

Phylogenetic relationships of five genera of Xylariales and *Rosasphaeria* gen. nov. (Hypocreales)

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Abstract Eight inconspicuous non-stromatic perithecial fungi immersed in plant tissue are assessed with respect to their morphology, ecology and phylogenetic position. Emphasis is laid on two genera now and then placed in the family Hyponectriaceae, Xylariales: *Leiosphaerella* with its type species *L. praeclara* occurring on *Vaccinium*, and *Pseudomassaria* with its type species *P. chondrospora* occurring on *Tilia*. In molecular phylogenetic analyses of LSU and ITS sequences, the generic types of these genera are closely related, but their familial affiliation within Xylariales remains unresolved. *Pseudomassaria sepincoli-formis* clusters with *P. chondrospora*, whereas *P. fallax* is not congeneric with *Pseudomassaria* and *P. lycopodina* is combined in *Leiosphaerella* despite its apiospores. Three species thought to belong to *Leiosphaerella* are re-assessed: *L. moravica* that occurs on *Rosa*, is disposed in the new genus *Rosasphaeria*, which is close to *Eucasphaeria* in the Niessliaceae (Hypocreales), according to multi-gene phylogenetic analyses (ITS, LSU, *rpb2* and *tef1*). For *L. vexata* the genus *Pseudomassariella* is revived. A *Leiosphaerella*-like fungus on *Lycopodium* is described as the new species *Monographella lycopodina*. In addition the phylogenetic relationships of two fungi forming superficial stromata are here clarified: *Collodiscula japonica* belongs to the Xylariaceae, while *Melogramma campylosporum* may currently be interpreted as representing a family of its own, the Melogrammataceae.

Keywords *Collodiscula* · *Eucasphaeria* · *Leiosphaerella* · *Massarina* · *Melogramma* · *Metasphaeria* · New genus · Phylogenetic reconstruction · *Pseudomassaria* · *Pseudomassariella* · Systematics

Introduction

This paper is part of a series devoted to the study of peri- or pseudothecial ascomycetes that occur on dead plant material in an early stage of succession (see e.g. Voglmayr and Jaklitsch 2008, 2011). Starting point of this study was the collection of a minute, hitherto unknown fungus on *Lycopodium annotinum* in Bavaria. The fungus was first identified morphologically as a species of *Leiosphaerella*. However, the molecular phylogenetic position of *Leiosphaerella* has been unknown, therefore the type species of this genus was collected, cultured and sequenced. Further searches revealed *Pseudomassaria lycopodina*, which made collection and sequencing of the type species of *Pseudomassaria*, *P. chondrospora*, necessary. Eventually eight species earlier thought to belong to *Leiosphaerella* or *Pseudomassaria* were included in morphological and molecular phylogenetic analyses, the results of which are here presented. At first sight these fungi are all similar to each other in forming minute, inconspicuous dots on the surface of their hosts. These dots may at first sight be interpreted as clypei, but in fact they are only translucent perithecia, because no clypeate structures, i.e. stromatised substrate parts, can be seen in microtome sections. Several morphological differences of the fungi studied here have been used for differentiation and classification on the specific, generic and even familial level. In particular we assess here whether a) amyloid vs. non-amyloid ascus apices and b) the position of the ascospore septum,

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eccentric vs. median, are useful criteria for distinction of taxa at the generic level.

One important objective of this paper is also to include as many generic types as possible. As many of the traditional morphology-based genera in Xylariales are revealed as polyphyletic in molecular phylogenetic analyses, this is a prerequisite for appropriate generic revisions and reclassification. In addition, phylogenetic relationships and placement of various lineages of Xylariales are still unresolved. For this reason we also included the type species of two genera of stromatic Xylariales, *Collodiscula japonica* and *Melogramma campylosporum*, which have not previously been included in molecular phylogenetic analyses.

Materials and methods

Isolates and specimens

Representative isolates have been deposited at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS). Specimens have been deposited in the Herbarium of the Institute of Botany, University of Vienna (WU).

Ascospore isolates were prepared as described by Jaklitsch (2009). Cultures were grown in 9-cm-diam Petri dishes with alternating 12 h cool white fluorescent light and 12 h darkness at 20–25 °C on 2% malt extract agar (MEA), potato dextrose agar (PDA), or corn meal dextrose agar (CMD; Jaklitsch 2009).

Morphological observations

Conidiation structures of *R. moravica* were examined, measured and photographed on a compound microscope after mounting in 3% KOH from PDA cultures grown for 1 month at 25 °C with alternating 12 h cool white fluorescent light and 12 h darkness and for 28 days at 15 °C in darkness. Dry perithecia were briefly rehydrated with water at room temperature in a Petri dish, treated with 3% KOH, embedded in Tissue-Tek O.C.T. Compound 4583 (Sakura Finetek Europe B.V., Zoeterwoude, The Netherlands) and sectioned at a thickness of 12–15 µm with a freezing microtome. Sections were measured and photographed in 50% glycerol. Asci and ascospores were measured in separate preparations in water or where noted in the figure legends, in 3% KOH. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses. Nomarski differential interference contrast (DIC) was used for observations and measurements. Images were recorded with the Nikon Coolpix 4500, DS-U2 or Zeiss AxioCam

ICc3 digital cameras. Measurements were carried out using the NIS-Elements D version 3.0 software.

DNA extraction, PCR and sequencing

Mycelium for DNA extraction was grown in 2% liquid malt extract culture, harvested, freeze-dried and ground according to Voglmayr and Jaklitsch (2008). Genomic DNA was extracted using the modified CTAB method described in Riethmüller et al. (2002) or by application of the DNeasy Plant Minikit (QIAGEN GmbH, Hilden, Germany). A 1.6–2 kb fragment containing partial nuSSU, ITS1, 5.8S, ITS2 and partial LSU rDNA was amplified with the primer pair V9G (de Hoog and Gerrits van den Ende 1998) and LR5 (Vilgalys and Hester 1990). For direct amplification of the ITS or ITS-LSU region from ascomata of *Monographella lycopodina*, ascomata were rehydrated and thoroughly washed with sterile distilled water, squashed with a sterile forceps to release their contents, which were directly transferred with a fine forceps to a reaction tube containing the PCR master mix with primers ITS4 and ITS5 (White et al. 1990) or V9G and LR5. PCR products were purified by an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) and an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems) with the same primers as in PCR; in addition, primers LR3 (Vilgalys and Hester 1990), ITS4 (White et al. 1990), F5.8Sf and F5.8Sr (Jaklitsch and Voglmayr 2011) were used as internal sequencing primers for the SSU-ITS-LSU fragment. For multi-gene analyses of *Rosasphaeria*, also the *tef1* and *rpb2* genes were amplified and sequenced as described in Jaklitsch and Voglmayr (2011). GenBank accession numbers of sequences generated in this study are given in the specimen information after each taxon description except for *Rosasphaeria*, where they are listed in Table 1.

Analysis of molecular data

All alignments were produced with Muscle version 3.6 (Edgar 2004), checked and refined using BioEdit version 7.0.9.0 (Hall 1999). To reveal the phylogenetic positions of the taxa investigated, representative LSU sequences of Xylariales, Hypocreales, Sordariales and Diaporthales were downloaded from GenBank; resulting phylogenetic trees were rooted with Hypocreales, Sordariales and Diaporthales as outgroups according to Zhang et al. (2006). For ITS, representative samples of Xylariales were downloaded, with three *Diaporthe* species as outgroup. After the exclusion of 7 and 261 ambiguously aligned characters,

Table 1 GenBank accession numbers of the sequences used for the multi-gene analyses of *Hypocreales* to reveal the phylogenetic position of *Rosasphaeria*

Taxon	Strain	LSU	ITS	<i>rpb2</i>	<i>tef1</i>
<i>Bionectria ochroleuca</i> (Schwein.) Schroers & Samuels	CCFC 226708	AY283558	—	—	—
	CBS 376.55	—	AF358239	—	—
	AFTOL-ID 187	—	—	DQ862013	DQ862029
<i>Bionectria pityrodes</i> Schroers	CBS 102033	AY489728	AF210672	—	AY489623
<i>Cosmospora coccinea</i> Rabenh.	A.R. 2741	AY489734	—	—	AY489629
	CBS 114050	—	FJ474072	DQ522438	—
<i>Cosmospora episphaeria</i> (Tode) Rossman & Samuels	G.J.S. 98-160	—	FJ474073	—	—
	G.J.S. 88-29	AY015625	—	—	—
<i>Cosmospora vilior</i> (Starbäck) Rossman & Samuels	Guardbridge 20	—	GU726755	—	—
	G.J.S. 96-186	AY015626	—	—	—
<i>Eucasphaeria capensis</i> Crous	CBS 120027	EF110618	EF110618	—	—
<i>Haematonectria haematococca</i> (Berk. & Broome) Samuels & Rossman	voucher 83364	DQ119558	—	—	—
	not specified	—	—	Genome ^a	Genome ^a
	ATCC MYA-4622	—	GU327638	—	—
<i>Hydropisphaera peziza</i> (Tode) Dumort.	G.J.S. 92-101	AY489730	—	—	AY489625
	CBS 102038	—	—	DQ522444	—
<i>Hypocrea rufa</i> (Pers.) Fr.	CBS 114374	AY489726	—	EF692510	—
	C.P.K. 1998	—	DQ677656	—	DQ672616
<i>Hypocrea schweinitzii</i> (Fr.) Sacc.	ICMP1694	AY283549	—	—	—
	C.P.K. 2454	—	FJ860832	—	—
	CBS 121275	—	—	FJ860586	—
	C.P.K. 2005	—	—	—	FJ860694
<i>Hypomyces aurantius</i> (Pers.) Fuckel	TFC 95-171	FN859425	FN859425	FN868679	FN868743
<i>Hypomyces lactifluorum</i> (Schwein.) Tul. & C. Tul.	TAAM 170476	EU710768	FN859432	EU710773	FN868751
<i>Nectria aquifolii</i> (Fr.) Berk.	CBS 127381	HM534891	HM534891	HM534881	HM534870
<i>Nectria aurantiaca</i> (Tul. & C. Tul.) Jacz.	CBS 236.29	HM534892	HM534892	HM534882	HM534871
<i>Nectria berolinensis</i> (Sacc.) Cooke	CBS 127382	HM534893	HM534893	HM534883	HM534872
<i>Nectria cinnabarina</i> (Tode) Fr.	CBS 127383	HM534894	HM534894	HM534884	HM534873
<i>Nectria coryli</i> Fuckel	CBS 127384	HM534895	HM534895	HM534885	HM534874
<i>Nectria eustomatica</i> Jaklitsch & Voglmayr	CBS 121896	HM534896	HM534896	HM534886	HM534875
<i>Nectria lamyi</i> (Desm.) De Not.	CBS 127385	HM534898	HM534898	HM534888	HM534877
<i>Nectria pseudotrichia</i> (Schwein.) Berk. & M.A. Curtis	CBS 641.83	HM534899	HM534899	HM534889	HM534878
<i>Nectria sinopica</i> (Fr.) Fr.	CBS 127386	HM534900	HM534900	HM534890	HM534879
<i>Neonectria coccinea</i> (Pers.) Rossman & Samuels	CBS 237.29	AY677327	—	—	—
	CBS 29181	—	FJ474075	—	—
	CBS 119159	—	—	DQ789819	—
	CBS 118914	—	—	—	DQ789688
<i>Neonectria ditissima</i> (Tul. & C. Tul.) Samuels & Rossman	CBS 226.31	AY677330	—	—	—
	CBS 117752	—	DQ178168	—	—
	G.J.S. 94-12	—	—	DQ789823	—
	CBS 118927	—	—	—	DQ789743
<i>Neonectria punicea</i> (J.C. Schmidt) Castl. & Rossman	CBS 124262	HM534901	HM534901	—	HM534880
	CBS 119724	—	—	DQ789753	—
<i>Niesslia exilis</i> (Alb. & Schwein.) G. Winter	CBS 560.74	AY489720	—	AY489647	AY489614
<i>Pseudonectria rousseliana</i> (Mont.) Wollenw.	A.R. 2716	U17416	—	—	AF543780
	not specified	—	FJ555527	—	—
	CBS 114049	—	—	DQ522459	—
<i>Rosasphaeria moravica</i> (Petr.) Jaklitsch & Voglmayr	CBS 124270	JF440985	JF440985	JF440986	JF440987

Table 1 (continued)

Taxon	Strain	LSU	ITS	<i>rpb2</i>	<i>tef1</i>
<i>Roumegueriella rufula</i> (Berk. & Broome) Malloch & Cain	G.J.S. 91-164	EF469082	–	EF469116	EF469070
<i>Sphaerostilbella aureonitens</i> (Tul. & C. Tul.) Seifert, Samuels & W. Gams	TFC 96-77	AF160246	–	–	–
	G.J.S. 74-87	–	FJ442633	FJ442763	DQ834452
<i>Stromatonectria caraganae</i> (Höhn.) Jaklitsch & Voglmayr	CBS 125579	HQ112288	HQ112288	HQ112290	HQ112286

^a Retrieved from the JGI database (<http://genome.jgi-psf.org/>)

the final LSU and ITS matrices contained 864 and 520 characters, respectively.

To reveal the phylogenetic position of *Rosasphaeria* within Hypocreales, multi-gene analyses were performed with a combined matrix of four genes (SSU, LSU, *rpb2*, *tef1*). Sequences were selected from Jaklitsch and Voglmayr (2011); in addition, sequences of *Bionectria pityrodes*, *Eucasphaeria capensis*, *Hypomyces aurantius*, *H. lactifluorum*, *Niesslia exilis*, *Rosasphaeria moravica* and *Stromatonectria caraganae* were added. The final combined four-gene matrix contained 3,628 characters; the GenBank accession numbers of sequences used are given in Table 1.

Maximum parsimony (MP) analyses of the three matrices (LSU, ITS and combined) were performed with PAUP* version 4.0 b10 (Swofford 2002), using 1,000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, COLLAPSE=MAXBRLEN, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data. Bootstrap analysis with 1,000 replicates was performed in the same way, but using ten rounds of random sequence addition and subsequent TBR branch swapping during each bootstrap replicate. Each bootstrap replicate was limited to 1 million rearrangements in the LSU and ITS matrices.

