Fungal Diversity (2013) 58:61–72
DOI 10.1007/s13225-012-0179-4

ORIGINAL PAPER

Molecular data place the hyphomycetous lichenicolous genus Sclerococcum close to Dactylospora (Eurotiomycetes) and S. parmeliae in Cladophialophora (Chaetothyriales)

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Received: 6 January 2012 / Accepted: 23 May 2012 / Published online: 17 June 2012

Abstract The lichenicolous anamorphic fungus *Sclerococcum parmeliae* was isolated in pure culture, and ITS, nuLSU and mtSSU sequences were obtained from these isolates. For comparison, sequences from *S. sphaerale*, the generic type, were obtained directly from freshly collected specimens. Phylogenetic analyses place *S. sphaerale* with species of *Dactylospora* and an unidentified lichen-inhabiting isolate in a strongly supported clade that is sister to a lineage comprising members of the Chaetothyriales and Pyrenulales. In contrast, *S. parmeliae* is inferred as a member of the Herpotrichiellaceae (Chaetothyriales) and belongs to a robustly supported clade that also includes species of *Cladophialophora*, *Capronia semiimmersa*, and *Phialophora verrucosa.* Within the Herpotrichiellaceae, *S. parmeliae* most closely resembles members of the anamorph genus *Cladophialophora*. Accordingly, we propose the transfer of *S. parmeliae* and the morphologically similar species *S. cladoniae*, *S. hawksworthii* and *S. normandinae* to *Cladophialophora*. A new lichenicolous species, *Clad. megalosporae*, collected twice on *Megalospora* in Florida and Papua New Guinea, is also described.

Keywords Anamorphic fungi · *Capronia* · Conidial fungi · Hyphomycetes · Parasitic fungi

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Introduction

The genus *Sclerococcum* Fr. was originally described for the single lichenicolous hyphomycete *S. sphaerale* (Ach. : Fr.) Fr., a species that forms conspicuous, blackish, compact, stromatic sporodochia on the thallus of *Pertusaria* gr. *corallina*, in which dark brown, 2–6-celled conidia are produced in irregular chains (Hawksworth 1975, 1979). Fifteen additional species of *Sclerococcum* have been described since; all but one member of the genus are lichenicolous, and most species are confined to a single host species or genus (see http://www.lichenicolous.net). In addition to a number of species with blackish, compact sporodochia (keyed out by Etayo and Calatayud 1998), several other species with quite loose, greyish or brown sporodochia and irregularly catenate 0–1-septate conidia that separate easily in squash preparations have been described in *Sclerococcum*, including *S. cladoniae* Diederich, *S. hawksworthii* Etayo & Diederich, *S. normandinae* Diederich & Etayo and *S. parmeliae* Etayo & Diederich (Etayo and Diederich 1995, 1996). *Sclerococcum griseosporodochium* Etayo was described as possibly lichenicolous over saxicolous lichens (Etayo 1995) but it is now regarded as a lichenized, non-lichenicolous species associated with a *Trentepohlia* photobiont (Smith 2009).

Although morphological variation among species of *Scleroccocum* suggests that the genus is strongly heterogeneous,

molecular data are needed to assess the phylogenetic position of *S. sphaerale*, the generic type, and other representatives of the genus. The aim of this study was to investigate for the first time the position of *S. sphaerale* within the Ascomycota using molecular data and to determine if this species is closely related to *S. parmeliae*, a member of the genus that produces catenate, disarticulating conidia.

Materials and methods

Morphological study

Herbarium specimens are deposited in BR, DBN, LG, M and NY, and in the private collections of P. Diederich and F. Berger. Microscopical examination (including all microscopical measurements) was carried out using thin handmade sections mounted in water. Conidial measurements of the new species are indicated as (minimum–)$\overline{X} - \sigma_X - \overline{X}+ \sigma_X$(–maximum), followed by the number of measurements (n); the length/breadth ratio of conidia is indicated as l/b and given in the same way.

Isolation of fungal cultures

Conidiomata of *S. parmeliae* were isolated from lichen host tissues in 70 % ethanol, dried on a glass slide, and crushed in sterile water; conidia were then collected in water and plated onto Maltose Extract agar or Potato Dextrose agar (Difco, Detroit, Michigan, USA) lacking antibiotics, where they were observed to germinate within 7 days. Conidiomata of freshly collected material of *Sclerococcum sphaerale* were crushed in sterilized water, and conidia transferred to Malt-Yeast Extract agar following Yoshimura et al. (2002). Germination occurred within a few days, but the resulting cultures ceased growing on this medium after a week.

Molecular techniques

Cultures of *Sclerococcum parmeliae* (CBS 129337) were grown on Sabouraud's agar with maltose (SMA, Difco, Detroit, Michigan, USA) for 2 weeks. Genomic DNA was extracted from harvested mycelia using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogen, Illkirch, France) according to the manufacturer's protocol with slight modifications. Approximately 10 ng of extracted DNA were subjected to a standard PCR in a 25 µL reaction volume using either Taq Gold polymerase (Applied Biosystems, Foster City, California, USA) or Bio-X-Act Long Mix (Bioline USA, Inc., Taunton, Massachusetts, USA). We amplified and sequenced approximately 1,700 bp of the nuLSU using primers LR0R, LR3R, LR8R, LR5, LR7 and LR9, and for *S. parmeliae* (CBS 129337), the ITS using primers ITS5 and ITS4 (available on the Duke Mycology website, http://www.biology.duke.edu/fungi/mycolab/primers.htm). Primers for amplification and sequencing of the mtSSU rDNA were mrSSU1 and mrSSU3R (Zoller et al. 1999). Hyphae of the second culture of *S. parmeliae* (CBS 132232) were added to a tube containing the PCR reaction mixture and amplified directly, for the nuLSU using LIC15R and LR6 (available on the Duke Mycology website, http://www.biology.duke.edu/fungi/mycolab/primers.htm) and for the mtSSU rDNA using mrSSU1 and mrSSU3R. The method of direct PCR as explained in Lawrey et al. (2007: 780) was performed on the conidiomata of three freshly collected specimens of *Sclerococcum sphaerale* using the same primers as for *S. parmeliae* (CBS 132232).

PCR products were visualized in a 1 % agarose gel with ethidium bromide, purified with Ampure magnetic beads (Agencourt Biosciences, Beverly, Massachusetts, USA) following the manufacturer's instructions, and used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). Reactions were then purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, Missouri, USA), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems), and sequenced using a 3130xl Genetic Analyzer (Applied Biosystems). Sequences were analyzed using Sequence Analysis v5.4 (Applied Biosystems) and were manually edited using Sequencher v4.7 (Gene Codes Corporation, Ann Arbor, Michigan, USA), and overlapping fragments were assembled in larger consensus sequences.

