS region of variable length up to about 3,200 bp (Choi et al. 2007b; Komjáti et al. 2007; Thines et al. 2005; Thines 2007a), which is remarkable compared to the usually about 800 bp in downy mildews (Voglmayr 2003; Göker et al. 2004). Size increase mainly concerns the ITS2 region and originates from repetitive elements, the number and length of which appear to be taxon-specific (Choi et al. 2007b; Thines 2007a, c). The number and sequence of repetitive elements is usually conserved within different lineages. Choi et al. (2007b) investigated *Bremia* accessions from different hosts and identified nine repetitive elements showing high sequence heterogeneity between the accessions from different hosts, which indicates that they may represent distinct species. Thines (2007a) investigated repetitive elements for several species and recorded a highly variable number. He concluded that these repetitive elements could be useful for investigation of speciation and radiation processes. Based on sequence variability observed in the ITS2 region, Spring et al. (2006) could separate and characterise two groups of pathotypes of *Plasmopara halstedii* from sunflower.

Repetitive elements are also present in the ITS2 region of some *Hyaloperonospora* species (Voglmayr 2003; Göker et al. 2004); however, they are confined to a few species for which they may be diagnostic. Thines (2007a) recorded similarities to the repetitive elements of the *Plasmopara-Bremia* clade and suggested that this may be indication of a closer relationship for these two lineages.

Repetitive elements are also observed within *Peronospora* sensu stricto, where they have been reported within the ITS1 region (Voglmayr 2003). The number of repetitive elements has recently been investigated and analysed in detail for *Peronospora* on *Trifolium* (García Blázquez et al. 2008). The number of additional copies of a region about 70 bp long ranged from one to 11 and was, with few exceptions, diagnostic for different host-specific lineages. Therefore, different lengths of ITS may, with limits, be suitable for species identification. However, little is yet known on the mechanisms governing the copy number of repetitive elements, and taxon specificity needs additional detailed investigations.

Discovery of morphologically distinct species by molecular data

Molecular phylogenetic data also stimulated closer morphological examinations at the species level, which showed that the non-critical but widely applied species identification solely on host association can be misleading. Several new species could be described which were morphologically well distinct but remained remarkably unnoticed (e.g., Voglmayr et al. 2006; Choi et al. 2007a). *Plasmopara* on Geraniaceae provides an excellent example for this. Traditionally, two morphologically distinct species, *Plasmopara pusilla* on European and *P. geranii* on North American *Geranium* species, were accepted and recognised (Constantinescu 2004). Detailed molecular and morphological investigations confirmed distinctness of another previously described but neglected species and revealed two additional undescribed species which are morphologically distinct, widespread and rather common (Voglmayr et al. 2006). This indicates that biodiversity of downy mildews is still imperfectly known even in regions which are considered well-studied.

Unresolved questions and future perspectives

Detailed phylogenetic relationships and evolutionary scenario within downy mildews

It remains still unresolved whether downy mildews are monophyletic and how the major groups of downy mildews are related to each other. Clarification of phylogenetic relationships will necessitate additional molecular markers, new methods of phylogenetic inference as well as improved taxon sampling. The graminicolous downy mildews and representatives of

remote, little sampled areas (Africa, South America, East and Southern Asia) are especially underrepresented in the present studies, and additional taxa need to be sampled. Albuginales are also little-investigated, and recent investigations on the *Albugo candida* complex indicate a high level of, as yet, undescribed biodiversity (Choi et al. 2007d, 2008a, b, which could also be true in other lineages. In other cases such as the downy mildews with pyriform haustoria *Plasmopara*, *Bremia* and relatives, additional sequence data are needed to confirm more robust phylogenetic relationships, a precondition for a sound delimitation of genera. In addition, the investigations need to be embedded in a wider taxonomic context. *Phytophthora* especially needs to be included, which is a key genus for the evolution of downy mildews. In the recent multigene phylogeny of *Phytophthora* by Blair et al. 2008), obtained from a data matrix of 8,700 bp from seven genes, various *Phytophthora* groups were highly supported; however, internal support for the tree backbone was still only moderate to low in maximum parsimony and likelihood analyses, despite the large data matrix, which may indicate that it may be difficult to obtain highly supported gene phylogenies for *Phytophthora* and its relatives. To test this, these sequence data should be complemented and analysed with corresponding sequence data of representative downy mildews as well as from the genus *Pythium*. Sound phylogenetic hypotheses are a precondition for detailed insights into the processes of character evolution, adaptive radiation and speciation of downy mildews on different host groups. The species-rich genera *Peronospora* and *Plasmopara* especially require extended, representative taxon sampling as well as additional molecular markers to provide a sound phylogenetic framework. It is still little investigated whether polyploidy is involved in speciation, and detailed studies on nuclear genome size may provide information on genome evolution (Voglmayr and Greilhuber 1998).