For Bayesian analyses, the GTR+I+G model was implemented for all matrices. Bayesian analyses were performed with the computer program MrBayes (version 3.1.2; Huelsenbeck and Ronquist 2001). Three parallel runs of four incrementally heated, simultaneous Markov chains were performed over 5 million generations from which every 500th tree was sampled in each run. For the combined four-gene alignment, substitution model parameters were calculated separately for the different gene regions. The first 1,000 trees sampled were discarded, and a 90% majority rule consensus of the remaining trees was computed to obtain estimates for the probabilities that groups are monophyletic given the sequence data (posterior probabilities). To test convergence of runs, the results were analysed using AWTY (Nylander et al. 2008); no indication of lack of convergence was detected.

Results

Molecular phylogenetic analyses

Amplification of the partial nuSSU-ITS1-5.8S-ITS2-LSU rDNA revealed sequences of ca 1,600 or 2,000 bp; length increase in the latter cases was due to insertions of ca 400 bp towards the 3' ends of the LSU fragments amplified.

Of the 864 included characters of the LSU matrix, 217 were parsimony informative. Maximum parsimony analyses revealed three most parsimonious trees consisting of 1,301 steps, one of which is shown in Fig. 1. Topology of the MP trees differed slightly within the Xylariaceae and at the base of the clade containing Apiosporaceae. The two nodes collapsing in the strict consensus tree are marked with an arrowhead in Fig. 1.

Of the 520 included characters of the ITS matrix, 219 were parsimony informative. Maximum parsimony analyses revealed two most parsimonious trees consisting of 1,632 steps, one of which is shown in Fig. 2. Topology of the MP trees differed only within the clade containing *Monographella lycopodina*, *Microdochium phragmitidis* and *Microdochium* sp., which were contained in a polytomy in the strict consensus tree.

Of the 3,628 characters in the combined four-gene (SSU, LSU, *rpb2*, *tef1*) alignment, 1,006 were parsimony informative. MP analyses revealed one MP tree consisting of 5,178 steps which is shown in Fig. 3.

In all sequence datasets, the three Bayesian runs revealed almost identical posterior probabilities, and the results were compatible with the MP trees, except for a minor difference in the combined analyses, where *Nectria aurantiaca* was sister taxon to the other three *Nectria* species of that clade with a PP of 95% (Fig. 3; conflicting node marked with arrowhead). Corresponding MP bootstrap support above 50% and Bayesian posterior probabilities above 90% are given in Figs. 1, 2, 3 at first and second position above or below the branches.

In the phylogenetic analyses of the LSU and ITS datasets, only few nodes received significant support (Figs. 1, 2). In the LSU dataset the following main groups received high support: Xylariales, Xylariaceae, Diatrypeaceae, Apiosporaceae, the *Monographella* / *Microdochium* clade, Hypocreales,

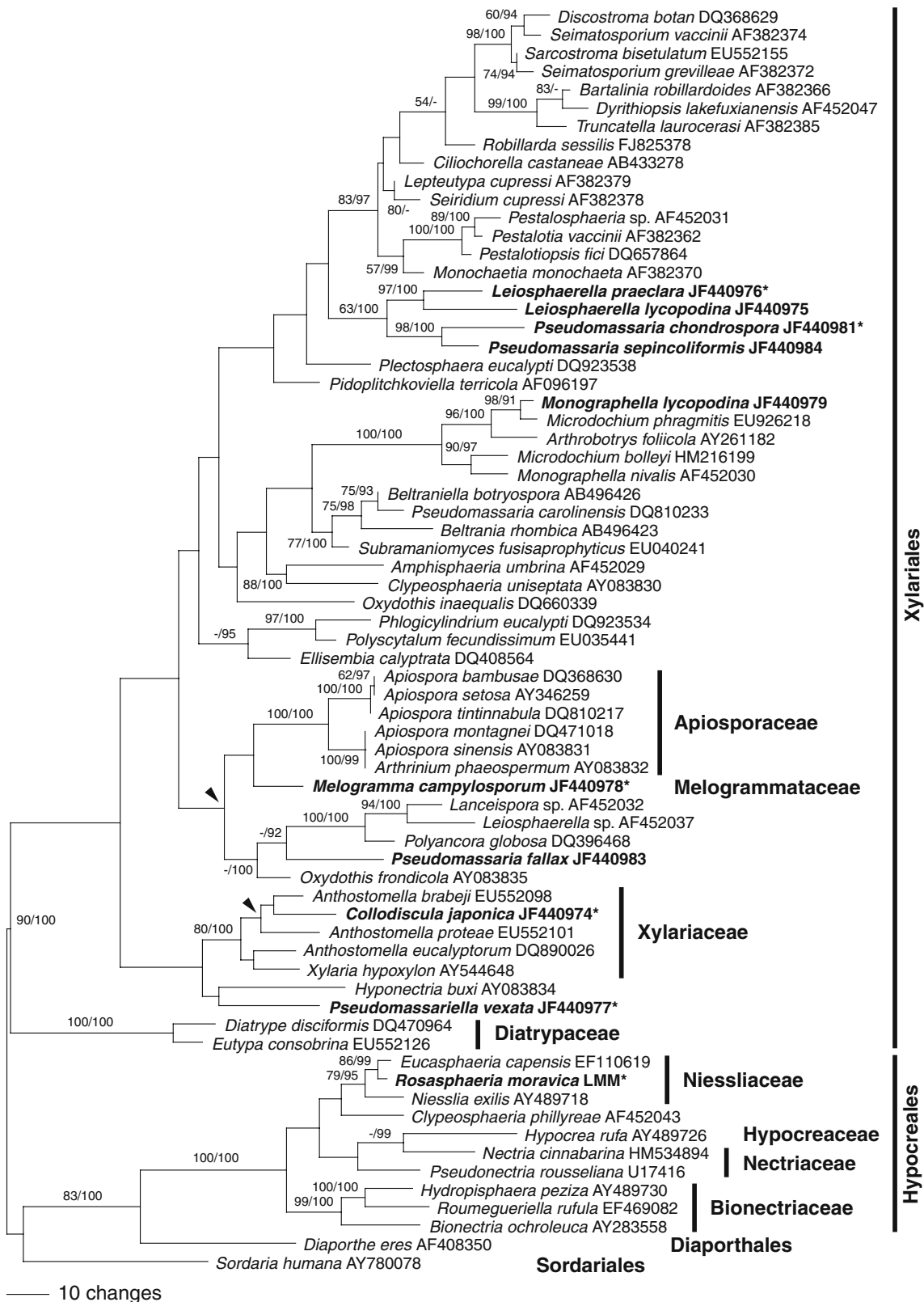


Fig. 1 Phylogram showing one of three most parsimonious trees of 1,301 steps revealed by PAUP from an analysis of the LSU rDNA of selected Xylariales, Hypocreales, Sordariales and Diaporthales. Numbers following the taxon names represent GenBank accession numbers; arrowheads denote nodes collapsing in the strict consensus

tree of the three MP trees. MP bootstrap support above 50% and Bayesian posterior probabilities above 90% are given at first and second position above or below the branches. Taxa sequenced and documented in the present study are in bold face; those marked with asterisks represent generic type species

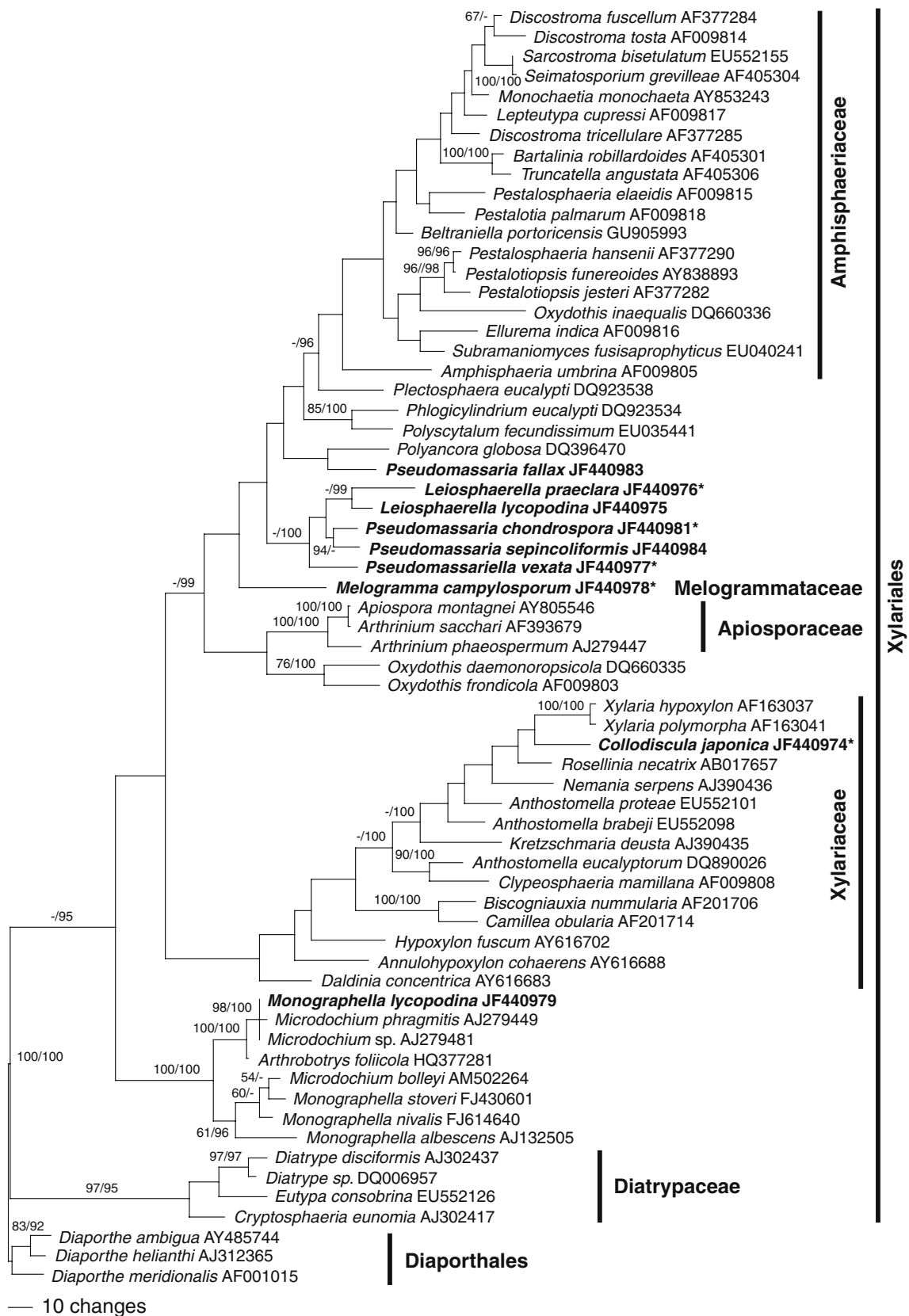
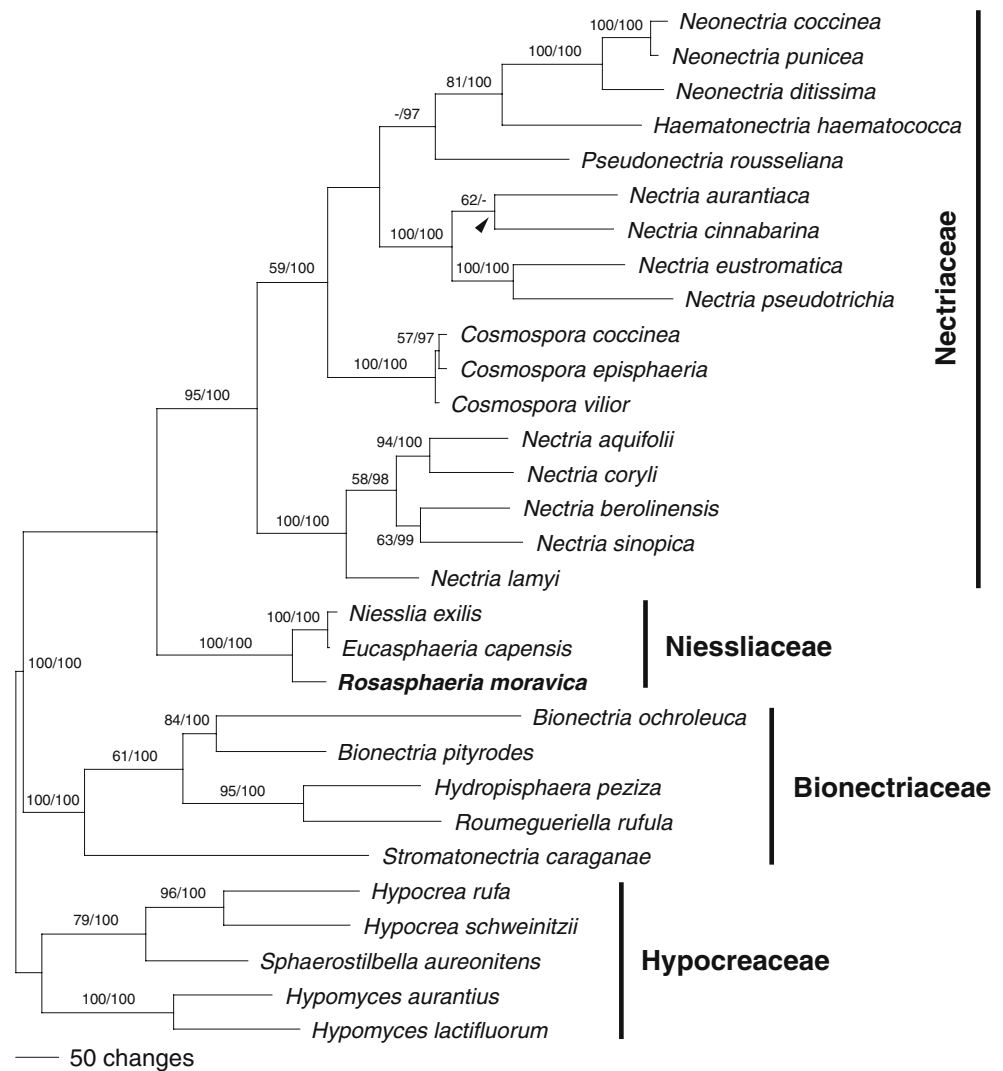


Fig. 2 Phylogram showing one of two most parsimonious trees of 1,632 steps revealed by PAUP from an analysis of the ITS rDNA of selected Xylariales. Numbers following the taxon names represent GenBank accession numbers. MP bootstrap support above 50% and

Bayesian posterior probabilities above 90% are given at first and second position above or below the branches. Taxa sequenced and documented in the present study are in bold face; those marked with asterisks represent generic type species

Fig. 3 Phylogram of the single most parsimonious tree of 5,178 steps revealed by PAUP from an analysis of the multi-gene (LSU, ITS, *rpb2*, *tef1*) matrix of selected Hypocreales, showing the phylogenetic position of *Rosasphaeria* within Niessliaceae. MP bootstrap support above 50% and Bayesian posterior probabilities above 90% are given at first and second position above or below the branches. The arrowhead denotes a topological difference to the Bayesian analyses, where *N. aurantiaca* is sister taxon to the other three *Nectria* species of this clade



Bionectriaceae and Niessliaceae. In the ITS dataset, Xylariales, Diatrypaceae, Apiosporaceae and the *Monographella* / *Microdochium* clade received high support as well. *Leiosphaerella* sensu stricto (containing *L. praeclara* and *L. lycopodina*) and *Pseudomassaria* sensu stricto (containing *P. chondrospora* and *P. sepincoliformis*) were highly supported in the LSU analyses (Fig. 1), but did not consistently receive significant support in the ITS analyses (Fig. 2). Sister group relationship of *Leiosphaerella* and *Pseudomassaria* was consistently present in the analyses of both datasets and received low to high internal support in the LSU but lacked significant support in the ITS analyses. *Monographella lycopodina* was closely related to *Microdochium phragmitidis* in the LSU and ITS datasets with high support. *Collodiscula japonica* was consistently embedded within Xylariaceae, with high support in the LSU dataset, whereas *Pseudomassaria fallax* was placed close to *Polyancora globosa* (Figs. 1, 2). *Rosasphaeria moravica* was consistently placed in the Hypocreales, and close relationship to *Niesslia exilis* and *Eucasphaeria capensis* received high support in the LSU

(Fig. 1) and combined four-gene analyses (Fig. 3). Phylogenetic positions of *Melogramma campylosporum* and *Pseudomassariella vexata* were less consistent in the LSU and ITS trees, but they were consistently placed within the Xylariales.