Taxon selection and phylogenetic analyses

Taxa selected for phylogenetic analyses (Table 1) included members of the Chaetothyriales published by Crous et al. (2007), Gueidan et al. (2008), Ruibal et al. (2005, 2008), and Untereiner and Naveau (1999), as well as those retrieved from GenBank based on searches of the newly generated sequences of *Sclerococcum* using BLAST (Altschul et al. 1990). Major groups represented in these phylogenies include the Herpotrichiellaceae, a number of the strongly supported lineages basal to this family that were recognized by Badali et al. (2008) and Untereiner et al. (2011), and clades corresponding to the Coryneliales, Eurotiales, Lichinales, Mycocaliciales, Onygenales and Pyrenulales.

Alignments were generated with Clustal X 1.82 (Jeanmougin et al. 1998) and edited using Se-Al 2.0a11 (Rambaut 2002). Multiple base indels were reduced to single characters, and all ambiguously aligned sequences were excluded. The positions of *Sclerococcum parmeliae* and *S. sphaerale* within the Ascomycota were inferred based on

Table 1 Sources and accession numbers of the isolates examined in this study

Taxon	Source[a]	GenBank Accession Numbers[b]	
		ITS	nuLSU
Anthracothecium nanum (Zahlbr.) R.C. Harris	J.C.L. 4525	–	FJ358271
Arachniotus littoralis (G.F. Orr) Arx	CBS 454.73	–	FJ358272
Aspergillus fumigatus var. *acolumnaris* J.N. Rai et al.	NRRL 5587	–	AFU28465
Caliciopsis pinea Peck	CBS 139.64	–	DQ678097
Cal. orientalis A. Funk	CBS 138.64	–	DQ470987
Capronia epimyces M.E. Barr	DAOM 216385	–	AF050244
Cap. peltigerae (Fuckel) D. Hawksw.	UAMH 11090	–	HQ613813
Cap. pilosella (P. Karsten) E. Müller et al.	DAOM 216387	AF050254	DQ823099
Cap. pulcherrima (Munk) E. Müller et al.	DAOM 216384	AF050256	AF050256
Cap. semiimmersa (Cand. & Sulmont) Unter. & F.A. Naveau	MUCL 40572	AF050259	FJ358226
Cap. semiimmersa	MUCL 39979	AF050260	–
Chaenothecopsis savonica (Räsänen) Tibell	Tibell 15876	–	AY796000
Cladophialophora australiensis Crous & A.D. Hocking	CBS 112793	EU035402	–
Clad. boppii (Borelli) de Hoog et al.	CBS 126.86	EU103997	FJ358233
Clad. boppii	CBS 110029	EU103998	–
Clad. boppii	CBS 124175	GQ426956	–
Clad. carrionii (Trejos) de Hoog et al.	CBS 160.54	EU137266	FJ358234
Clad. carrionii	CBS 406.96	EU137317	–
Clad. carrionii	CBS 859.96	AY857520	–
Clad. chaetospira (Grove) Crous & Arzanlou	CBS 115468	EU035404	EU035404
Clad. chaetospira	CBS 491.70	EU035405	–
Clad. minourae (Iwatsu) Hasse & de Hoog	CBS 556.83	AY251087	FJ358235
Clad. modesta McGinnis et al.	CBS 985.96	–	FJ358236
Clad. potulentorum Crous & A.D. Hocking	CBS 112222	EU035409	–
Clad. potulentorum	CBS 114772	EU035410	–
uncultured *Cladophialophora* clone RELIS K1 D02	–	JF519044	–
uncultured *Cladophialophora* clone RELIS K1 E03	–	JF519056	–
uncultured *Cladophialophora* clone RELIS K6 G06	–	JF519312	–
Coniosporium perforans Sterfl.	CBS 885.95	–	FJ358237
Dactylospora haliotrepha (Kohlm. & E. Kohlm.) Hafellner	Kohlmeyer 5129B	–	FJ713617
D. mangrovei E.B.G. Jones et al.	CBS 110444	–	FJ176890
Eupenicillium limosum S. Ueda	CBS 339.97	–	EF411064
Exophiala dermatitidis (Kano) de Hoog	CBS 207.35	–	DQ823100
Ex. jeanselmei (Langeron) McGinnis & A.A. Padhye	CBS 664.76	–	AF050271
Ex. nigra (Issatsch.) Haase & de Hoog	d.H. 12296	–	FJ358244
Ex. pisciphila McGinnis & Ajello	CBS 537.73	–	NG027583
Ex. salmonis J.W. Carmichael	CBS 157.67	–	EF413609
Fonsecaea monophora (M. Moore & F.P. Almeida) de Hoog et al.	CBS 102243	EU938579	FJ358247
F. pedrosoi (Brumpt) Negroni	CBS 274.66	EU938587	–
Peltula auriculata Büdel et al.	DUKE 47648	–	DQ832330
Pel. umbilicata (Vainio) Swinscow & Krog	DUKE 47527	–	DQ832334
Phaeococcomyces catenatus (de Hoog & Herm.–Nijh.) de Hoog	CBS 650.76	–	AF050277
Phialophora verrucosa Medlar	MUCL 9760	AF050281	AF050281
Phial. verrucosa	MUCL 9768	AF050282	–
Pleopsidium chlorophanum (Wahlenb.) Zopf.	V.R. 8-VIII-02/8	–	DQ842017
Pyrenula aspistea (Ach.) Ach.	CBS 109078	–	EF411063
Pyr. cruenta (Mont.) Vainio	DUKE 47880	–	AF279407

Table 1 (continued)

Taxon	Source[a]	GenBank Accession Numbers[b]	
		ITS	nuLSU
Pyr. pseudobufonia (Rehm) R.C. Harris	DUKE 47599	–	AY640962
Pyrgillus javanicus (Mont. & Bosch) Nyl.	DUKE 47748	–	DQ823103
Sarcinomyces petricola Wollenz. & de Hoog	CBS 101157	–	FJ358249
Sclerococcum parmeliae Etayo & Diederich	CBS 129337	**JQ342180**	**JQ342182**
Scl. parmeliae	CBS 132232	–	**JX081671**
Scl. sphaerale (Ach. : Fr) Fr.	Diederich 17279	–	**JX081672**
Scl. sphaerale	Diederich 17283	–	**JX081673**
Scl. sphaerale	Ertz 17425	–	**JX081674**
Shanorella spirotricha Benjamin	CBS 304.56	–	FJ358288
Sphinctrina turbinata (Pers. : Fr.) De Not.	DUKE 47956	–	EF413632
endolichenic isolate 99002b	HMAS-L 99002b	EU139132	–
endolichenic isolate 99003c	HMAS-L 99003c	EU139129	–
endolichenic isolate 99003e	HMAS-L 99003e	EU139131	–
lichen-inhabiting isolate ALr-1	G.B. ALr-1	–	EF210108
rock-inhabiting isolate TRN4	TRN4	–	FJ358251
rock-inhabiting isolate TRN115	TRN115	–	FJ358254
rock-inhabiting isolate TRN210	TRN270	–	GU323982
rock-inhabiting isolate TRN270	TRN270	–	GU323982
rock-inhabiting isolate TRN486	TRN486	AY843171	FJ358261
rock-inhabiting isolate TRN508	TRN508	AY843187	FJ358265
rock-inhabiting isolate TRN515	TRN515	AY843190	FJ358266
rock-inhabiting isolate TRN522	TRN522	AY843193	–
rock-inhabiting isolate TRN528	TRN528	AY843199	–
rock-inhabiting isolate TRN531	TRN531	AY843201	FJ358267