Whole genome analysis in an evolutionary context

For a better understanding of the evolutionary processes and of phylogenetic relationships, the integration of whole genome data is also promising, especially where phylogenetic relationships remain unsettled with the current molecular phylogenetic analyses (e.g. monophyly of downy mildews, detailed relationships of graminicolous downy mildews, detailed relationships of genera). The availability of whole genome sequences of several species of *Phytophthora* should accelerate our understanding of molecular evolution in plant pathogenic oomycetes (Lamour et al. 2007). However, for progress in comparative genome analysis, it is critical that high quality genome sequence assemblies and gene models are developed, which is a next, urgently needed step (Lamour et al. 2007). When such high-quality data become available for *Phytophthora*, they could provide the basis for new evolutionary and phylogenetic investigations on downy mildews. As *Phytophthora* is the closest relative of downy mildews, the genetic models of host specificity and host jumps could be adapted to and tested in downy mildews. The *Hyaloperonospora-Arabidopsis* pathosystem would be an ideal candidate, as both host and pathogen are genetically well studied, and as the genus *Hyaloperonospora* contains numerous host-specific, genetically distinct entities commonly recognised as separate species (Choi et al. 2003; Göker et al. 2004). In addition, comparative analysis of genome organisation could provide detailed evolutionary insights. However, it should be mentioned that these investigations are methodologically difficult and require the development of sophisticated techniques as downy mildews cannot be cultivated on artificial media. It is evident that sequencing of the whole genome or even large quantities of the genome can practically only be done for a few selected representatives. In addition, suitable computational methods of phylogenetic analysis of such large data sets need to be developed. Despite these limitations, whole genome data could contribute to more robust phylogenetic hypotheses than investigations based on one or a few sequences regions only, and could provide help for the selection of promising proper genome regions for phylogenetic analyses on an extended taxon selection.

Applicable species definitions and the need for taxonomic and nomenclatural revisions

The most important issues of downy mildew systematics outside the taxonomic community concern the species concept. Nomenclatural stability as well as sound applicable species circumscription are eagerly anticipated. For this, more detailed and conclusive

molecular studies are required to resolve the species boundaries, especially in the species-rich genera *Peronospora*, *Hyaloperonospora* and *Plasmopara*. In addition, to achieve nomenclatural stability, numerous taxonomic and nomenclatural problems involving host range and correct typification need to be solved. Although molecular data provide strong evidence that a narrow species concept as advocated by Gäumann (1918, 1923) may in many cases be appropriate, there are numerous problems in his classification in detail. Whereas some of his species are apparently conspecific, others are heterogeneous. As he did not designate type collections, lectotypification is necessary which for heterogeneous assemblages has great impact on species nomenclature. This has been recently discussed for *Peronospora* on Fabaceae (García Blázquez et al. 2008), but is true also for other lineages of *Peronospora* and *Plasmopara*. Therefore, thorough investigations of types and proper typification whenever necessary are tedious but unavoidable prerequisites to receive correct and stable circumscription for many species. In addition, as shown above, numerous distinct species remained undetected up to now, which require additional thorough studies involving morphological as well as molecular data. Even lineages containing important plant pathogens such as the *Plasmopara halstedii* group are in need of critical taxonomic and nomenclatural revision to reveal an appropriate species delimitation.

Molecular bar coding systems for improved and reliable species identification

Another important issue is the development of methods for reliable and easy species identification. As discussed above, morphology is often not the best basis for identification due to a lack of morphological distinctness of numerous genetically well distinct lineages. In addition, all morphological structures necessary for identification are not always present on a specimen to be identified. Especially for the plant pathology community, molecular methods are much easier to use, and are nowadays routine procedures, and provide reliable identification even with the lack of morphological structures required for identification (e.g. sporangiophores, oospores). Therefore, a species identification system based on sequence data is highly desirable. Numerous sequence data are already available in sequence databases like Genbank; however, these data usually suffer from the lack of standards concerning correct identification, documentation, nomenclature but also the sequence data quality. Therefore, molecular bar coding initials have been recently proposed and started for many taxonomic groups of organisms to facilitate identification (see the publications of the themed issue of the Philosophical Transactions of the Royal Society, Biological Sciences 360, Number 1462, 2005). Molecular bar coding requires strict quality standards for the laboratory routine, the sequence data as well as identification, documentation, specimen deposition and nomenclature; for details see the homepage of the Consortium for the Barcode of Life (http://barcoding.si.edu/index.htm). Evidently, a bar coding approach needs to be accompanied by thorough taxonomic revisions to provide a proper taxonomic framework.

Crucial for the resolution of a molecular bar coding system is the selection of the sequence region used. Ideally, species identification should be possible with a single and the same sequence over a wide range of organisms, at best with the same primers, which is problematic for downy mildews. Therefore, a compromise between applicability for as many different taxa as possible and sufficient taxonomic resolution needs to be found. One possible candidate is the ITS rDNA: it is a multicopy region easy to amplify in most downy mildews, can detect pathogens with high sensitivity, specific as well as universal primers are available, and it is also a region of choice in *Phytophthora*, the closest relative of downy mildews. In addition, numerous data on ITS are already available for downy mildews which is important for testing the discriminatory power of the data. However, amplification and sequencing can be troublesome in lineages affected by substantial size increases of the ITS region (see above), limiting universal applicability. Alternatively, sequences from the mitochondrial DNA should be considered. It is usually much easier to amplify from historic material than nuclear DNA due to a higher copy number, and therefore herbarium collections can be used. A promising candidate for bar coding is cox2, which offers high resolution in some closely related species groups (e.g. Choi et al. 2006, 2007d), also mtDNA sequence stretches less variable than cox2 should be considered, e.g. ribosomal mtDNA and cox1 or cox3. Additional detailed,

comparative studies are necessary before the most appropriate sequence region can be selected. However, the development of a molecular bar coding system is now within reach.

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