Taxonomy

The ten species investigated in detail are described below in alphabetical order.

Collodiscula japonica I. Hino & Katum., Bull. Faculty Agricult., Yamaguchi Univ. 6:55 (1955). Fig. 4

Stromata scattered or gregarious, solitary or laterally fused, superficial, pulvinate to nearly semiglobose, 0.5–1(–1.3) mm diam, 0.3–0.6 mm high, containing 1(–3) perithecia. Surface convex or flattened, silvery grey to dark brown, smooth, with a central papilla of black ostiole(s). External stromatal layer grey to black, carbonaceous, apically disintegrating and forming a sharp coronate margin. Base often surrounded by

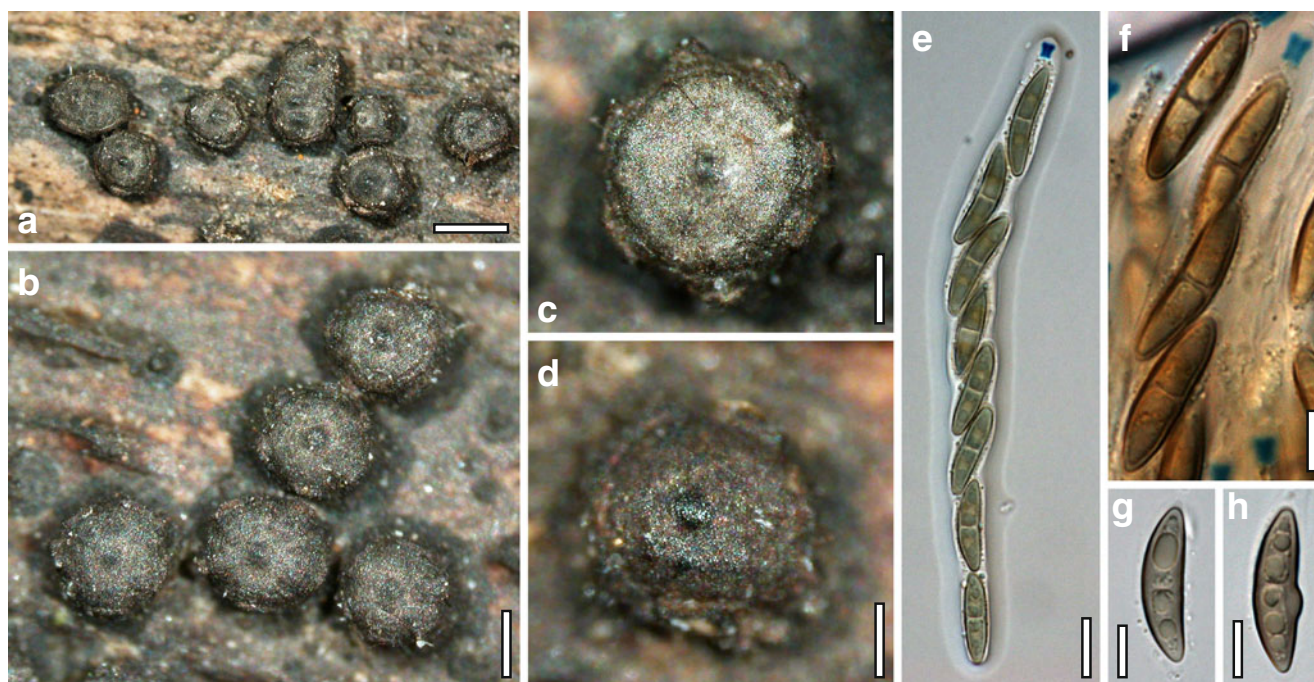


Fig. 4 *Collodiscula japonica* (WU 31311). **a–d** Stromata. **e** Ascus (in Lugol). **f** Ascospores in ascus showing amyloid apical plug (in Lugol). **g, h** Ascospores in 3% KOH. Scale bars: **a** = 0.8 mm. **b** = 0.4 mm. **c, d** = 0.2 mm. **e** = 10 μ m. **f–h** = 7 μ m

a black crustose ring on the host surface. Perithecia globose, with a soft brown hyphal peridium. Apically free paraphyses persistent. Asci cylindrical, with eight overlapping uniseriate ascospores, ca. $105\text{--}140 \times 6\text{--}10\text{ }\mu\text{m}$; apex containing a funnel-shaped, amyloid plug, $(2.5\text{--})2.8\text{--}3.3\text{--}(3.5)\text{ }\mu\text{m}$ high, $(2.0\text{--})2.3\text{--}2.5\text{--}(3.0)\text{ }\mu\text{m}$ diam ($n=30$). Ascospores $(15\text{--})17\text{--}21\text{--}(23) \times (3.5\text{--})4\text{--}5\text{--}(6)\text{ }\mu\text{m}$, l/w $(3.3\text{--})3.7\text{--}4.7\text{--}(5.2)$ ($n=35$), with one median, not or slightly constricted septum, fusoid, inaequilateral, with rounded ends, rarely one end pinched, yellowish brown to dark brown, initially with a hyaline minute globose basal cell, smooth, with two guttules in each cell and thin hyaline sheath; in 3% KOH ascospores partly swelling, septum becoming indistinct.

Anamorph: *Acanthodochium collodisculae* Samuels, J. D. Rogers & Nagas., Mycotaxon 28(2): 457 (1987).

Distribution: East Asia (China, Japan, Russia)

Habitat: dead culms of bamboos.

Material examined: CHINA, Zhejiang, Hangzhou, Jiu Xi, on dead culms of *Phyllostachys* sp., 20 Oct. 2008, W. Jaklitsch and H. Voglmayr, W.J. 3226 (WU 31311, culture CJ = CBS 124266; ITS-LSU sequence JF440974).

Notes: See Samuels et al. (1987) for a detailed description of the holomorph. As noted by Læssøe and Spooner (1994), this fungus agrees in all aspects with species of *Astrocystis* except for the septate ascospores. *Collodiscula* has been assigned either to the Amphisphaeriaceae or to the Xylariaceae. LSU and ITS sequences place this taxon clearly in the latter family. This fungus was also reported from Russia (Vasiljeva 1998; on *Sasa*) and Taiwan (Ju and Rogers 1999).

Leiosphaerella Höhn., Sitzungsber. Akad. Wiss. Wien, mathem. naturwiss. Kl., Abt. 1 128:579 (1919).

Type species: *Leiosphaerella praeclara*.

Leiosphaerella is a genus of unitunicate pyrenomycetes of uncertain affinities within the Xylariales, Sordariomycetes. Barr (1990) placed it in the Hyponectriaceae. The genus is characterised by immersed perithecial ascomata with apically free paraphyses, octosporous unitunicate asci with amyloid apical rings, and elongate, bicellular, hyaline ascospores. It consisted of approximately 16 species, morphology-based, without any available molecular data of named species useful for phylogenetic reconstruction.

Leiosphaerella lycopodina (P. Karst.) Jaklitsch & Voglmayr, comb. nov. Fig. 5

Mycobank MB 561138

Basionym: *Sphaerella lycopodina* P. Karst., Bidr. Känn. Finl. Nat. Folk 23:184 (1873).

= *Pseudapiospora lycopodina* (P. Karst.) L. Holm, in Lundell & Nannfeldt, Fungi Exsiccati Suecici 45–46 (Sched.):41 (1954).

= *Pseudapiospora moravica* Petr., Ann. Mycol. 29:356 (1931).

= *Pseudapiospora necans* (Rehm) Petr., Ann. Mycol. 32(5/6):420 (1934).

= *Pseudomassaria lycopodina* (P. Karst.) Arx, in Müller & Arx, Beitr. Kryptogamenfl. Schweiz 11 (no. 2):686 (1962).



Fig. 5 *Leiosphaerella lycopodina*. (WU 31312). **a** Habit on vital leaves. **b, c** Ascomata on leaves in face view (**c** in 3% KOH). **d** Perithecium in section (including asci). **e** Peridium in section. **f, g** Asci in Lugol. **h–j** Ascospores (**h** in Lugol). **k** Peridium in face view.

l Young paraphyses. **m, n** Amyloid ascus rings in Lugol. Scale bars: **a** = 1.5 mm. **b** = 0.1 mm. **c** = 50 μ m. **d** = 15 μ m. **e–h, l** = 10 μ m. **i–k, m, n** = 5 μ m

Ascomata amphiphylous, inconspicuous, appearing on the plant surface as brown, grey to black, circular areas 65–150 μ m diam, containing black ostiolar dots 15–30 μ m diam in the centre. Perithecia immersed below the epidermis of the host, scattered or in short rows of 3–5, (sub-)globose, (80–)105–135(–150) μ m high and wide ($n=42$). Ostioles slightly papillate, (28–)32–40(–46) μ m long, (14–)15–30(–40) μ m wide at the apex ($n=20$), filled with 1–1.5 μ m wide paraphyses; turning pale in 3% KOH. Peridium (8–)10–14(–16) μ m thick ($n=42$), sometimes thickened at the ostiole, composed of mostly long and narrow, strongly compressed, medium to dark brown, thin-walled cells (3.5–)5–15(–20) \times (1.5–)2–4(–4.5) μ m in section ($n=30$); in face view appearing as a *t. epidermoidea* of light to

medium brown cells (5–)6–15(–22) \times (2.5–)4–8(–10) μ m ($n=30$); inside lined by a layer less than 10 μ m thick of narrow hyaline hyphae up to 2.5 μ m wide; outside covered by brown, thin-walled, branched, (2.0–)2.5–5.0(–7.0) μ m wide ($n=60$) hyphae becoming lighter to hyaline with distance from the perithecium. Apically free paraphyses scant, indistinct broad bands, (2–)3–5 μ m wide, basally thickened to 5–8 μ m. Asci basal, (57–)61–85(–98) \times (8.5–)9–12(–14) μ m ($n=30$), fusoid to subclavate, straight or curved, with eight biseriate spores to the base, and a thickened apex with a flat amyloid (Lugol-blue) ring, 1.5–2 \times ca. 0.5 (<1) μ m. Ascospores (17–)18–23(–28) \times (3.2–)3.5–4.5(–4.8) μ m ($n=60$), narrowly fusoid, often curved, hyaline, smooth, comprising a large cell with 1–2

guttules and a small acute cell (3.5–)4.5–7.0(–8.5) μm long, mostly at the lower end, with several small guttules; surrounded by an inconspicuous swelling sheath 1–3 μm wide.

Cultures on slight nutrient agar (SNA; Nirenberg 1976) produced abundant black fertile perithecia after 2 months at room temperature.

Anamorph: not known.

Distribution: Europe, North America.

Habitat: Recently dead shoots of *Lycopodium* spp.; in Europe mainly recorded from *L. annotinum*.

Material examined: **Austria**, Oberösterreich, St. Willibald, Großer Salletwald, on *Lycopodium annotinum*, 25 Oct. 2009, H. Voglmayr (WU 31312, culture PL = CBS 125717; ITS-LSU sequence JF440975).

Notes: Ascomata of *Leiosphaerella lycopodina* are similar to those of *Monographella lycopodina* (see below), but even more inconspicuous and smaller. The ascospores of *L. lycopodina* resemble those of *L. praeclara* in shape, but differ by a distinctly eccentric septum and a swelling sheath. In other characters, *L. lycopodina* is similar to *L. praeclara*, supporting congeneric classification. *Pseudapiospora necans* (Rehm) Petr. is another taxon described from the same host with smaller asci (30–55 \times 7–9 μm) and ascospores (12.5–17 \times 3–4 μm). Petrak (1934, p. 420) reported that Rehm's material is immature and poorly developed and suspected conspecificity of this taxon with *L. lycopodina* (as *Pseudapiospora moravica*). Also Müller and von Arx (1962) and Barr (1964) treated these taxa as synonyms. Considering the distinct variation in spore size depending on the developmental state, we concur with these authors.

Leiosphaerella praeclara (Rehm) Höhn., Sitzungsber. Akad. Wiss. Wien, mathem. naturwiss. Kl., Abt. 1 128:579 (1919). Fig. 6

Basionym: *Didymella praeclara* Rehm in Krieger, Ann. Mycol. 4:39 (1906).