[a] Cultures and vouchers are located in the following collections and herbaria: CBS, CBS Fungal Biodiversity Center, Utrecht, The Netherlands; d.H., collection of S. de Hoog, Utrecht, The Netherlands; DAOM, (CCFC/*DAOM),* Canadian *Collection* of Fungal *Cultures*, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; Diederich, collection of P. Diederich, National Natural History Museum, Luxembourg; DUKE, Duke Herbarium, Department of Biology, Duke University, Durham, NC, USA; Ertz, herbarium of D. Ertz, National Botanic Garden of Belgium, Meise, Belgium; G.B., collection of G. Brunauer, University of Salzburg, Salzburg, Austria; HMAS-L, lichen section of Herbarium of Mycology, Institute of Microbiology, Academia Sinica, Beijing, China; J.C.L., herbarium of J.C. Lendemer, Cryptogamic Herbarium, Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY, USA; Kohlmeyer, collection of J. Kohlmeyer, Marine Sciences Department, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL, ARS Culture Collection, Peoria, IL, USA; Tibell, herbarium of L. Tibell, Department of Systematic Botany, Uppsala University, Uppsala, Sweden; TRN, collection of C. Ruibal, Madrid, Spain; UAMH, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Canada; V.R., herbarium of V. Reeb, Department of Biological Science, University of Iowa, IA, USA. [b] Newly prepared sequences are in bold

the analysis of the nuLSU sequences of 51 taxa with *Peltula auriculata, P. umbilicata* (Lichinales) and *Pleopsidium chlorophanum* (Lecanoromycetes) as outgroup taxa. We also analyzed ITS sequences of 29 taxa to more accurately position *S. parmeliae*. Outgroup taxa for this analysis included *Capronia pilosella* and *C. pulcherrima*.

Maximum parsimony (MP) analyses were performed with PAUP* 4.0b10 (Swofford 2002). Heuristic searches of datasets were performed employing simple sequence addition and TBR branch swapping with the MULTREES option activated. Gaps were defined as a fifth character in all analyses. Bootstrap support (BS) for internal branches was evaluated from 1,000 full heuristic searches using TBR branch swapping but without the MULTREES option activated. Only groups with a frequency of greater than 50 % were retained in consensus trees.

Results and discussion

Phylogenetic analyses

The nuLSU dataset consisted of 1,358 characters of which 332 were parsimony-informative (PI). Analysis of this dataset produced 3 most parsimonious trees (MPTs) 1,195 steps in length (L) with a consistency index (CI) of 0.503 and a retention index and (RI) of 0.765. One of these trees is shown in Fig. 1.

In this phylogeny isolates of *Sclerococcum parmeliae* were inferred as members of the Chaetothyriales within a strongly supported clade (BS 99 %) that included two rock-inhabiting isolates (TRN486, TRN531), *Capronia semiimmersa*, *Cladophialophora carrionii*, *Clad. boppii*, *Clad. chaetospira*, and *Phialophora verrucosa*. The close relationship of *S. parmeliae* to these species is also supported by BLAST searches of the mtSSU sequences of CBS 129337 (JQ342181) and CBS 132232 (JX081675) (data not shown). *Sclerococcum sphaerale* was positioned with species of *Dactylospora* and an unidentified lichen-inhabiting isolate in a strongly supported clade (BS 100 %) that was basal to a lineage comprising members of the Chaetothyriales and Pyrenulales. BLAST searches of mtSSU sequences of three collections of *S. sphaerale* (JX081676, JX081677, JX081678) return low values for coverage query (76 %) and maximum identity (92 %) and do not further resolve the relationship of this species to other Eurotiomycetes.

The ITS datasets considered to more precisely position *S. parmeliae* within the Herpotrichiellaceae consisted of 543 (136 PI) characters. Comparison of ITS sequences for this dataset produced a single MPT (L= 370, CI=0.643, RI=0.815) (Fig. 2). This phylogeny confirmed the close relationship of *S. parmeliae* to TRN486 and TRN531 but it did not resolve the position of this species within a clade (BS <50 %) that also included *Clad. chaetospira*, three endolichenic isolates, and three unidentified, uncultured *Cladophialophora*. However, this clade was positioned within a robustly supported lineage (BS 90 %) that included a group comprising isolates of *Clad. carrionii*, *Clad minourae*, *C. semiimmersa*, and *P. verrucosa* (BS 100 %) and a clade that contained isolates of *Clad. boppii* (BS 100 %).

