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Sareomycetes cl. nov.: A new proposal for placement of the resinicolous genus *Sarea* (*Ascomycota, Pezizomycotina*)

C. Beimforde[1]*, A.R. Schmidt[1], J. Rikkinen[2], J.K. Mitchell[3]

[1]Department of Geobiology, University of Göttingen, Goldschmidtstraße 3, 37077 Göttingen, Germany
[2]Finnish Museum of Natural History, P.O. Box 7, 00014 University of Helsinki, Finland, and Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, P.O. Box 65, 00014 University of Helsinki, Finland
[3]Department of Physics, Harvard University, 17 Oxford Street, Cambridge, MA 02138 and Farlow Reference Library and Herbarium of Cryptogamic Botany, 22 Divinity Avenue, Cambridge, MA 02138, USA

*Corresponding author: christina.beimforde@uni-goettingen.de

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Abstract: Resinicolous fungi constitute a heterogeneous assemblage of fungi that live on fresh and solidified plant resins. The genus *Sarea* includes, according to current knowledge, two species, *S. resinae* and *S. difformis*. In contrast to other resinicolous discomycetes, which are placed in genera also including non-resinicolous species, *Sarea* species only ever fruit on resin. The taxonomic classification of *Sarea* has proven to be difficult and currently the genus, provisionally and based only on morphological features, has been assigned to the *Trapeliales* (*Lecanoromycetes*). In contrast, molecular studies have noted a possible affinity to the *Leotiomycetes*. Here we review the taxonomic placement of *Sarea* using sequence data from seven phylogenetically informative DNA regions including ribosomal (ITS, nucSSU, mtSSU, nucLSU) and protein-coding (*rpb1, rpb2, mcm7*) regions. We combined available and new sequence data with sequences from major *Pezizomycotina* classes, especially *Lecanoromycetes* and *Leotiomycetes*, and assembled three different taxon samplings in order to place the genus *Sarea* within the *Pezizomycotina*. Based on our data, none of the applied phylogenetic approaches (Bayesian Inference, Maximum Likelihood and Maximum Parsimony) supported the placement of *Sarea* in the *Trapeliales* or any other order in the *Lecanoromycetes*. A placement of *Sarea* within the *Leotiomycetes* is similarly unsupported. Based on our data, *Sarea* forms an isolated and highly supported phylogenetic lineage within the "*Leotiomyceta*". From the results of our multilocus phylogenetic analyses we propose here a new class, order, and family, *Sareomycetes*, *Sareales* and *Sareaceae* in the *Ascomycota* to accommodate the genus *Sarea*. The genetic variability within the newly proposed class suggests that it is a larger group that requires further infrageneric classification.

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INTRODUCTION

Many conifers and angiosperms have developed resin-based defence mechanisms to deter herbivores and microbial pathogens (Farrell *et al.* 1991, Gershenzon & Dudareva 2007, Howe & Schaller 2008). The sticky resin seals injuries in the trees and acts as a biochemical barrier due to terpenoid and phenolic compounds (Bednarek & Osbourn 2009, Rautio *et al*. 2011, Sipponen & Laitinen 2011, Seyfullah *et al.* 2018). However, certain fungi have developed resistance against toxic resin compounds (Rautio *et al.* 2011, Adams *et al*. 2013), and are able to colonize fresh and solidified resin (Tuovila *et al.* 2013). Resinicolous fungi represent a polyphyletic assemblage of ascomycetes which grow exclusively on tree resins (Tuovila 2013, Rikkinen *et al.* 2016).