Host bark containing perithecia lively reddish brown, slightly elevated and discontinued on its surface either by inconspicuous, circular, diffuse, greyish areas 140–270 μm diam, sometimes delimited by a diffuse black outer line, or by black dots (47–)50–135(–172) μm diam comprising ostioles and upper parts of ascomata. Dots more distinct in 3% KOH in face view, but invisible in thin sections. Ostioles (30–)37–54(–62) μm long, (20–)28–45(–60) μm wide at the apex ($n=18$), even with the bark surface or convex, with circular or oblong outline; interior with 1–2 μm wide periphyses. Ascomata perithecial, entirely immersed below the epidermis in bark, solitary to scattered, rarely aggregated in small numbers. Perithecia globose, (100–)140–205(–235) μm ($n=50$) μm high and wide, depressed and 80–160 μm high when dry. Ascomata surrounded by scant, thin-walled brownish hyphae (1.7–)

2.5–5.7(–8.2) μm wide ($n=20$). Perithecial surface tightly connected with bark cells. Peridium (9–)11–16(–19) μm wide ($n=50$), composed of strongly compressed, thin-walled, dark brown cells (4–)7–15(–19) \times (1.0–)1.7–4.0(–4.7) μm ($n=30$), lined inside by a thin hyaline layer of 1–2 μm wide hyphae; peridium in face view a *t. angularis* of distinct, thin-walled, medium to dark brown cells (6–)8–15(–18) \times (4–)5–10(–15) μm ($n=30$). Apically free, (2–)3–6(–7) μm wide paraphyses numerous, in a gel matrix, upwards thinner within a short distance to 2–3 μm . Asci basal, (67–)80–100(–112) \times (12–)14–18(–24) μm ($n=25$), broadly fusoid, thick-walled, with eight fasciculate ascospores and a flat, amyloid (Lugol-blue) apical ring 2–4 \times 0.7–1.5 μm . Ascospores (28–)34–40(–46) \times (4.5–)5–6(–7) μm , l/w (5.1–)5.8–7.7(–9.3) ($n=60$), fusoid, hyaline, two-celled, with a non-constricted median septum, with narrowly rounded to acute ends, straight, curved or sigmoid, smooth; rarely with two septa; contents cloudy in KOH.

Cultures on MEA produced olive to black sterile perithecia after 4–6 months.

Anamorph: not known.

Distribution: Europe.

Habitat: On recently dead stems of *Vaccinium myrtillus*; also reported from *V. vitis-idaea* in Sweden (Note 351 in <http://www8.umu.se/myconet/asco/litt/newNotes.html>).

Isotype: **Germany**, Hessen, near Königstein, on 2 mm thick twigs of *Vaccinium myrtillus*, 18 July 1902, H. Rehm (W11860; as *Didymella praeclara*). *Epitype*, here designated: **Austria**, Oberösterreich, St. Willibald, Großer Salletwald, on *Vaccinium myrtillus*, soc. *Valsa ceratosperma* and *Seimatosporium* sp., 25 Oct. 2009, H. Voglmayr (WU 31314; culture LP = CBS 125586; ITS-LSU sequence JF440976).

Other specimens examined: **Austria**, Niederösterreich, Litschau, Schönau, Rottalmoos, on *Vaccinium myrtillus*, 14 Sep. 2009, H. Voglmayr and W. Jaklitsch (WU 31313).

Notes: Freshly collected material is in perfect agreement with the isotype. To ensure nomenclatural stability, a recent collection for which a culture and sequences are available, is here designated as epitype. This fungus is extremely inconspicuous and accordingly difficult to spot. Apart from the type collection, it has only rarely been recorded.

Melogramma campylosporium Fr., Summa veg. Scand., Section Post. (Stockholm):386 (1849). Fig. 7

Basionym: *Sphaeria melogramma* (Bull.) Pers., Syn. meth. fung. (Göttingen) 1:13 (1801).

= *Melogramma bulliardii* Tul. & C. Tul., Selecta fung. carpol. (Paris) 2:87 (1863).

See LaFlamme (1976) for additional synonyms.

Stromata erumpent from bark, solitary, scattered or aggregated in lines, sometimes confluent; pulvinate or discoid, (0.6–)1–3(–4) mm diam, 0.5–1.1 mm high, with

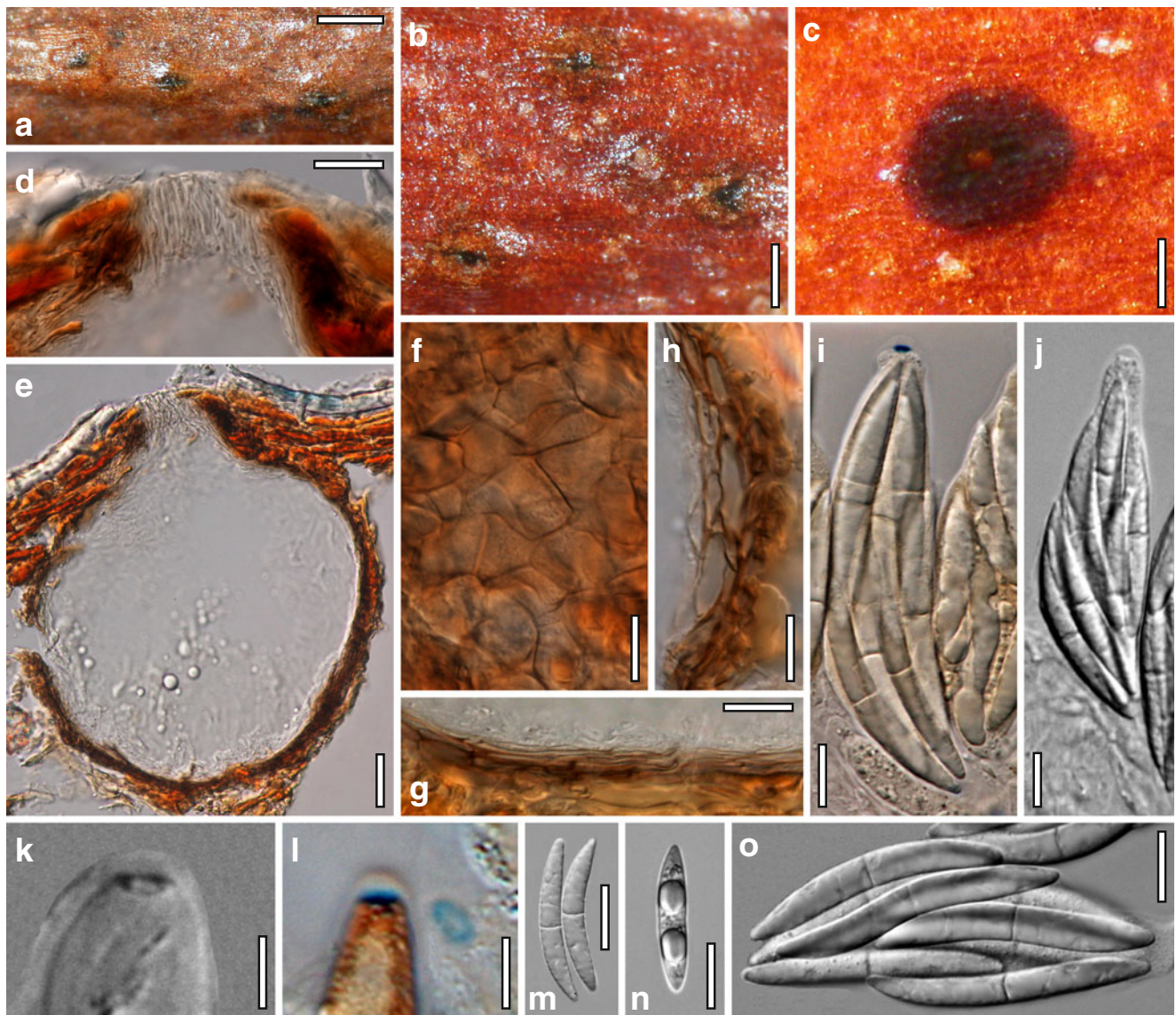


Fig. 6 *Leiosphaerella praeclara*. **a–c** Ostioles and perithecial dots on bark surface (**c** in 3% KOH). **d** Ostiole in vertical section. **e** Perithecium in section. **f** Peridium in face view. **g, h** Peridium in section (**g** at the base; **h** lateral). **i, j** Asci (**i** in Lugol, showing amyloid ring; **j** in water). **k, l** Apical ascus rings (**k** in water; **l** in Lugol). **m–o**.

Ascospores (**n** in Lugol). Sources: **a, k**, holotype W 11860; **b–j, l, m, o**, WU 31314; **n**, WU 31313. Scale bars: **a** = 0.3 mm. **b** = 0.15 mm. **c** = 0.1 mm. **d, g, m, n** = 15 μ m. **e** = 30 μ m. **f, h–j, o** = 10 μ m. **k, l** = 5 μ m

nearly smooth to slightly velutinous brown surface and slightly papillate black ostiolar dots (52–)67–154(–183) μ m diam or with a tubercular surface due to nearly free, grey to nearly black perithecia 300–500 μ m diam on top of the stromata, often with a collapsed concave top. Less commonly free or laterally fused perithecia in small groups erumpent through bark fissures. Stroma outline circular, angular, fusoid or oblong. Apically free paraphyses numerous, conspicuously long, 1.5–4 μ m wide. Asci clavate or fusoid, straight, curved or sigmoid, (90–)98–121(–134) \times (12–)13–15(–16.5) μ m ($n=30$), compressed in mounts up to 25 μ m wide, with short and narrow stipe, unitunicate,

with an inamyloid, undifferentiated, thickened apical wall ca. 1–3 μ m wide, containing eight bi- to tetraseriate ascospores, uni- or biseriata towards the base. Stipe sometimes elongated (20–66 μ m), then asci up to 157 μ m long. Ascospores falcate, often strongly curved, (35–)38–45(–48) \times (4–)4.5–5.7(–6.5) μ m, l/w (5.8–)7.2–9.4(–10.3) ($n=50$), compressed in mounts in 3% KOH to 7 μ m wide; with three equidistant septa, brown, end cells slightly lighter entirely or only at their tips, tips narrowly rounded to subacute, with a smooth narrow hyaline perispore; often with one large guttule in each cell; when young hyaline, straight and 0–1 septate.



Fig. 7 *Melogramma campylosporum*. **a–d** Stromata (**d** with collapsed perithecia on top). **e** Group of three aggregated perithecia. **f** Ascus apex. **g, h** Ascospores (**g** with slightly lighter tips, **h** with lighter

terminal cells). **i, j** Asci (**i** compressed). **f–j** in 3% KOH. **a, b, g.** WU 31317. **c, d, f, h–j.** WU 31316. **e.** WU 31315. Scale bars: **a** = 1 mm. **b, e** = 0.3 mm. **c, d** = 0.6 mm. **f** = 5 μ m. **g–j** = 10 μ m

Anamorph: not known.

Habitat: On corticated branches and logs of *Carpinus betulus* and *Corylus avellana*.

Specimens examined: **Austria**, Burgenland, Neusiedl/See, Edelstal, Spitzerberg, map grid 7967/2, on branches of *Carpinus betulus*, 10 Apr. 2010, I. Greilhuber and H. Voglmayr (culture MBU; ITS-LSU sequence JF440978). Kärnten, Klagenfurt Land, St. Margareten im Rosental, village area, grid square 9452/4, on branches of *Corylus avellana*, 19 Nov. 1994, W. Jaklitsch W.J. 291 (WU 31315). Niederösterreich, Mauerbach, near the cemetery, grid square 7763/1, on branches of *Carpinus betulus*, 12 Oct. 1996, W. Jaklitsch W.J. 971 (WU 31316). Steiermark, Pöllau, Schönaufklamm, grid square 8760/2, on branches of *Carpinus betulus*, 12 Sep. 2002, W. Jaklitsch W.J. 1953 (WU 31317).

Notes: See Munk (1957), LaFlamme (1976) and Hilber *et al.* (1982) for good descriptions and Breitenbach and Kränzlin (1984) for another illustration. LaFlamme (1976) examined types of other epitheta of *Melogramma* and accepted only *M. campylosporum* and *M. caucasicum* Jacz. in the genus. He transferred *M. spiniferum* (Wallr.) De Not. to his genus *Melanamphora*. Vasilyeva (1998) distinguished her *Melogramma corylina* on *Corylus heterophylla* by slightly longer ascospores (up to 56 μ m long).

Monographella lycopodina Jaklitsch, Siepe & Voglmayr, sp. nov. Fig. 8

Mycobank MB 561137

Perithecia immersa in foliis et caulibus Lycopodii, globosa, fusce brunnea. Peridium *textura angularis* ad *epidermoidea*. Hamathecium periphysibus et paraphysibus. Asci oblongi vel anguste clavati, (37–)43–57(–66) \times (5.0–)5.2–6.3(–7.5) μ m, octospori, amyloidei. Ascospores fusoidae vel oblongae, (9–)12–17(–24) \times (2.0–)2.2–3.0(–3.5) μ m, 1-(raro 2- vel 3-)septatae, hyalinae vel dilute brunneae, saepe curvatae, glabrae.