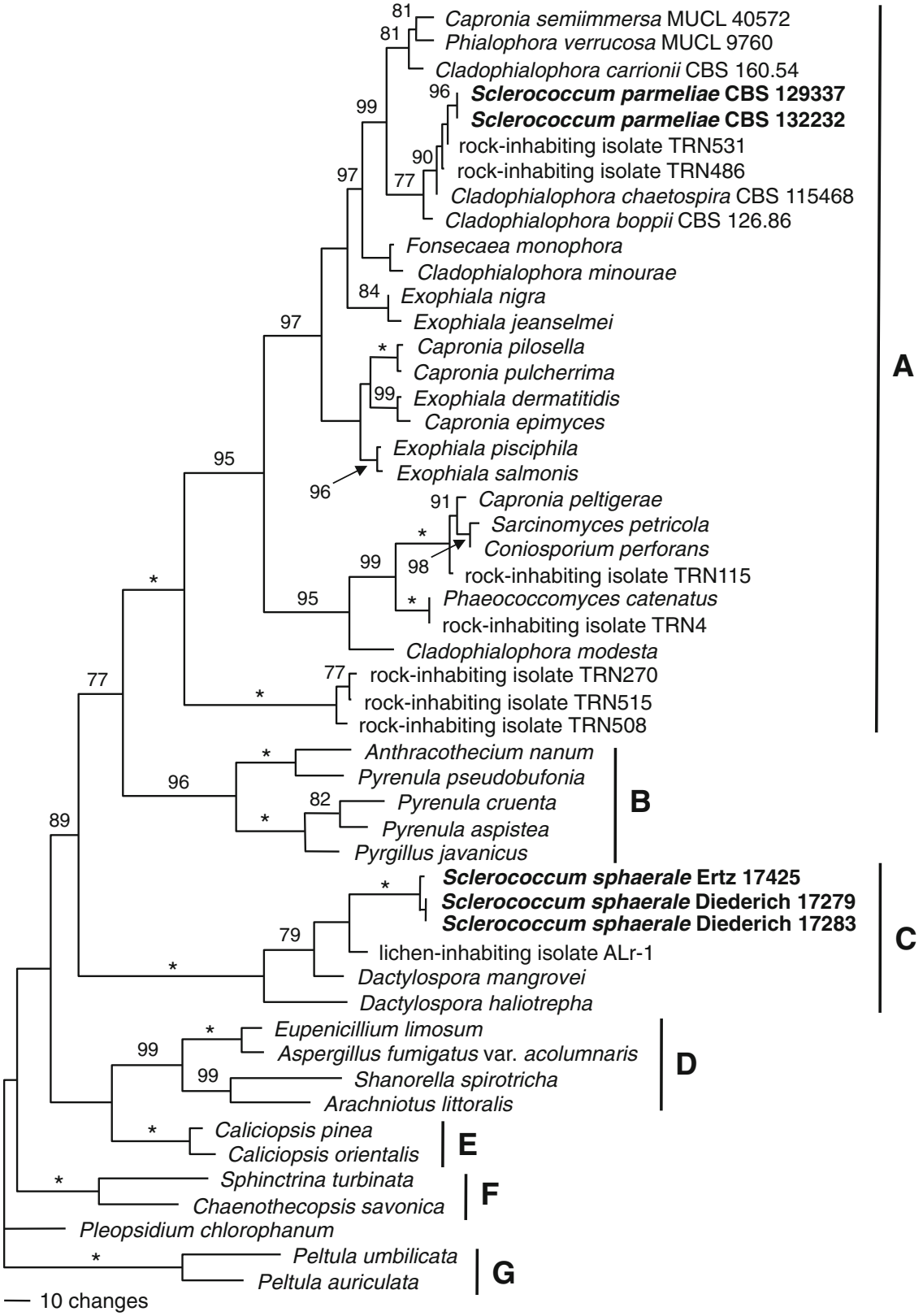
Discussion

Phylogenetic analysis of nuLSU sequences (Fig. 1) resolves *Sclerococcum sphaerale*, the generic type of *Sclerococcum*, as a member of a strongly supported clade basal to the Eurotiomycetes that also includes *Dactylospora* spp. and an unidentified lichenicolous rock isolate (ALr-1). The latter was obtained from sterile dark colored mycelia on the thallus of *Lecanora rupicola* (Brunauer et al. 2007), and a habit photograph (Fig. 1 in Brunauer et al. 2007) suggests *Sclerococcum montagnei* Hafellner, a species confined to *L. rupicola* (Hafellner 1996) that is morphologically similar to *S. sphaerale*. Unfortunately, the voucher specimen for ALr-1 could not be located in GZU.

The *Dactylospora* sequences used in our analysis were found to belong to the Eurotiomycetes by Schoch et al. (2009), a result confirmed by us. *Dactylospora* is therefore not a member of the Lecanorales as has been indicated recently in Index Fungorum, but is obviously heterogeneous, representing at least two distinct clades, one containing *D. lobariella* and *D. imperfecta*, and another more basal clade containing the marine species *D. mangrovei* and *D. haliotrepha* (Schoch et al. 2009, suppl. Figure 6c). Resolving the phylogenetic position of Dactylosporaceae in the Eurotiomycetes will therefore require information about which of these two clades contains the generic type, *D. floerkei* Körb. [= *D. parasitica* (Flörke ex Sprengel) Zopf].

Sclerococcum parmeliae is as a member of the Herpotrichiellaceae (Chaetothyriales, Eurotiomycetes, Ascomycota), a family that encompasses lignicolous and fungicolous *Capronia* Sacc. and a large number of taxa assigned to the anamorph genera *Cladophialophora* Borelli, *Exophiala* J.W. Carmich., *Fonsecaea* Negroni, and *Phialophora* Medlar (Müller et al. 1987; Gueidan et al. 2008; Untereiner et al. 2011). Within this family, *S. parmeliae* belongs to a lineage that includes TRN486 and TRN531, two unnamed rock-inhabiting isolates inferred previously as members of a well-supported group within the "*Capronia* clade" based on the analysis of ITS sequences (Ruibal et al. 2008). Although our analysis of an expanded set of ITS sequences (Fig. 2) does not identify the closest relatives of *S. parmeliae*, it unequivocally positions this species within a strongly supported clade that includes *Clad. carrionii* (= *Clad. ajelloi* Borelli), the type species of *Cladophialophora*.

Sclerococcum parmeliae is not closely related to *Capronia peltigerae*, another lichenicolous species included in our analysis. Our nuLSU phylogeny is consistent with a previous investigation (Untereiner et al. 2011) that resolved *C. peltigerae* as a member of a well-supported lineage basal to the Herpotrichiellaceae. *Capronia*, as circumscribed currently, is polyphyletic but it will not be possible to determine if *C. peltigerae* represents a novel taxon within the Chaetothyriales until additional lichenicolous representatives of the genus are cultured and included in molecular phylogenetic studies. That being said, it is noteworthy that lichenicolous species of *Sclerococcum* have repeatedly been observed on lichen thalli that also bear ascomata of lichenicolous *Capronia*. For example, *C. hypotrachynae* Etayo & Diederich and *S. parmeliae* occur on a thallus of *Hypotrachyna* collected in the Isle of Pico, Azores (Diederich 17092), and Etayo (2002) reported both *C. hypotrachynae* and *S. parmeliae* on the same lichen thalli from five localities in Colombia. *Capronia normandinae* R. Sant. & D. Hawksw. and *S. normandinae* were also found on the same thallus of *Normandina pulchella* collected in the French Pyrénées-Atlantiques (gorges de Kakouetta) (Diederich 9560). Although it is interesting to speculate that *S. normandinae* and *S. parmeliae* are the anamorphs of species of *Capronia*, these connections have yet to be established based on morphological observations and molecular data.



Our phylogenetic analyses demonstrate that *Sclerococcum parmeliae* is not congeneric with the type of *Sclerococcum* and should be transferred to another genus. *Sclerococcum parmeliae* and *S. sphaerale* can also be differentiated morphologically. Conidiomata of *S. sphaerale* are blackish, weakly stromatic sporodochia that produce irregular, nearly

◀**Fig. 1** Phylogenetic relationships of *Sclerococcum parmeliae* and *S. sphaerale* inferred from nuLSU sequences. This is one of two MPTs inferred from an heuristic search of 1,358 characters for 51 taxa (L= 1195, CI=0.503, RI=0.765). Bootstrap values greater than 50 % calculated from 1,000 full heuristic searches are provided above or adjacent to the corresponding nodes. An asterisk (*) indicates branches supported in 100 % of BS replicates. Vertical bars indicate well-supported lineages within the Eurotiomycetes, Lecanoromycetes, and Lichinomycetes (A = Chaetothyriales, B = Pyrenulales, C = *Sclerococcum sphaerale* - *Dactylospora* clade, D = Eurotiales / Onygenales, E = Coryneliales, F = Mycocaliciales, G = Lichinales)

biseriate chains of subglobose to ellipsoidal, multicellular conidia (Fig. 3). Hawksworth and Jones (1981) obtained *S. sphaerale* in axenic culture and reported the in vitro production of conidia to be comparable to what is observed on the host. In contrast, *S. parmeliae* forms loose, non-stromatic sporodochia that give rise to masses of irregularly catenate, ellipsoidal, 1-septate conidia that adhere in short chains (Fig. 5). Among the related anamorphic Herpotrichiellaceae, *S. parmeliae* most closely resembles *Cladophialophora*, a genus characterized by the production of poorly differentiated conidiophores that give rise to branched chains of dry, strongly coherent, moderately melanized, 1(–3) celled, ellipsoidal to fusiform conidia (Badali et al. 2008, 2009; de Hoog et al. 2007). Most members of the genus cause opportunistic