Resinicolous fungi occur scattered throughout many classes within the *Ascomycota*. Most resinicolous fungi described to date are ascomycetes within the order *Mycocaliciales* (*Eurotiomycetes*) (*e.g.* Rikkinen 2003, Rikkinen *et al.* 2014, Tuovila *et al.* 2011a, b, 2012, Tuovila 2013). *Sorocybe resinae* (*Chaetothyriales, Herpotrichiellaceae*) and its synasexual morph *Hormodendrum resinae* (Seifert *et al.* 2007), and *S. oblongispora* (Crous *et al.* 2019), represent asexual *Eurotiomycetes* that are also often found on resin. The association of these fungi with conifer resin has existed for at least 35 M years as evidenced by fossilized specimens in Palaeogene amber (Rikkinen & Poinar 2000, Tuovila *et al.* 2013, Beimforde *et al.* 2014, Rikkinen & Schmidt 2018). While other resinicolous fungi have not received as much recent attention, a significant number occurs in other classes. *Dothideomycetes* contains at least six resinicolous species: *Helicoma resinae*, *Mytilinidion resinae*, *M. resinicola*, *Strigopodia batistae*, *S. resinae*, and *Torula resinicola*. *Leotiomycetes* boasts a similar number, with at least six resinicolous species: *Bisporella resinicola*, *Claussenomyces kirschsteinianus*, *C. olivaceus*, *Hymenoscyphus resinae-piceae*, *Lachnellula resinaria*, and *Micropodia resinicola*. A similar number of fungi are also currently not satisfactorily placed. Fungi such as *Gyrocerus resinae* and *Moriola resinae* have not been collected in over a century, while more recently collected fungi such as *Bruceomyces castoris* and *Resinogalea humboldtensis* are classified based on morphological characters due to the lack of molecular data (Rikkinen *et al.* 2016). Among this group of

poorly placed fungi, two widely collected discomycetes in the genus *Sarea* are also found.

Sarea resinae and *S. difformis* are both found fruiting exclusively on conifer resins and often co-occur on the same substrate. These two fungi are the only presently known species in the genus *Sarea*, which was erected by Fries in 1825. In contrast to other resinicolous discomycetes, which are placed in genera also including wood rotting species or parasites, *Sarea* species only ever fruit on resin. Both species are common in northern latitudes where they are usually found on resins of *Picea* and *Pinus* species, but also on other genera of *Pinaceae* including *Abies, Larix* and *Pseudotsuga* (Hawksworth & Sherwood 1981), *Cedrus* (Malençon 1979) and *Tsuga* (Baranyay 1966). They have also been reported from exudates of *Cupressaceae s. l.* such as *Chamaecyparis* (Ayers 1941, Suto 1985), *Cupressus* (Hawksworth & Sherwood 1981, Garrido-Benavent 2015), *Cryptomeria* (Suto 1985) and *Juniperus* (Petrini & Carrol 1981) indicating a relatively broad host range.

Little has been conclusively shown about the ecology and evolutionary origin of the genus *Sarea* so far. Species of the genus have variously been treated as lichen symbionts (Mudd 1861, Koerber 1865, Nylander 1866, Ohlert 1870, Hasse 1898, 1908, Cappelletti 1924, Fink 1935, Watson 1948, Etayo 1996, Bartkowiak & Bennett 2015) or mild to serious parasites (Kujala 1950, Conners 1967, Smerlis 1973, Funk 1981, Kobayashi & Zhao 1989, Kuz'michev *et al.* 2001, Safronova & Palnikova 2010, Bazhina & Aminev 2012, Safronova & Sorokin 2013). Currently they are mostly treated as saprobes (Hawksworth & Sherwood 1981, Wirth 1995, Gadgil & Dick 1999, Suto 2000, Robertson 2002, Czyżewska *et al.* 2005, Kukwa *et al.* 2008, Lõhmus *et al.* 2012, Łubek & Jaroszewicz 2012, Szymczyk *et al.* 2014, Garrido-Benavent 2015, Motiejūnaitė 2015, Yatsyna 2015, Himelbrant 2016, Kuznetsova *et al.* 2016, McMullin & Lendemer 2016), but additionally have been regarded as endophytes (Petrini & Carroll 1981, Petrini & Fisher 1988, Kowalski & Kehr 1992, Giordano *et al.* 2009, Koukol *et al.* 2012, Sanz-Ros *et al.* 2015).