Perithecia amphiphylous, immersed below and visible through the translucent epidermis lifting it from the leaf tissue, solitary or in groups, with central, papillate and often acute ostiole projecting up to 100 μ m. Perithecia globose to bluntly conical, (80–)115–160(–190) μ m high and wide ($n=78$), often collapsed from above when dry, light brown to black. Ostioles usually distinctly darker than the perithecial body, black and shiny, bluntly conical or cylindrical, (20–)27–43(–51) μ m long, (8–)10–25(–35) μ m wide at the apex inside, (23–)39–72(–87) μ m ($n=53$) outside, filled with slightly clavate, 1–2.5 μ m wide periphyses. Peridium (10–)13–18(–22) μ m wide ($n=46$), composed of thin-walled, longish, light to medium brown cells (2.2–)3–11(–19) \times 2.0–4.5(–9) μ m in section ($n=35$),

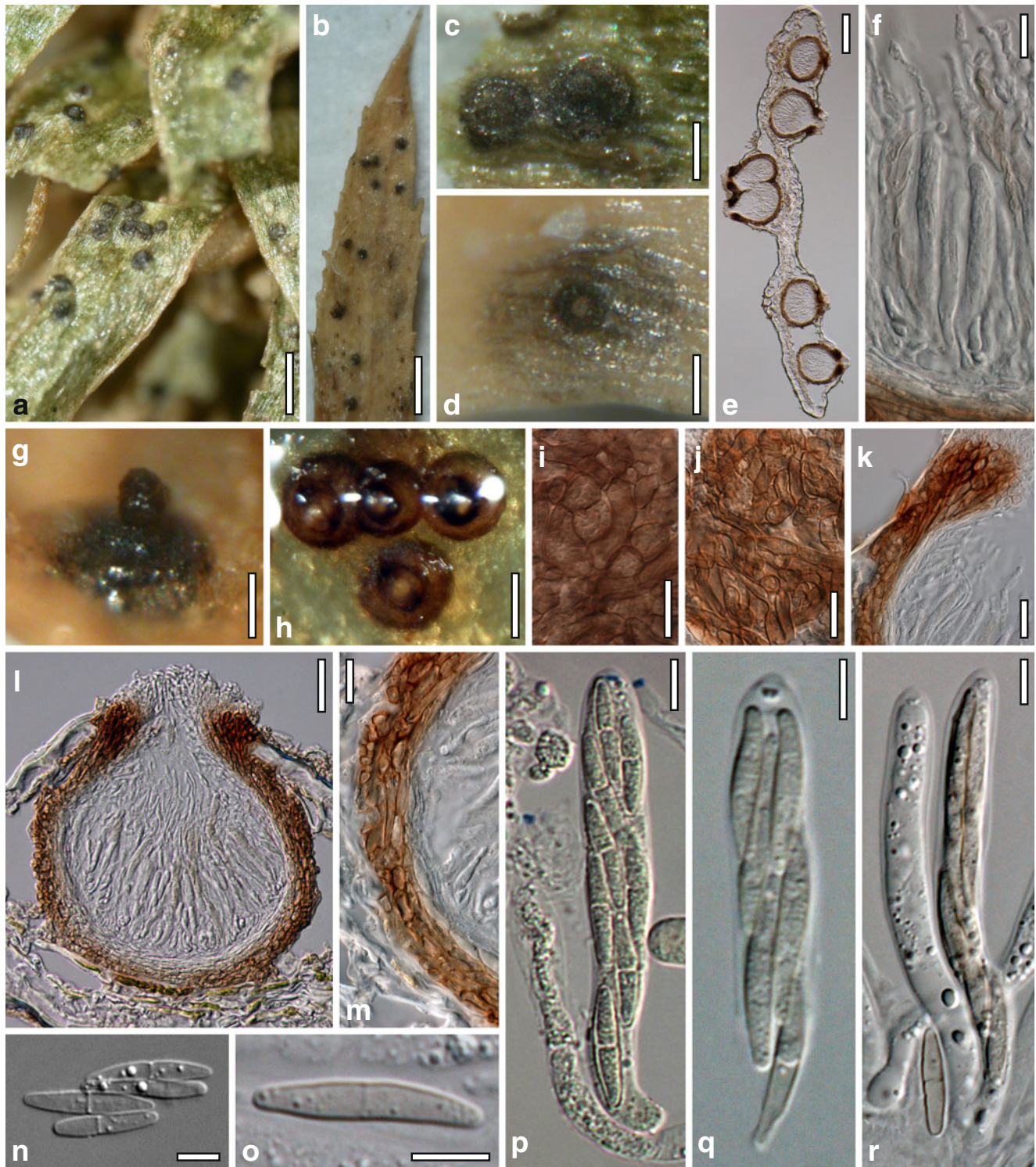


Fig. 8 *Monographella lycopodina*. **a, b** Habit on vital and dead leaves. **c, d, g, h** Ascomata on leaves in face view (**g** showing papillate ostiole; **h** in 3% KOH). **e** Amphiphylous ascomata in section. **f** Centrum characteristics. **i, j** Peridium in face view. **k, m** Peridium in section (**k**

thickened around ostiole). **l** Perithecium in section. **n, o** Ascospores. **p–r** Asci (**p** in Lugol, showing amyloid rings). Sources: **a–h, k–m**. (WU 31318); **i, j, n–r**. (WU 31319). Scale bars: **a** = 0.4 mm. **b** = 0.6 mm. **c, e, h** = 0.1 mm. **d, g** = 50 μ m. **f, i–k, m** = 10 μ m. **l** = 20 μ m. **n–r** = 5 μ m

lined inside by a thin layer to 7 μ m thick, of 0.7–1.5 μ m wide hyaline hyphae, partly shorter-celled and wider, to 3.5 μ m, at the base. Peridium thickened and darker around

the ostiole, comprising more reddish brown, thicker-walled, isodiametric to globose cells; cells towards the surface also smaller and more isodiametric. Peridium in face view a *t*.

angularis-epidermoidea of thin-walled brown cells (3–)6–13(–15)×(2.5–)4–9(–12) µm ($n=35$), sometimes with some brown hyphae on the surface. Apically free paraphyses scant, 1.5–4 µm wide. Asci basal, (37–)43–57(–66)×(5.0–)5.2–6.3(–7.5) µm ($n=40$), oblong or narrowly clavate, with 8 biseriate ascospores, short stipe and a refractive, amyloid (Lugol-dark blue), flat, funnel-shaped apical ring 1–1.5×0.5–0.8 µm ($n=20$). Ascospores (9–)12–17(–24)×(2.0–)2.2–3.0(–3.5) µm, l/w (3.6–)4.7–7(–10.4) ($n=115$), fusoid or oblong, hyaline to faintly brownish, straight or often slightly curved, smooth, 2-celled (rarely 3- or 4-celled) with a thin median, non-constricted septum becoming indistinct in KOH. Ascospore size much dependent on the state of development, from 8×1.5 µm and 1-celled when young.

Anamorph: not known; cultures on MEA and SNA remained sterile.

Distribution: Central Europe (Austria, Germany).

Habitat: on living and recently dead stems and leaves of *Lycopodium annotinum*.

Holotype: Austria, Oberösterreich, St. Willibald, Großer Salletwald, in leaves of *Lycopodium annotinum*, 19 July 2009, H. Voglmayr (WU 31319, culture LL = CBS 125585; ITS-LSU sequence JF440979). Paratype: Germany, Bavaria, Bayerischer Wald, Großer Arber, slope forest, grid square 6844/44, on partly living leaves and stalks of *Lycopodium annotinum*, N. Heine, comm. K. Siepe SI 44/2006, 8 Oct. 2006 ('LLB'; WU 31318); ITS sequence JF440980 (DNA extracted from ascomata).

Notes: Most ascospores of *Monographella lycopodina* did not germinate in pure culture on MEA or stopped growth after transfer from the isolation plate, and repeated attempts were necessary to obtain pure cultures. Asci were transferred upon ascospore germination to minimise potential contamination. To confirm the identity of the cultures, PCR was also directly performed on perithecial contents from both collections, which consistently revealed ITS sequences identical with those from pure cultures.

Remarkably, ITS sequences of *M. lycopodina* are identical with those from an undescribed *Microdochium* sp. (GenBank sequences AM502255, AM502256, AM502257, AJ279458, AJ279470, AJ279481). The latter was commonly isolated from flooded roots and stems of *Phragmites australis* at Lake Constance in southern Germany (Wirsal et al. 2001), and subsequent investigations revealed that this fungus is a common endophyte in *Phragmites*, especially in the rootstock of inundated plants (Ernst 2005). Neither *Phragmites* nor wetland habitats were present in the nearer vicinity of the collecting sites of *M. lycopodina*. Considering the highly distinct ecology, it appears unlikely that the *Microdochium* sp. from *Phragmites* is conspecific with *M. lycopodina*, and ITS sequences may not be suitable for distinction at the species level.

Another fungus with hyaline, 2-celled ascospores described from *Lycopodium*, *Metasphaeria lycopodina* Petr. (Czech Republic, Moravia, Hranice (Mährisch Weisskirchen), Paršovice (Parschowitz), F. Petrak, May 1929, holotype W 12052), was examined in this study. It has clavate, fissitunicate asci (50–)53–61(–65)×(8.5–)9–10.5(–11.5) µm ($n=20$) with ascospores (16–)17–21(–23)×(3.2–)3.5–4.5(–5.2) µm, l/w (3.5–)4.1–5.3(–6) ($n=20$). Morphologically, this is clearly a species of *Massarina* and was revised by A. Aptroot (specimen labels) as *M. chamaecyparidis* (Rehm) L. Holm & K. Holm.

Pseudomassaria Jacz., Bull. Herb. Boissier 2:663 (1894).
= *Apiospora* Höhn., Sitzungsber. Akad. Wiss. Wien, mathem. naturwiss. Kl., Abt. 1 118:1215 (1909).
=? *Apiosporina* Petr., Ann. Mycol. 23(1/2):18 (1925).
=? *Pseudapiospora* Petr., Hedwigia 68:233 (1928).

Type species: *Pseudomassaria chondrospora*.

This genus differs from *Leiosphaerella* mainly by apiospores and usually more clavate asci, which contain amyloid or inamyloid apical rings. Approximately thirty species are currently classified in this genus, but no molecular phylogenetic data were available. Several generic synonyms are known. Petrak obviously missed the generic name *Pseudomassaria* and erected his genus *Apiosporina* (Petrak 1925) for *Pseudomassaria corni* (Sowerby) Arx (basionym *Sphaeria corni* Sowerby). Later Petrak (1928) realised homonymy with *Apiosporina* Höhn. (Höhnelt 1910) and therefore replaced his genus *Apiosporina* by *Pseudapiospora* Petr., which accordingly also became a synonym of *Pseudomassaria*. However, in the absence of molecular data for *P. corni*, the generic type of *Apiosporina* Petr. and *Pseudapiospora*, and considering low correlation of phylogenetic and morphological genus circumscriptions in Xylariales, synonymy with *Pseudomassaria* cannot be ascertained.

Pseudomassaria chondrospora (Ces.) Jacz., Bull. Herb. Boissier 2:663 (1894). Fig. 9

Basionym: *Sphaeria chondrospora* Ces., Hedwigia: tab. 11, Fig. 2 (1855).

Perithecia depressed globose to ellipsoid, (180–)220–300(–340) µm high ($n=25$), (220–)300–400(–500) µm wide ($n=33$), distinctly depressed and 95–220 µm high when dry; scattered, gregarious or aggregated in groups in pale bark substance, immersed in bark below small bumps often with longish fissures and black dots. In 3% KOH bark surface with distinct black convex pustules 150–550 µm. Ostioles short cylindrical, (38–)40–50(–56) µm long, (24–)28–50(–54) µm wide at the apex ($n=13$), even with the bark surface, containing 1–2 µm wide periphyses in a gel matrix. Peridium (14–)18–25(–28) µm thick ($n=40$),

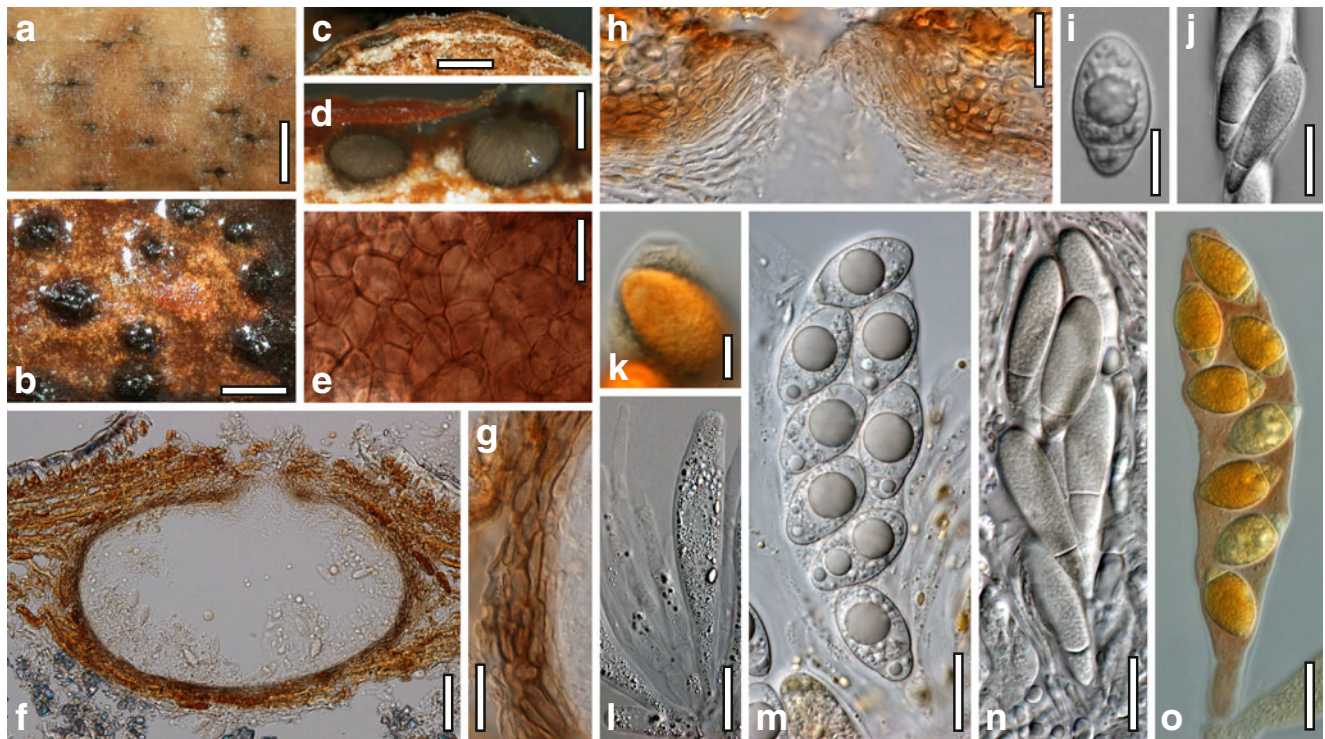


Fig. 9 *Pseudomassaria chondrospora*. **a, b** Habit of ascomata in bark (**b** in 3% KOH). **c, d** Ascomata immersed in bark, in section (**c** before, **d** after rehydration). **e** Peridium in face view. **f** Perithecium in section. **g** Peridium in section. **h** Ostiole in section. **i, j** Ascospores. **k**

Inamyloid ascus apex in Lugol. **l** Immature ascus with paraphyses. **m–o**. Asci (**o** in Lugol). Sources: **a–i, l, n**. (WU 31324); **k, m**. (WU 31320); **j, o**. (WU 31322). Scale bars: **a–c** = 0.5 mm. **d** = 0.2 mm. **e, g, h, j, l–o** = 15 μ m. **f** = 50 μ m. **i** = 10 μ m. **k** = 5 μ m

composed of strongly compressed, narrow, variable, light to medium brown, thin-walled cells (3.5–)7–17(–22) \times (1.2–)2.5–4.5(–5.5) μ m in section ($n=35$), lined inside by a hyaline layer up to 10 μ m thick of few thin parallel hyphae, outside tightly connected and mixed with bark cells. Peridium around ostioles 30–45 μ m thick including cells embedded in bark, of light brown to hyaline, thick-walled, isodiametric cells (3–)4–7(–11) \times (2.0–)2.5–5.0(–7.0) μ m ($n=30$). Peridium in face view a medium brown, thin-walled *t. angularis* of cells (4.5–)8–17(–23) \times (3.5–)6–12(–14) μ m ($n=35$), covered by some amorphous matter. Hyphae below perithecia scant, hyaline, (1.0–)1.5–3.0(–4.0) μ m wide ($n=30$). Apically free paraphyses numerous, 3–9 μ m wide, basally to 12 μ m, often indistinct broad bands, usually more distinct between asci. Asci basal, (80–)96–118(–132) \times (19–)22–28(–32) μ m ($n=45$), clavate to fusoid, often with a long stipe, with eight bi-, or partly obliquely uniseriate ascospores; apex inamyloid, also after KOH-treatment, slightly thickened, without a ring. Ascospores (18–)21–31(–41) \times (7–)10–13(–15) μ m, l/w (1.4–)1.6–3.1(–5.6) ($n=150$), hyaline to yellowish, broadly ellipsoid, sometimes oblong or clavate, apiosporous, with a rounded to subconical small lower cell 2.5–8(–11) μ m long, straight or curved, thick-walled, smooth, contents granular, with one large guttule per cell, or small cell multiguttulate;

contents granular or cloudy in KOH; with a swelling sheath when young; rarely with a septum in the large cell or with a small cell at each end.