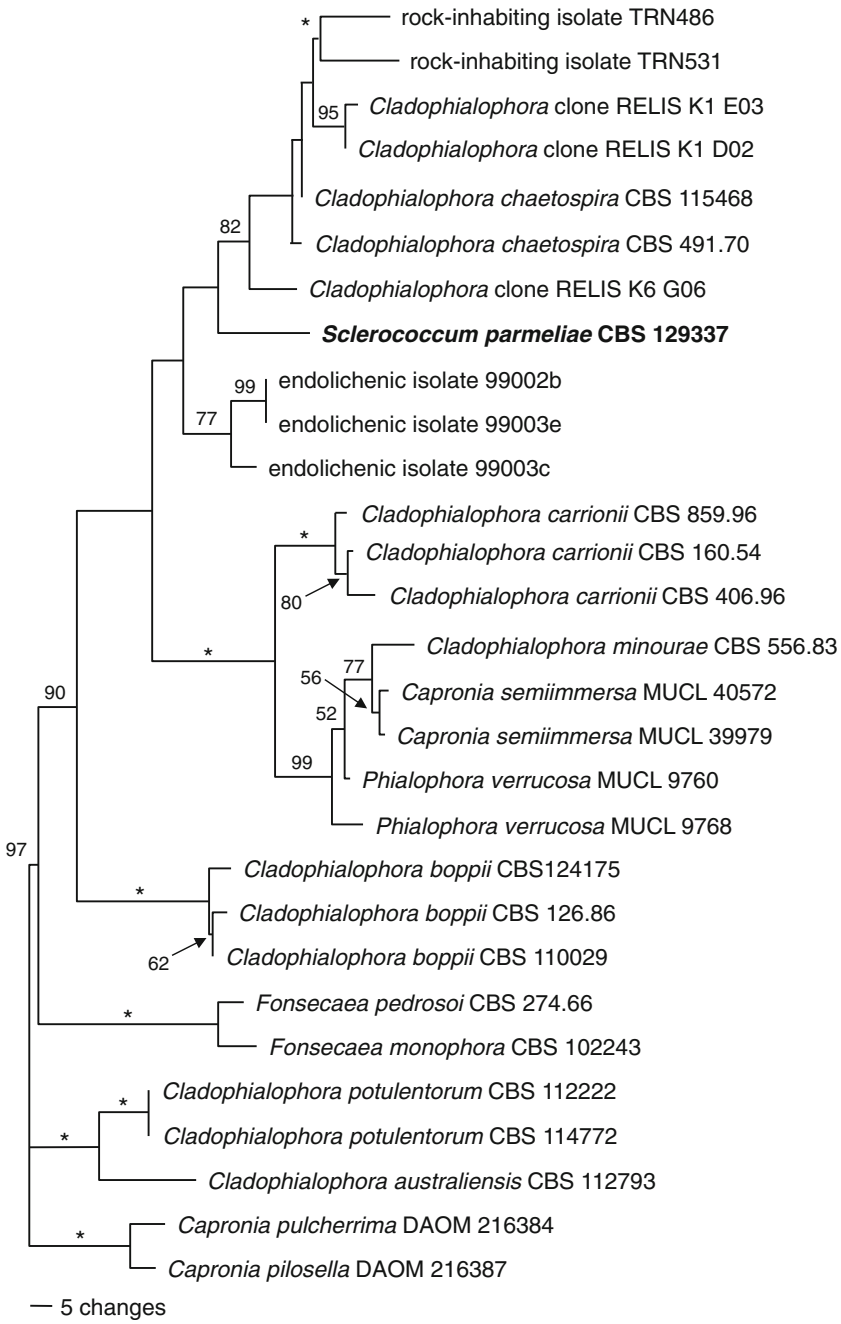


Fig. 2 Phylogenetic relationship of *Sclerococcum parmeliae* inferred from ITS sequence data. This is the single MPT inferred from an heuristic search of 543 characters for 29 taxa (L=370, CI=0.643, RI= 0.815). Bootstrap values greater than 50 % calculated from 1,000 full heuristic searches are provided above or adjacent to the corresponding nodes. An asterisk (*) indicates branches supported in 100 % of BS replicates

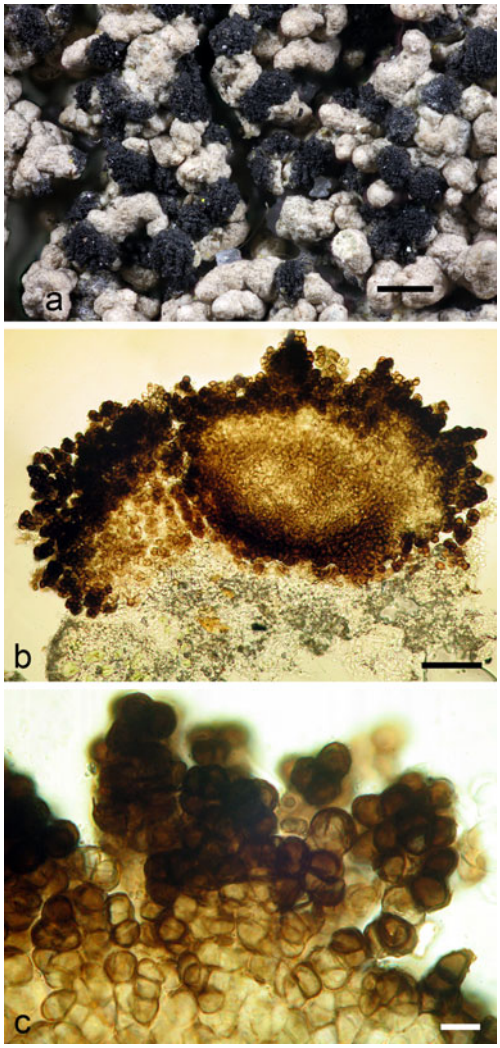


Fig. 3 *Sclerococcum sphaerale* (Diederich 5745). **a** Sporodochia developing on the thallus of *Pertusaria corallina*. **b** Section through stromatic sporodochium. **c** Multicellular conidia. Scale bars: a= 500 μm; b=50 μm; c=10 μm

infections of vertebrates but a number of phytopathogenic and saprobic species have been described (Crous et al. 2007; de Hoog et al. 2007; Badali et al. 2009). *Sclerococcum parmeliae* can be distinguished from the type species *Clad. carrionii* (with aseptate conidia) and from *Clad. chaetospira* (with 1(–3)-septate conidia), the two members of the genus to which it is most closely related (Fig. 2), by its 1-septate, verrucose conidia that frequently remain attached to each other laterally (Fig. 5d–f). The resulting chains of conidia are typically strongly curved and flexuous rather than straight. Several other species of *Sclerococcum*, viz. *S. cladoniae*, *S. hawksworthii* and *S. normandinae*, resemble *S. parmeliae* in the morphology of their conidiomata and mode of conidiogenesis, but they differ in forming aseptate, often smaller and smoother conidia. Although molecular data are not yet available for these species, they are sufficiently similar to *S. parmeliae* to warrant their combination in *Cladophialophora*.

Taxonomy

Sclerococcum Fr. : Fr.

Syst. mycol. **1**: xl (1821). Type: *S. sphaerale* (Ach. : Fr.) Fr.

Sclerococcum sphaerale (Ach. : Fr.) Fr. (Fig. 3)

Ach., *Syn. Lich.*: **2** (1814); Fries, *Syst. mycol.* **3**: 257 (1832).

MycoBank 206581

For typification, description and illustrations, see Hawksworth (1975).

Selected specimens examined: **Belgium:** Ardennes, SW of Nadrin, Le Hérou, 2012, Ertz 17425 (BR). **Luxembourg:** Berdorf, Binzeltschlëff, on *Pertusaria corallina*, 1984, Diederich 5745 (hb Diederich); ibid., 2012, Diederich 17279 (BR, hb Diederich); Berdorf, Wanterbaach, 2012, Diederich 17283 (BR, hb Diederich).

Cladophialophora Borelli

Proc. 5th Int. Conf. on the Mycoses, PAHO Publ. **396**: 335 (1980). Type: *C. ajelloi* Borelli [= *C. carrionii* (Trejos) de Hoog et al.].

For illustrations, notes and references, see Seifert et al. (2011). As discussed in the previous section, we are proposing the transfer of *Sclerococcum parmeliae* and three morphologically similar species of *Sclerococcum* to *Cladophialophora*. A fifth species, collected twice on *Megalospora*, will be described as new. The genus *Cladophialophora* should therefore be emended to include also species with loose sporodochia. We contemplated transferring these species to *Capronia* Sacc. (1883), a generic name that precedes *Cladophialophora* Borelli (1980). However, until the phylogenetic position of the generic type, *Capronia sexdecimspora* (Cooke) Sacc., is determined, combining these species of *Sclerococcum* in *Capronia* is considered as premature.

Cladophialophora cladoniae (Diederich) Diederich **comb. nov.**

MycoBank MB800397

Basionym: *Sclerococcum cladoniae* Diederich, *Bull. Soc. Natur. Luxemb.* **111**: 57 (2010). Type: Luxembourg, W of Kayl, Monument des mineurs, alt. 370 m, over mosses in a disused quarry, on *Cladonia pocillum* and *C. subulata*, 1 Sep. 2009, P. Diederich 16826 (BR–holotype!; HAL, hb Diederich–isotypes).

For a description and illustrations, see Diederich (2010).

Cladophialophora hawksworthii (Etayo & Diederich) Diederich **comb. nov.** (Fig. 4h–k)

MycoBank MB800398

Basionym: *Sclerococcum hawksworthii* Etayo & Diederich, in Daniels et al., Flechten Follmann: 217 (1995). Type: France, Pyrénées-Atlantiques, Ibarre, near St-Jean-Pied-de Port, on *Quercus robur*, on *Megalospora tuberculosa*, 25 June 1992, J. Etayo 2647 & C. Printzen (MA-Lichen–holotype; hb Etayo–isotype).

For a description and illustrations, see Etayo and Diederich (1995).

Specimen examined: **France:** *Pyrénées-Atlantiques*: au sud de Tardets-Sorholus, Ste-Engrâce, vers Pierre-St-Martin, à 3 km après la dernière maison, on *Fagus*, on *Megalospora tuberculosa*, 1991, Diederich 12221 & Etayo (hb Diederich).