Anamorph: not known.

Distribution: Europe, North America.

Habitat: Common on recently dead thin twigs of *Tilia* spp. often still attached to the tree.

Material examined: **Austria**, Vienna, Döbling, Grinzing Friedhof, grid square 7763/2, on twigs of *Tilia platyphyllos*, 20 Feb. 2000, W. Jaklitsch W.J. 1413 (WU 31321). Same place and host, soc. *Amphiportha hranicensis* and *Herco-spora tiliae*, 21 Aug. 2010, W. Jaklitsch and O. Sükösd (WU 31325, culture PC1; ITS-LSU sequence JF440982). Döbling, Aslangasse, grid square 7763/2, on twigs of *Tilia tomentosa*, 8 May 1999, W. Jaklitsch, W.J. 1316 (WU 31320). Same area, on twigs of *Tilia platyphyllos*, 19 May 2001, W. Jaklitsch W.J. 1759 (WU 31322). Floridsdorf, ‘Neu-Stammersdorf’, at Kummergasse, grid square 7764/2, on twigs of *Tilia tomentosa*, 10 June 2006, W. Jaklitsch W.J. 2918 (WU 31323). Landstraße, Botanical Garden, 2 June 2009, H. Voglmayr (WU 31324, culture PC = CBS 125600; ITS-LSU sequence JF440981).

Notes: Ascospores of this common fungus vary considerably in size and shape; although they are mostly broadly ellipsoid and 18–27 \times 10–15 μ m, with the small cell 2.5–

6 μm long, long and narrow ascospores may be present in the same perithecium. Both collections sequenced revealed identical ITS-LSU sequences.

Pseudomassaria fallax (Petr.) Arx, Ber. schweiz. bot. Ges. 62:349 (1952). Fig. 10

Basionym: *Apiosporina fallax* Petr., Ann. Mycol. 25(3/4):279 (1927)

Perithecia immersed below pallid, round or elongate patches or minute greyish brown dots in the slightly elevated epidermis of the host, scattered, subglobose, (70–)100–160(–220) μm wide, corresponding to the width of the dots, hyaline to yellowish, with a (sub-)hyaline pseudoparenchymatous peridium, surrounded by white hyphae, or with the outermost peridial cell layer brown when immersed beneath darkened dots. Perithecial contents comprising an amorphous rubber-like gel partly turning yellow in 3% KOH when fresh. Apically free paraphyses embedded in gel, indistinct, 1.5–6 μm wide. Asci agglutinated in the gel, unstable when fresh, disintegrated by pressure, clavate, (54–)62–75(–82) \times (13–)14–18(–19) μm ($n=30$), curved in the perithecium, fusoid or clavate, with eight biseriate ascospores and short narrow stipe; apex usually attenuated, thickened to ca. 2.5 μm , containing an amyloid ring 2.5–3.5 \times 0.7–1.5 μm . Ascospores broadly ellipsoid, fusoid or oblong, straight or slightly curved, symmetric or slightly inequilateral, (15.5–)18–21(–24) \times (5.5–)6.5–8(–9) μm , l/w (2–)2.4–3.2(–3.8) ($n=80$), hyaline, yellow in Lugol, apiosporous with a small lower cell (2–)3–4(–5) μm long, ends narrowly rounded; wall smooth, 0.5 μm wide; contents multiguttulate or granulose

in water, with a large guttule in the main cell when dead; surrounded by a gelatinous sheath distinctly swelling in 3% KOH to 2.5–5(–7) μm .

Anamorph: not known.

Distribution: Europe.

Habitat: On recently dead twigs of *Cornus sanguinea*.

Specimens examined: **Austria**, Kärnten, Klagenfurt Land, St. Margareten im Rosental, village area, grid square 9452/4, on recently dead twigs of *Cornus sanguinea*, 7 Aug. 1993, W. Jaklitsch (WU 15592). St. Margareten im Rosental, Wograde, grid square 9452/3, on recently dead twigs of *Cornus sanguinea*, 31 May 2000, W. Jaklitsch W.J. 1476 (WU 31327). Vienna, 3rd district, Botanical Garden, on corticated twigs of *Cornus sanguinea*, 9 Jan. 2011, H. Voglmayr (WU 31326, culture PSC = CBS 129020; ITS-LSU sequence JF440983).

Notes: Another species of *Pseudomassaria* occurring on *Cornus* spp. that we have not found recently, is *P. corni*. Based on descriptions of this taxon (Barr 1964; Corlett 1978, 1981; Petrak 1925), which was originally described from *Cornus suecica*, it differs morphologically from *P. fallax* by the length of the small ascospore cell (ca. 6–8 μm) comprising one third or even more of the whole ascospore length in *P. corni*. Furthermore, *P. fallax* is characterised by a hyaline perithecial wall that only turns brown in the outer layer with age. Petrak (1927) described *P. fallax* (as *Apiosporina fallax*) in detail and distinguished it from *P. corni* (as *A. corni*) by the traits mentioned, and also by a slower maturation of ascospores. Unlike other species of *Pseudomassaria* studied here, perithecia of *P.*



Fig. 10 *Pseudomassaria fallax*. **a** Habit in bark. **b** Ascus apex containing amyloid ring in Lugol. **c–f** Asci (**e**, **f** in Lugol). **g**, **h** Ascospores in 3% KOH showing swelling sheath (**g** showing yellow

gel). Sources: **a**, **b**, **g**, **h**. WU 31326. **c**. WU 15592. **d–f**. WU 31327. Scale bars: **a** = 0.2 mm. **b**, **g** = 5 μm . **c–f**, **h** = 10 μm

fallax contain indistinct paraphyses immersed in a gel. A distinctly swelling ascospore sheath has also been seen in *Leiosphaerella lycopodina*. Phylogenetically, *P. fallax* does not belong to the genus *Pseudomassaria*. However, owing to the poor representation of other genera in terms of gene sequences, we refrain from a taxonomic change at this time.

Pseudomassaria sepincoliformis (De Not.) Arx, Ber. schweiz. bot. Ges. 62:350 (1952). Fig. 11

Basionym: *Sphaerella sepincoliformis* De Not. [as *sepincolaeformis*], Comm. Soc. crittog. Ital. 2(no. 3):486 (1867).

Ascomata immersed below the epidermis of the host, scattered or in small groups, appearing on the surface as grey

to black rings with a minute central hole to ca. 40 μm diam, or as brown, greyish to black ‘clypei’ (150–)220–300(–330) μm diam ($n=40$). ‘Clypei’ after rehydration conspicuous, uniformly black, surrounded by narrow yellow zones in the bark. Perithecia when dry black, depressed globose, subglobose, ellipsoid or nearly lenticular, erumpent through the epidermis of the host, lifting it and tearing it longitudinally or in stellate fissures, eventually centrally collapsing discoid from above when old, 230–380 μm diam and max. 130 μm high. In section perithecia ellipsoid or subglobose, (100–)130–185(–205) μm high, (200–)230–285(–300) μm diam ($n=20$); on the top often partly covered by a light amorphous substance mixed with narrow hyaline hyphae. Ostioles

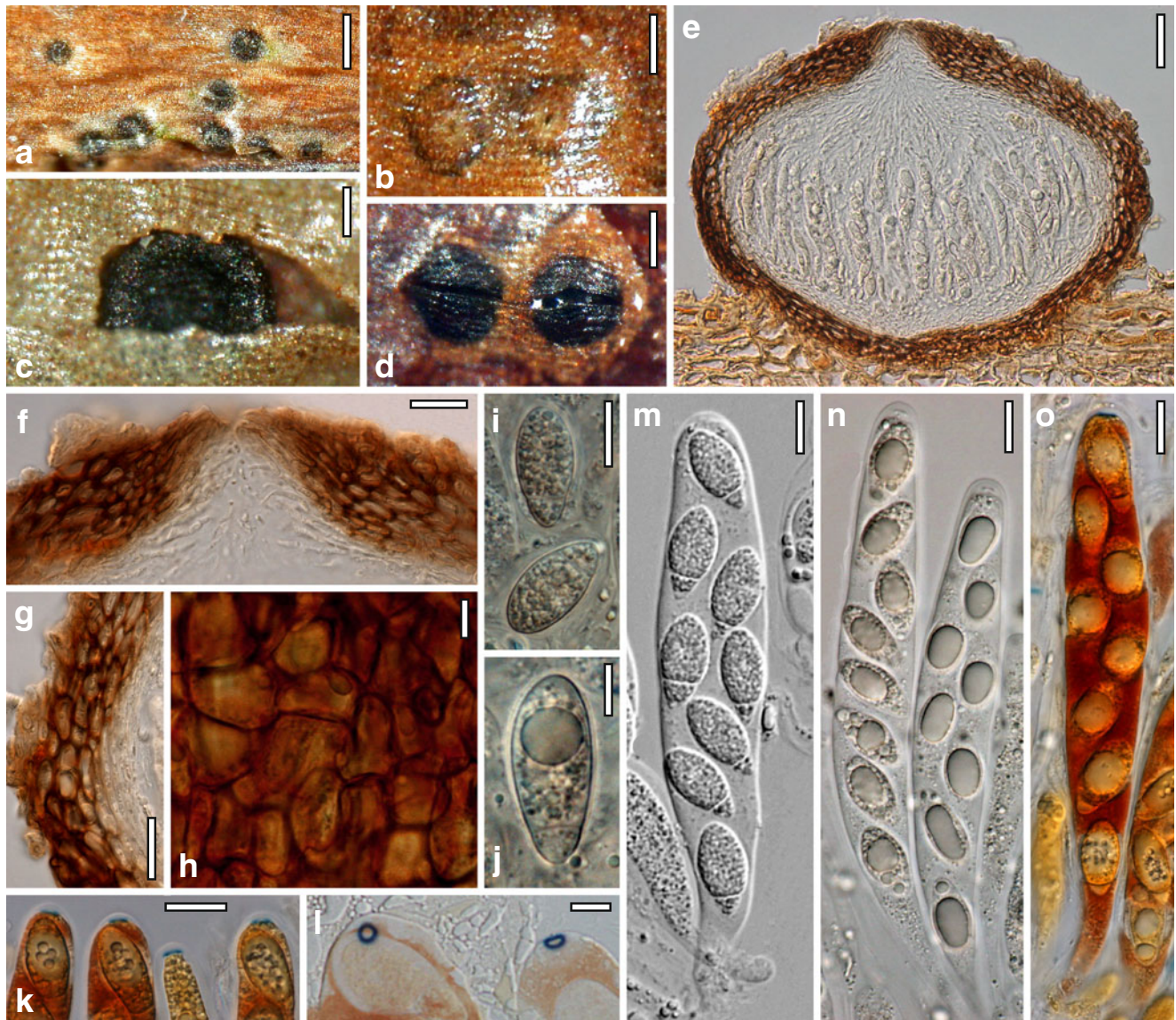


Fig. 11 *Pseudomassaria sepincoliformis*. **a** Habit in bark. **b** Ring-like ‘clypeus’ in bark. **c** Erumpent discoid perithecium. **d** Perithecial dots after rehydration. **e** Perithecium in section. **f** Ostiole in section. **g** Peridium in section. **h** Peridium in face view. **i, j** Ascospores (**i** vital; **j**

dead). **k, l** Amyloid ascus rings in Lugol. **m–o** Asci (**m, n** in 3% KOH; **o** in Lugol). Sources: **a, k, o**. (WU 31331); **b–h, n**. (WU 31330); **i, j, l, m**. (WU 31332). Scale bars: **a** = 0.4 mm. **b, d** = 150 μm . **c** = 70 μm . **e** = 30 μm . **f, g** = 15 μm . **h, j, l** = 5 μm . **i, k, m–o** = 10 μm

inconspicuous, not projecting, (32–)36–44(–48) μm long ($n=20$), (20–)27–48(–56) μm wide outside ($n=11$), filled with periphyses 1–2.5 μm wide. Peridium in section (15–)19–28(–30) μm thick at the base, (21–)27–35(–37) μm at the sides ($n=20$), often thinner at the base than at the sides and upper part; composed of medium to dark brown angular cells (3.5–)6–15(–19) \times (2–)3–7(–8) μm ($n=35$) with common walls up to 2 μm thick; inside lined by a narrow, max. 15 μm thick layer of hyaline hyphae 1–2.5 μm wide (layer included in measurements). Peridium in face view a medium to dark brown *t. angularis* of cells (6–)8–14(–18) $\mu\text{m} \times$ (3.5–)5–10(–13) μm ($n=30$) with common walls up to 2 μm thick; covered by few brown hyphae 2.5–4(–5) μm wide ($n=25$), becoming lighter with distance from the peritheci-um. Hyphae in the substrate scant, hyaline, thin-walled, 2–4(–5) μm wide, corresponding to those situated on top of perithecia. Apically free paraphyses numerous, 1.5–4 μm wide, thickened to 6–8 μm towards base. Asci (87–)100–120(–127) \times (11.5–)13–20(–26) μm ($n=50$), subclavate, fusoid or oblong with a stipe variable in length; containing 6–8 biserial or obliquely uniserial ascospores, with a KOH-refractive, amyloid (Lugol-blue) apical ring 2.2–4 \times 0.4–0.8 μm ; contents lively red-brown in Lugol. Ascospores (12.5–)16–20(–21.5) \times (6.5–)7.8–9.5(–10.5) μm , l/w (1.5–)1.8–2.3(–2.9) μm ($n=100$); ellipsoid, with a non-constricted septum near the lower end, separating a small cell (2.5–)3–5(–7) μm long ($n=82$), hyaline or yellowish, multiguttulate when vital, with one large and several small guttules in the larger cell when dead; initially with a narrow inconspicuous sheath.