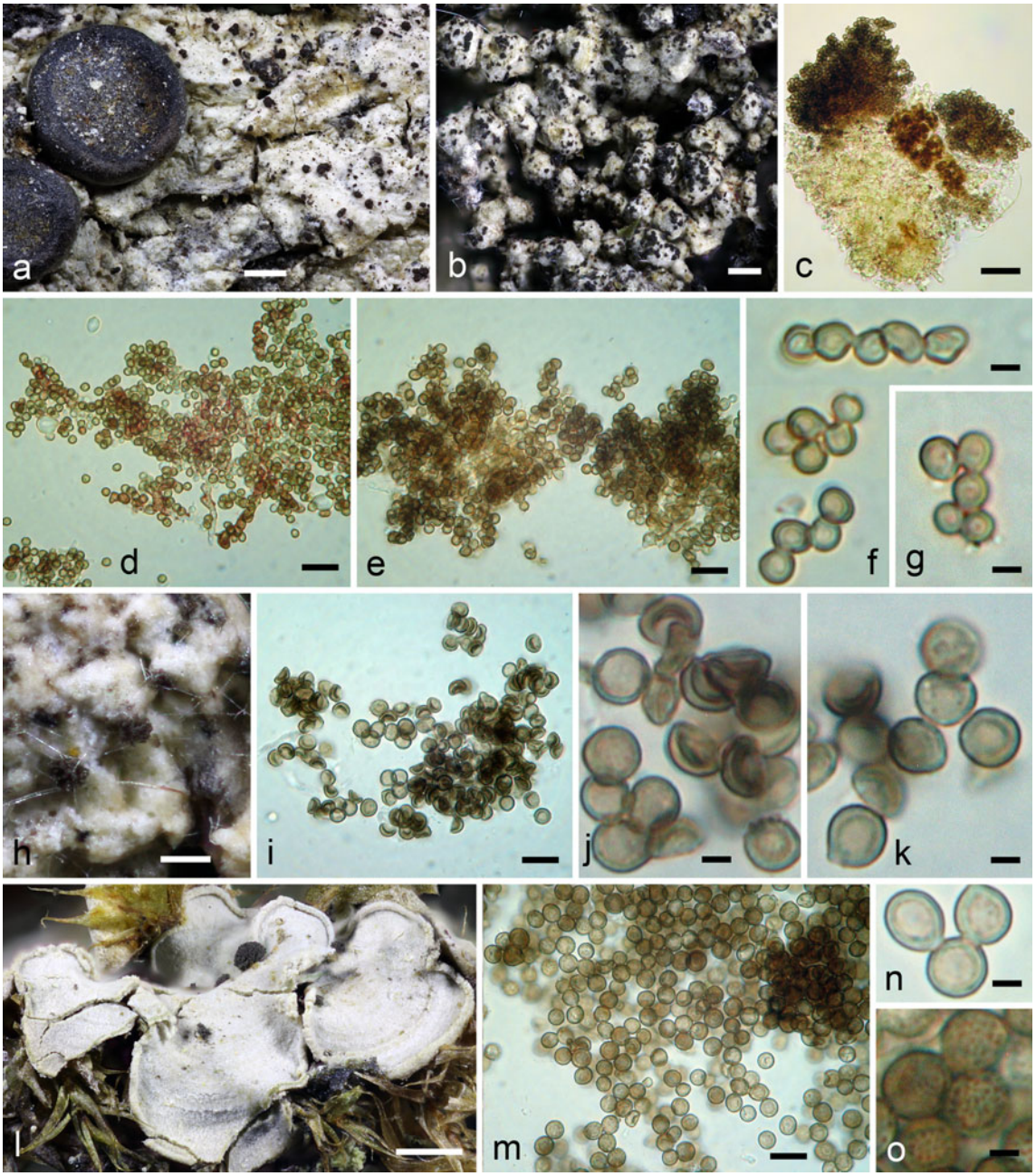


Fig. 4 **a**–**g**. *Cladophialophora megalosporae* (a,d: Aptroot 32016; b–c, e–g: holotype). **a**. Sporodochia developing on the thallus of *Megalospora* sp. b. Sporodochia developing on the thallus of *M. pachycheila*. c. Section through sporodochium. **d**–**e**. Irregularly catenate conidia arising from conidiophores (below the centre of d). **f**. Chains of conidia. **g**. Conidia in surface view, showing the smooth wall. **h**–**k**. *Cladophialophora hawksworthii* (Diederich 12221). **h**. Minuscule sporodochia on the thallus of *Megalospora tuberculosa* (note the acicular crystals of zeorin appearing in herbarium specimens). **i**–**k**. Irregularly catenate conidia, some collapsed in old herbarium specimen, in k surface view, showing a smooth to slightly ornamented wall. **l**–**o**. *Cladophialophora normandinae* (Diederich 9567). **l**. Sporodochium in lateral view on thallus of *Normandina pulchella*. m–o. Irregularly catenate conidia, in o surface view, showing a strongly verrucose wall. Scale bars: a, l=500 μm; b=200 μm; h=100 μm; c=20 μm; d–e, i, m=10 μm; f–g, j–k, n–o=2 μm

Cladophialophora megalosporae Diederich **sp. nov.** (Fig. 4a–g)

MycoBank MB800399

Type: U.S.A., Florida, Liberty Co., hardwoods on W side of Ochlockonee River at Forest Hwy. 13 bridge, 21.5 mi E of Wilma, on *Megalospora pachycheila*, 28 Dec. 1990, R. C. Harris 26148 (NY–holotype; hb Diederich–isotype).

Colonies forming discrete, minuscule conidiomata on the host thallus; mycelium immersed, brownish, macroscopically not visible. Conidiophores semi-macronematous, pale to medium brown or reddish brown, aggregated into irregularly rounded, convex, medium to dark brown or greyish brown, simple, partly immersed to superficial sporodochia of 20–100(–200) µm diam. Conidiogenous cells monoblastic or polyblastic, integrated, terminal, brown, not clearly defined, and the terminal cells acting in turn as conidiogenous cells. Conidia irregularly adhering in short, branched, acropetal chains, dry, subspherical, pale to medium brown, color not changing in 5 % KOH (or becoming slightly olivaceous), aseptate, smooth-walled, thin-walled, (2.2–)2.4–2.9(–3.3)× (1.9–)2.1–2.6(–3.0) µm, ratio l/b (1.0–)1.0–1.2(–1.4) (n=40).

Microscopically, this species is very similar to *Cladophialophora cladoniae*. It is distinguished by the much larger conidiomata, those of *C. cladoniae* being 7–20(–30) µm diam. As both known specimens of this species occur on hosts belonging to the genus *Megalospora*, we initially expected that they might belong to *C. hawksworthii*, a species known only from *M. tuberculosa*. Macroscopically, conidiomata of both taxa are similar in size, but those of *C. megalosporae* are more blackish (Fig. 5a–b), while those of *C. hawksworthii* appear more greyish brown with a slight reddish tinge (Fig. 5h). Microscopically, conidia of *C. hawksworthii* are distinctly larger (2.5–4 µm diam.) than those of the new species. Furthermore, conidia of *C. hawksworthii* are more greyish brown, often with a darker wall in optical section (Fig. 5i–k), whereas those of the new species are reddish brown, with a less visible wall (Fig. 5d–g).

Additional specimen examined: **Papua New Guinea:** *Madang Province*: Huon Peninsula, Finisterre range, Yupna valley, Teptep village, 5°57′ S, 146°33′ E, alt. 2,500 m, disturbed mountain forest, mossy mountain forest and gardens near village, on *Megalospora*, 1992, Aptroot 32016 (M–0045424, hb Diederich).

Cladophialophora normandinae (Diederich & Etayo) Diederich **comb. nov.** (Fig. 4l–o)

MycoBank MB800400

Basionym: *Sclerococcum normandinae* Diederich & Etayo, in Etayo & Diederich, in Daniels et al., Flechten Follmann: 218 (1995). Type: France, Pyrénées-Atlantiques, au sud de Tardets-Sorholus, Ste-Engrâce, vers Pierre-St-Martin, à 3 km après la dernière maison, on *Fagus*, on *Normandina pulchella*, 26 July 1990, P. Diederich 9388 (LG–holotype; hb Diederich–isotype!).

For a description and illustrations, see Etayo and Diederich (1995). Specimens from S Chile reported by Etayo and Sancho (2008) as *Sclerococcum* cf. *normandinae*, mainly growing on *Nephroma*, differ by smaller conidiomata, 50–150 µm diam., and by the presence of 20 % of 1-septate conidia; they might represent a distinct, yet undescribed species.

Additional specimens examined: **France:** *Pyrénées-Atlantiques*: S of Tardets-Sorholus, Ste-Engrâce, gorges de Kakouetta, on *Crataegus*, on *Normandina pulchella*, 1991, Diederich 9560, 9567 & Etayo (hb Diederich).—**United Kingdom, Scotland:** *Mid Ebudes*, *VC 103*: Isle of Mull, overlooking Sound of Ulva, hazel wood SE of Oskamull, NM463398, on *N. pulchella*, 1999, H. Fox 247 (DBN).

Cladophialophora parmeliae (Etayo & Diederich) Diederich & Untereiner **comb. nov.** (Fig. 5)

MycoBank MB800401

Basionym: *Sclerococcum parmeliae* Etayo & Diederich, *Mycotaxon* **60**: 425 (1996). Type: Spain, Navarra, valle de Basaburúa Mayor, between Aizároz and Arrarás, track to Bergañe, alt. 550 m, on *Fagus*, on *Parmelia saxatilis*, 20 Sep. 1994, J. Etayo 12688 (MA-Lichen–holotype; hb Etayo–isotype, non vid.).

For a description and illustrations, see Etayo and Diederich (1996).

Specimens examined: **Austria:** *Oberösterreich*: Donautal, Schlögener Schlinge, Steiner Fels, 2185/66, MTB 7549, alt. 380 m, on *Flavoparmelia caperata*, 1994, Berger 8149 (hb Berger).—**France:** *Pyrénées-Atlantiques*: col de Lizuniaga, c. 5 km from Bera de Bidasoa, on *F. caperata*, 1994, Etayo 12674 (hb Diederich).—**Spain, Azores:** *Pico*: S of Sao Roque do Pico, forest remnants on the shore of Lagoa Capitao, 38°29′ 9″ N, 28°18′58″ W, alt. 780 m, on *Juniperus brevifolia*, on *Hypotrachyna imbricatula*, 2010, Diederich 17055 (hb Diederich) (culture JL-477, CBS 129337); ibid., on *Hypotrachyna*, 2011, Ertz 16591 (BR) (culture CBS 132232); N of Lajes do Pico, near Lagoa do Paúl, 38°25′40″ N, 28°13′ 58″ W, alt. 790 m, on *J. brevifolia*, on *H. imbricatula*, 2010, Diederich 17028 (hb Diederich); between Lajes do Pico and Sao Roque do Pico, Bosque da Junqueira, 1 km S of crossing with road going to the east, 38°27′56″ N, 28°17′57″ W, on *J. brevifolia* in laurisilva, on *Hypotrachyna*, 2010, Diederich 17092 (hb Diederich).—**United Kingdom, Scotland:** *Isle of Skye*: Forest SW of Tokavaig, close to the sea, alt. 5 m, on *Lobaria pulmonaria*, 2003, Diederich 15659 (hb Diederich).

Key to the lichenicolous species of *Cladophialophora*

1 Conidia mainly 1-septate, ellipsoidal, slightly to distinctly verrucose; conidiomata 50–120 µm diam.; on parmelioid lichens, *Lobaria*, *Normandina*, *Pannaria* and *Sticta* .....**C. parmeliae**

1 Conidia aseptate, subspherical to short-ellipsoidal.....2

2 Conidia 4–6×3.5–4 µm, verrucose; conidiomata 150–300 µm diam.; on *Normandina pulchella*.....**C. normandinae**

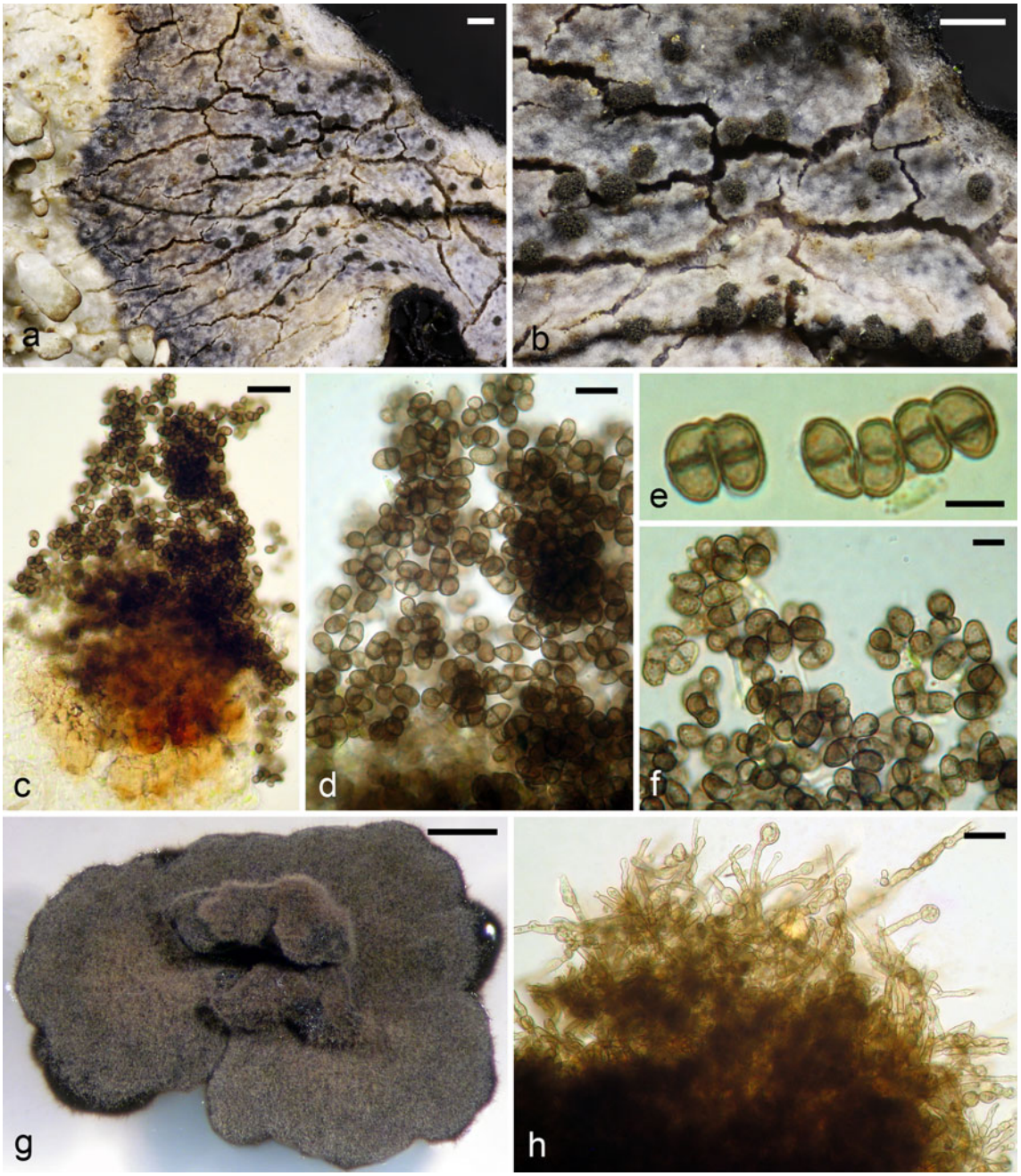


Fig. 5 *Cladophialophora parmeliae*. **a**–**b**. Sporodochia developing on the thallus of *Hypotrachyna imbricatula*. **c**. Section through sporodochium. **d**. Irregularly catenate, 1-septate conidia. **e**. Chains of conidia, showing the lateral attachment. **f**. Conidia in surface view, showing the verrucose ornamentation. **g**. 10–week-old culture. h. Section through culture. (a, c–d: Diederich 17055; b: culture JL–477 from Diederich 17055). Scale bars: g=1 mm; a–b=200 μm; c, h=20 μm; d=10 μm; e–f=5 μm

2 Conidia smaller, smooth or indistinctly ornamented; conidiomata smaller.....3
3 Conidia 2.5–4 μm diam., greyish brown; conidiomata 30–100(–130) μm diam.; on *Megalospora tuberculosa* .....**C. hawksworthii**
3 Conidia less than 3 μm diam., reddish brown.....4
4 Conidiomata 7–20(–30) μm diam.; conidia 2.2–3 μm; on *Cladonia*.....**C. cladoniae**
4 Conidiomata 20–100(–200) μm diam.; conidia 2.5–3.0× 2.0–2.5 μm; on *Megalospora*.....**C. megalosporae**

Acknowledgments We thank the curators of herbaria and the owners of private collections for the loan of specimens in their care, and the curator of GZU for searching for the voucher specimen of culture ALr-1. This study was supported by NSF grant DEB 0841405 to JDL and a Discovery Grant to WAU from the Natural Science and Engineering Research Council (NSERC) of Canada.

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