Anamorph: not known.

Distribution: Europe.

Habitat: Recently dead twigs of *Rosa* spp.

Material examined: **Austria**, Niederösterreich, Böheimkirchen, Maierhöfen, road to Hegerberg, grid square 7860/4, on dead twigs of *Rosa canina* s.l., 5 Aug. 2000, W. Jaklitsch W.J. 1515 (WU 31328). Gießhübl, Kleiner Sattelberg, on *Rosa* sp., 12 Sep. 2010, H. Voglmayr (WU 31329, culture PS = CBS 129022; ITS-LSU sequence JF440984). Neunkirchen, Wechsel, Mariensee, on *Rosa* sp., 17 Sep. 2008, H. Voglmayr (WU 31330); ibidem, 23 Sep. 2009, H. Voglmayr (WU 31331). Vienna, 21st district, Marchfeldkanalweg, east from Brünner Straße, on dead twigs of *Rosa canina* s.l., 14 Nov. 2010, W. Jaklitsch and O. Sükösd (WU 31332, culture PS1).

Notes: Based on phylogenetic analyses (Figs. 1 and 2), *P. sepincoliformis* is closely related to the type species of *Pseudomassaria*, *P. chondrospora*, although the latter differs in a non-amyloid ascus apex.

Pseudomassariella vexata (Sacc.) Petr., Sydowia 9:603 (1955). Fig. 12

Basionym: *Didymella vexata* Sacc., Michelia 2(no. 6):58 (1880).

= *Leiosphaerella vexata* (Sacc.) E. Müll., in Müller & Arx, Beitr. Kryptfl. Schweiz 11 (no. 2):674 (1962).

Perithecia immersed in or directly below the slightly elevated epidermis of the host, scattered, depressed globose and <200 μm high when dry, appearing on the bark surface as circular, flat or convex, light brown to dark greyish brown dots, 130–250(–320) μm diam; in section subglobose or ellipsoid, (150–)180–255(–285) μm high, (180–)230–310(–340) μm diam ($n=30$). Ostioles (39–)48–70(–83) μm long, (15–)22–34(–46) μm inside ($n=30$), inconspicuous, conical or cylindrical, central or slightly eccentric, periphysate. Peridium in section (12–)15–21(–24) μm wide at the base, (16–)18–26(–31) μm at the sides ($n=30$), wider laterally and particularly apically, comprising medium brown and elongate, thin-walled cells (4–)6–17(–25) \times (2–)3–6(–8) μm ($n=30$), wider and isodiametric in outer layers, particularly at the perithecial top. Peridium in face view a light to medium brown *t. angularis* of thin-walled cells (6–)9–19(–26) \times (5–)7–13(–16) μm ($n=30$), surrounded externally by scant subhyaline or light brown, thin-walled hyphae (1.5–)2.5–4.5(–5.5) μm wide ($n=35$). Apically free paraphyses numerous, 3–9 μm wide, tapering upwards. Asci in small numbers in the peritheci-um, clavate, ellipsoid or subglobose, (75–)84–108(–133) \times (23–)30–40(–43) μm ($n=21$), with (4–)8 fasciculate ascospores and a short narrow stipe; apex attenuated, not differentiated but wide when young, inamyloid. Ascospores broadly ellipsoid or oblong, rarely reniform, (22–)28–33(–38) \times (9.5–)11–14(–15) μm , l/w (1.7–)2.1–2.8(–3.4) ($n=100$), subhyaline or yellowish, light brown when old, with one median or slightly eccentric, not or slightly constricted septum, rarely with two septa, smooth; contents finely multiguttulate or granulose, with 1–2 guttules when dead; often becoming hyaline and unstructured internally in 3% KOH; width of vital ascospores tending to be at the upper range.

Fertile perithecia up to ca. 0.8 mm diam with contents identical to perithecia on the natural host formed on PDA and MEA after 2 months (1 month at room temperature plus 1 month at 15°C).

Anamorph: not known.

Distribution: Europe.

Habitat: On recently dead twigs of *Cornus sanguinea*.

Specimens examined: **Austria**, Niederösterreich, Tulln, Donau-Auen, on corticated twigs of *Cornus sanguinea*; Oct. 1952, F. Petrak (W). **Austria**, Vienna, 3rd district, Botanical Garden, on corticated twigs of *Cornus sanguinea*, 9 Jan 2011, H. Voglmayr (WU 31333, culture LVE = CBS 129022; ITS-LSU sequence JF440977).

Notes: Petrak (1955) removed *P. vexata* from *Didymella* and erected the genus *Pseudomassariella* for it. He interpreted this genus as being closely related to *Pseudo-*

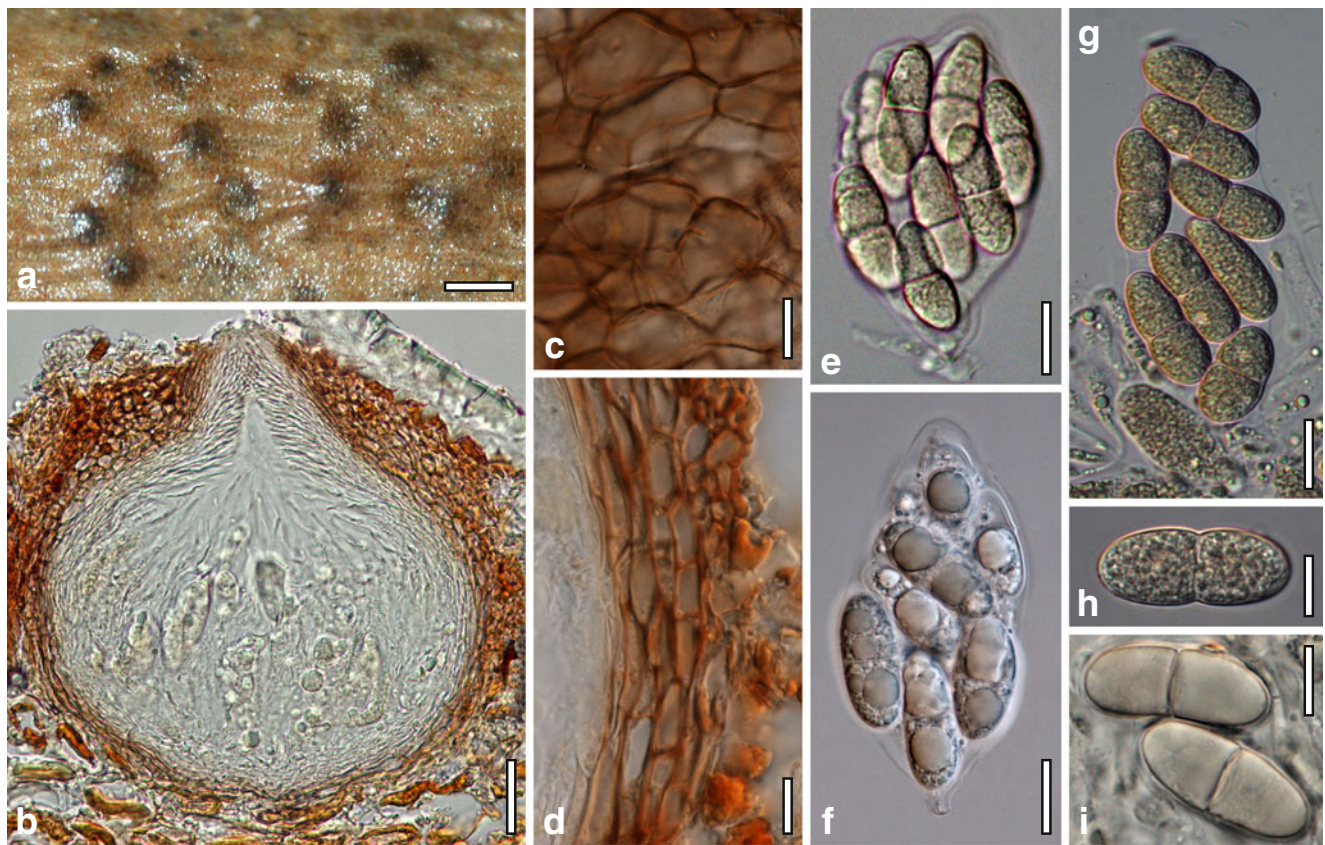


Fig. 12 *Pseudomassariella vexata* (WU 31333). **a** Habit in bark. **b** Perithecium in section. **c** Peridium in face view. **d** Peridium in section. **e–g** Asci (**f** in 3% KOH). **h, i** Ascospores (**h** in water; **i** in 3% KOH).

Sources: a, e. Petrak 1952 (W); b–d, f–i. WU 31333. Scale bars: a = 0.3 mm. b = 30 μ m. c, d, h, i = 10 μ m. e–g = 15 μ m

massaria, only differing in ascospore septation. Phylogenetic analyses of LSU and ITS are inconclusive about its exact phylogenetic placement: while ITS sequences (Fig. 2) support Petrak's view of a closer relationship to *Pseudomassaria*, LSU sequences (Fig. 1) suggest clustering with *Hyponectria*. Based on ascoma and ascus features, Müller and von Arx (1962) transferred the species to *Leiosphaerella*, which however is not supported by molecular phylogenies. It is therefore best classified in a distinct monotypic genus *Pseudomassariella*.

***Rosasphaeria* Jaklitsch & Voglmayr, gen. nov.**

Mycobank MB 561139

Genus novum Hypocrealeum. Ascomata perithecialia, substrato immersa. Hamathecium periphysibus et paraphysibus apicalibus. Asci inamyloidei. Ascosporeae hyalinae, septatae.

Species typica: *Rosasphaeria moravica*.

A new genus of Hypocreales. Ascomata perithecial, immersed in the substrate. Hamathecium composed of periphyses and apical paraphyses. Asci inamyloid. Ascospores hyaline, septate.

Type species: *Rosasphaeria moravica*.

Etymology: *Rosasphaeria* means a *Sphaeria* (ascomycete forming perithecia) on the host *Rosa*, analogous to the closely related *Eucasphaeria*, which occurs on *Eucalyptus*.

***Rosasphaeria moravica* (Petr.) Jaklitsch & Voglmayr, comb. nov. Fig. 13**

Mycobank MB 561140

Basionym: *Stigmatella moravica* Petr., Ann. Mycol. 12:473 (1914).

= *Nectriella moravica* (Petr.) Petr., Ann. Mycol. 17:78 (1920) [1919].

= *Leiosphaerella moravica* (Petr.) Petr., Sydowia 15:209 (1962) [1961].

Presence of ascomata indicated on smooth greyish bark surface by plane to convex, brown to black circular dots comprising ostioles, darkened areas or translucent perithecia (50–)130–190(–205) μ m diam ($n=25$), scattered or in lines; often with a dark centre (ostiole) and a lighter zone with its outer margin delimited by a black line. Ostioles (33–)44–72(–90) μ m long ($n=28$), (16–)27–48(–65) μ m wide at the apex ($n=50$), central or eccentric, cylindrical, filled with numerous periphyses. Perithecia entirely immersed in bark, scattered, gregarious or 2–3 aggregated,

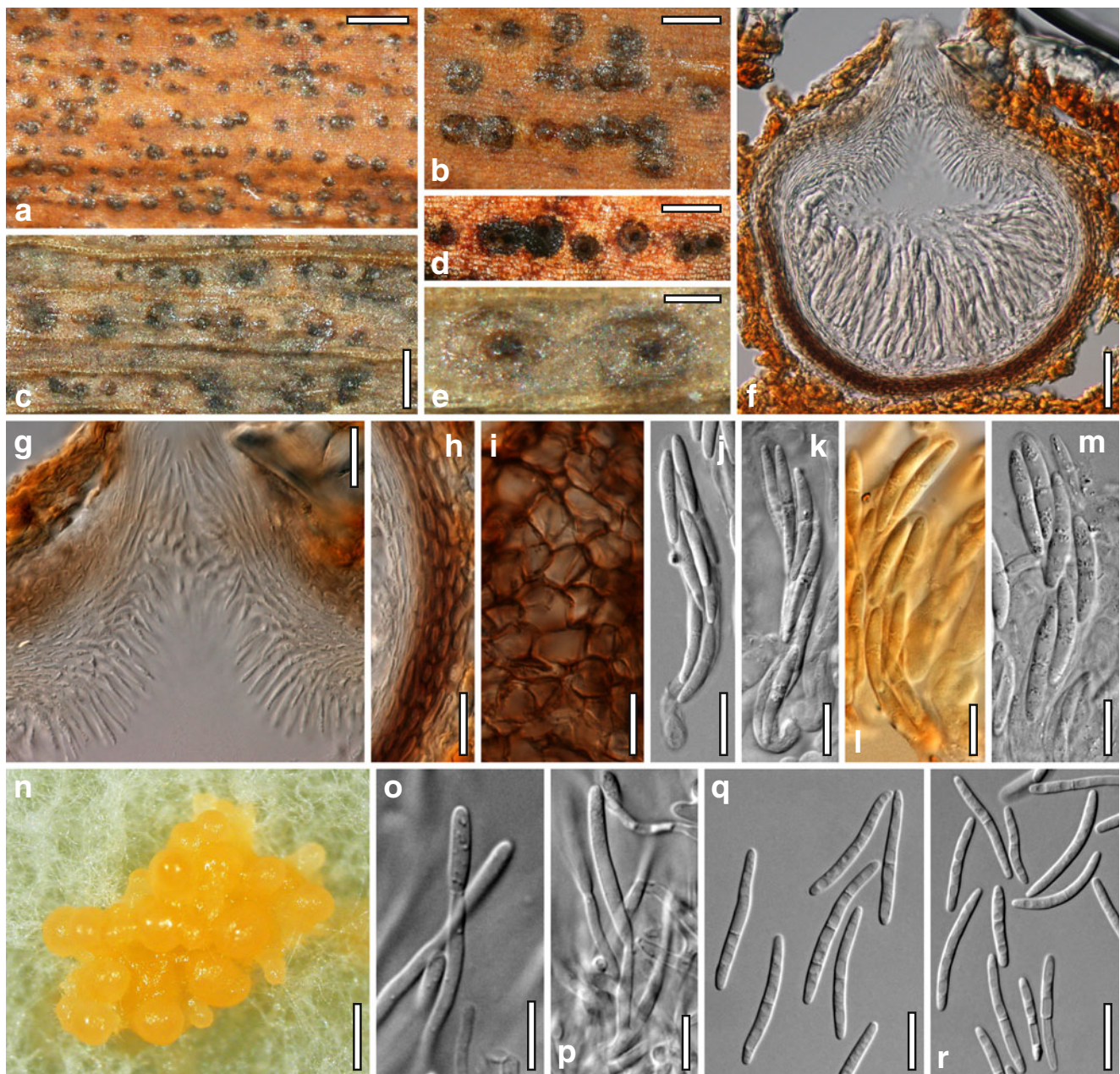


Fig. 13 *Rosasphaeria moravica* (WU 31334). **a–m** Teleomorph. **a–e** Perithecial dots on bark surface (**d** in 3% KOH). **f** Perithecium in section. **g** Hamathecium (periphyses and apical paraphyses). **h** Lateral peridium in section. **i** Peridium in face view. **j–m** Asci and ascospores (**l** in Lugol). **n–r** Anamorph. **n** Pycnidia (PDA, 54 days, 25°C). **o–r**

Conidia (**o**, **p** young, on phialides). Sources: **a**, **b**, **k**. Petrak 1919 (W). **c**, **e**, **m**. lectotype Petrak 1912 (W). **d**, **f–i**. WU 31334. **j**, **l**. Petrak 1913 (W). **n–r**. CBS 124270. Scale bars: **a**, **n** = 0.5 mm. **b–d** = 0.2 mm. **e** = 75 μ m. **f** = 30 μ m. **g**, **j**, **r** = 15 μ m. **h**, **i**, **k–m**, **o–q** = 10 μ m

(sub-)globose, (92–)135–200(–235) μ m diam ($n=60$), depressed when dry. Peridium in section (10–)12–18(–24) μ m wide ($n=60$), consisting of few layers of strongly compressed, thin-walled, angular or oblong brown cells (2.5–)4–8(–11) \times (1.5–)2–3(–3.5) μ m ($n=30$), often lighter around the ostiole; lined inside by a layer <10 μ m thick of narrow hyaline hyphae. Peridium in face view a dark (reddish-)brown *t. angularis* of distinct, thin-walled, angular cells,

(3.5–)5–10(–14) \times (2.5–)4–8(–10) μ m ($n=60$), isodiametric around the ostiole; often with a pale layer 3–7 μ m thick on the surface, of hyaline, often collapsed cells and amorphous matter connected to bark cells. Hamathecium of periphyses and apical paraphyses, both to 1.5 μ m wide. Asci basally and laterally emergent, (47–)58–78(–91) \times (6.5–)7–11(–12.5) μ m ($n=29$), clavate to fusoid, with eight 2–3-seriate ascospores, uniseriate towards the base; apex inamyloid,

thickened to 1–2 µm, without a ring; stipe short or lacking. Ascospores (12–)17–24(–28)×(2.2–)3.0–3.7(–4.3) µm, l/w (3.4–)5.1–7.2(–10.2) ($n=175$), fusoid or oblong, hyaline, sometimes faintly rosy-brownish in mass; 2-celled with a median, non-constricted septum becoming indistinct in KOH, rarely with two septa, often three-septate when germinating; straight or curved, ends narrowly rounded, lower end often more acute, upper cell often slightly wider, smooth, usually with minute guttules in groups towards ends and septum. Size much depending on maturity, from 7×2 µm and 1-celled when immature.

Culture and anamorph on PDA after 54 days at 25°C: colony dense, white, thick, with numerous aerial hyphae forming tufts with tooth-like outgrowths. Orange pycnidia 0.3–0.5 mm diam densely aggregated in a fascicle of 2.3×1.4 mm once formed in the colony centre. Pycnidial wall of densely packed, parallel, 1–2 µm wide hyaline hyphae. Conidia (13–)18–28(–33)×(2.0–)2.5–3.0 µm, l/w (5.8–)7–10.7(–12.4) ($n=41$), hyaline, cylindrical, often curved, 2-celled, formed within pycnidia on mostly solitary cylindrical phialides ca. 9–24×2.3–3.3 µm.

Distribution: Europe (Austria, Czech Republic, Sweden)

Habitat: on dead twigs of *Rosa* spp.

Lectotype, here designated: **Czech Republic**, Moravia, Mährisch-Weißkirchen (now Hranice), Hrabuvka, on dead twigs of *Rosa canina*, Apr. 1912, F. Petrak (1978/W09773, Reliquiae Petrakianae); lectotype already indicated on the specimen label by R. Lowen in Jan. 1991. **Epitype**, here designated: **Austria**, Vienna, 21st district, Marchfeldkanalweg/east from Brünner Straße, on dead twigs of *Rosa canina* s.l., soc. *Sacothecium sepincola*; 15 Nov. 2008, W. Jaklitsch and O. Sükösd (WU 31334, culture LMM = CBS 124270).

Other material examined: **Czech Republic**, Moravia, Hranice, at forest edges at the northern railroad close to Bölden (now Bělotín), on *Rosa canina*, 5 Feb. 1913, F. Petrak (1978/W 14871). Hranice, Ludona brook, on dead twigs of *Rosa 'dumetorum'*, 8 Jan. 1919 and 17 Jan. 1920, F. Petrak (1978/W 14514). Reported by Lowen (1991): **Sweden**, Uppland, Bromma par., Stockholm, Drottningholm, on *Rosa canina*, 5 Dec. 1920, T. Vestergren (S; not seen).

Notes: Petrak first issued this fungus under the name *Stigmatea seriata* G. Winter in his exsiccata Flora Bohemiae et Moraviae exsiccata, Abt. 1. Pilze Nr. 172, but then recognised it as a distinct species and described it as *Stigmatea moravica* (Petrak 1914). Later Petrak (1920, p. 78) noticed the typically hypocrealean perithecial architecture of the fungus and combined it in *Nectriella*. Finally Petrak (1962) placed the fungus in *Leiosphaerella*. Lowen (1991 and pers. comm.) examined Petrak's collections and excluded the taxon from *Nectriella* without suggesting a final placement. As noted by her, *Rosasphae-*

ria moravica (as *Leiosphaerella moravica*) differs from species of *Nectriella* in the dark perithecial wall and the elongate ascospores. Ascospores of *R. moravica* may be typical of *Leiosphaerella*, but the asci are J- and lack apically free paraphyses. To ensure nomenclatural stability, a recent collection for which a culture and sequences are available, is here designated as epitype.

Rosasphaeria moravica is with certainty only known from Europe. Barr (1998) reported Macoun's collections of *L. moravica* from British Columbia, Canada, on wild rose stems, although with amyloid ascus apices. Thus this seems to be a different fungus. Also another collection from the same region on *Amelanchier*, that produced a holoblastic hyphomycete in culture (Lowen 1991), remains to be re-examined.

Phylogenetically and morphologically closely related to *R. moravica* is *Eucasphaeria capensis* Crous (Crous et al. 2007), a fungus with nearly identical ascospore and conidial size, which, according to the protologue, differs by amyloid ascus apices, an acervular, *Ascochytopsis*-like anamorph in vivo, and black pycnidia formed in oatmeal agar cultures. Furthermore, in culture ascomata developed setae in the apical region; setae were brown, 1–2-septate, smooth, up to 100 µm long, with pointed to bluntly rounded apices. BLASTn results of the ITS sequence of *E. capensis* did not reveal close relatives, except for distant similarity to species of *Paecilomyces* (Hypocreales). The LSU sequence revealed it to be allied to *Niesslia exilis*, species of *Chaunopycnis*, and *Fusarium lichenicola* (Crous et al. 2007). The morphological differences and clear phylogenetic separation (Fig. 3) suggest the erection of a new genus for *L. moravica*. No fungus matching the description was found in the holotype of *E. capensis* (CBSH 19764), therefore an amyloid ring in the ascus apex given in the protologue, a trait that is alien to the Hypocreales, could not be verified.

Discussion

We re-describe type species of five genera of the Xylariales and provide sequences of phylogenetic markers for them. Emphasis is laid on *Leiosphaerella* and *Pseudomassaria*, two genera of the Xylariales. Up to eleven families were assigned to this order (Barr 1990). Familial subdivision within the Xylariales has been artificial and mostly based on morphology; e.g. Barr (1994) separated families basically on ascospore characteristics. Thus incongruence between morphology-based classification and molecular phylogenetic clades is likely. Many genera including *Leiosphaerella* and *Pseudomassaria*, have been variously distributed among several families. Lumbsch and Huhndorf (2010) list *Pseudomassaria* in the Hyponectriaceae among

15 other genera and *Leiosphaerella* with a question mark in the Amphisphaeriaceae among 40 genera. Petrak (1923) originally established the Hyponectriaceae for the foliicolous genera *Anisostomula* (now a synonym of *Hyponectria*), *Hyponectria* and *Physosporella*. Winter (1887) based his family Amphisphaeriaceae (as Amphisphaerieae) on *Amphisphaeria* Ces. & de Not. and some genera with bitunicate asci. After a re-arrangement of the family by Müller and von Arx (1962) and numerous subsequent additions, Kang et al. (1998, 1999) narrowed the concept of the Amphisphaeriaceae to ten genera with *Pestalotia*/*Pestalotiopsis*-like anamorphs, based on ITS sequences and morphology. Using LSU sequences, Jeewon et al. (2003) concluded that a broad concept of the Amphisphaeriaceae should be accepted.

Phylogenetic reconstruction of the Xylariales is on one hand hampered by the lack of DNA sequences for most generic types. On the other hand, only LSU and to a lesser extent, ITS sequences can be retrieved from GenBank for a considerably limited number of species, for which often no culture is available. In contrast to some other groups of perithecial ascomycetes, e.g. the Diaporthales (Castlebury et al. 2002; Gryzenhout et al. 2006), LSU sequences in the Xylariales are not sufficiently informative to resolve the order into well-defined clades that may be soundly interpreted as families. Topologies of phylogenetic trees substantially depend on the selection of taxa as well as characters (data not shown). ITS provides only slightly better resolution. The trees based on LSU (Fig. 1) and ITS (Fig. 2) are therefore not congruent, and many nodes lack significant support, limiting conclusions about phylogenetic relationships. However, some clades in the order are well-defined in terms of forming stable groups in all analyses: these are a) the Diatrypaceae; b) the Xylariaceae. *Collodiscula* is confirmed by both analyses to be a member of the latter family, as was anticipated by several authors (e.g. Læssøe and Spooner 1994; Ju and Rogers 1999); c) the Apiosporaceae and d) *Monographella* with its *Microdochium* anamorphs. *Monographella lycopodina* is described as a new species of the genus. Barr (1990) placed *Monographella* in the Hyponectriaceae close to *Leiosphaerella*. Lumbsch and Huhndorf (2010) listed the genus in the Amphisphaeriaceae and IndexFungorum (<http://www.indexfungorum.org/Names/Names.asp>) with uncertain affinity in the Xylariales. In the present analyses *Monographella* / *Microdochium* forms a well-supported lineage, which does not correspond to the Hyponectriaceae, if the LSU sequence labelled *Hyponectria buxi* in GenBank is correct. No ITS sequence is available for *Hyponectria buxi*. In various LSU analyses the position of *H. buxi* was found to vary considerably. Thus a family Hyponectriaceae cannot be recognised at this time. Also the circumscription of the Amphisphaeriaceae is still unsettled. A clade at the top of the tree inferred from ITS (Fig. 2) encompassing *Amphisphaeria*

umbrina is supported by the Bayesian analysis and may currently be interpreted as Amphisphaeriaceae, but this is rather a matter of taste. ITS alone may not be suitable to resolve this issue, and multi-gene analyses including a substantial number of representative taxa are required.

In both LSU and ITS analyses *Leiosphaerella* s. str. and *Pseudomassaria* s. str. are closely related (Figs. 1, 2). However, familial placement of these genera cannot be inferred due to insufficient phylogenetic resolution and internal support. In the LSU analyses (Fig. 1) the *Leiosphaerella*-*Pseudomassaria* clade is sister group to the clade containing the genera with *Pestalotia*/*Pestalotiopsis*-like anamorphs, which are commonly classified in the Amphisphaeriaceae. However, this sister group relationship is not present in the ITS analyses where the *Leiosphaerella*-*Pseudomassaria* clade occupies an isolated position (Fig. 2). *Pseudomassaria sepincoliformis* (amyloid ascus rings) belongs to *Pseudomassaria* s. str. Thus the presence or absence of an amyloid ring cannot be recognised as a morphological criterion for differentiation on the generic level. Owing to the consistent sister group relationship of *Pseudomassaria lycopodina* and *Leiosphaerella praeclara*, *P. lycopodina* is transferred to *Leiosphaerella*. As a consequence, the position of the ascospore septum is not a reliable criterion for generic distinction. *Pseudomassaria fallax* is clearly not a species of *Pseudomassaria*, but we refrain from a taxonomic change due to uncertainty about generic affiliations. Interestingly, in both LSU and ITS phylogenies *P. fallax* is closely related to *Polyancora globosa*, a peculiar aquatic fungus isolated from peat swamps in tropical Malaysia (Voglmayr and Yule 2006). Also *P. carolinensis* clearly does not belong to *Pseudomassaria*, if we assume that the accession DQ810233 indeed represents this taxon. Likewise, *Leiosphaerella* sp. identified by the accession AF452037 and isolated from a marine leaf (Jeewon et al. 2003) is not a species of *Leiosphaerella*. *Leiosphaerella vexata* neither belongs to *Leiosphaerella* nor to *Pseudomassaria*. For this fungus we re-instate Petrak's (1955) genus *Pseudomassariella*. Based on a multi-gene analysis (Fig. 3), we establish the new genus *Rosasphaeria* for *Leiosphaerella moravica*, which is well-delimited from *Niesslia* and *Eucasphaeria* in the Niessliaceae, Hypocreales. *Melogramma campylosporum* currently forms a clade of its own, thus Barr (1990) was correct to place the Melogrammataceae Winter with the single genus *Melogramma* and its type species *M. campylosporum* in the Xylariales.

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