The taxonomy of *Sarea* and its systematic assignment within the *Pezizomycotina* is still poorly resolved. Previously, *Sarea* species were placed in genera belonging to *Lecanoromycetes*, *Leotiomycetes*, and *Pezizomycetes*, including *Biatora*, *Biatoriella*, *Lecidea*, *Tympanis*, *Biatoridium, Pezicula* and *Peziza* (Hawksworth & Sherwood 1981). Hawksworth & Sherwood (1981) solved nomenclatural issues and provided detailed morphological descriptions of both *Sarea* species and placed the genus within *Agyriaceae*. Successive molecular studies suggested a relationship of *Sarea* to clades presently placed in *Leotiomycetes* (Reeb *et al.* 2004, Wang *et al.* 2006, 2009, LoBuglio & Pfister 2010, Miadlikowska *et al.* 2014), as opposed to earlier morphological placement within *Lecanoromycetes*, but these authors could not satisfactorily place the genus into any class within *Pezizomycotina*. Based on morphological traits, Lumbsch & Huhndorf (2010) and Hodkinson & Lendemer (2011) provisionally placed *Sarea* within *Trapeliaceae* (*Lecanoromycetes*). However, the difficulty of excluding potential homoplasy of morphological traits is well known in fungal systematics (*e.g.* Berbee & Taylor 1992, Schmitt *et al.* 2005, Lumbsch *et al.* 2007) and many studies show that morphological synapomorphies do not consequently correspond to monophyletic groups (*e.g.* Lumbsch *et al.* 2007, Prieto *et al.* 2013).

In this study, we aim to revise the current placement of *Sarea* in *Trapeliales* (*Lecanoromycetes*) with molecular data. Additionally, we aim to test the earlier suggestions of a placement within *Leotiomycetes* and calculate a phylogenetic hypothesis of *Sarea* and representatives of most *Pezizomycotina* classes. Only ribosomal sequences (nucLSU, nucSSU and 5.8S rDNA) of *Sarea* were available for phylogenetic studies so far and these may have provided insufficient information for accurate classification into the *Pezizomycotina*. Here we use seven phylogenetically informative DNA regions represented by ribosomal (ITS, nucSSU, mtSSU, nucLSU) and protein-coding (*rpb1, rpb2, mcm7*) sequences, of which four are new to the research community. Most sequences were obtained from *in vitro* cultures of *Sarea resinae* and *S. difformis* isolated from resin flows of *Picea abies* (Norway spruce). We combined the new sequence data with present sequences from major classes in *Pezizomycotina* in three different taxon samplings and applied the most current approaches including Bayesian Inference, Maximum Likelihood and Maximum Parsimony for the phylogenetic calculations.

MATERIAL AND METHODS

Biological material

Specimens of *Sarea difformis* and *S. resinae* originate from resin soaked bark or fresh, semi-solidified resin flows of *Picea abies*, *Pseudotsuga menziesii* and *Abies* sp. from coniferous forests in Finland, Germany and New Zealand. Sampled trees produced resin in response to mechanical damage due to animal or human activity or in response to microbial infections causing resinous canker lesions. Analysed specimens were deposited in the New Zealand Fungarium (PDD), Landcare Research in Auckland and in Helsinki (H). The collection data are provided in Table 1. GenBank accession numbers are provided in the supplementary data Table S1.

Table 1. List of *Sareomycetes* examined in this study with information to their substrate, collection locality, voucher number and collection where the specimens are deposited.

Taxon	Voucher	Substrate	Locality	Collection
Sarea difformis s. l.	CB093	resin, *Picea abies*	Göttingen, Lower Saxony, Germany	University of Helsinki (H), Helsinki
Sarea difformis s. l.	JR6451	resin, *Picea abies*	Finland	University of Helsinki (H), Helsinki
Sarea resinae s. l.	CB094	resin, *Picea abies*	Göttingen, Lower Saxony, Germany	University of Helsinki (H), Helsinki
Sarea resinae s. l.	JR6450	resin, *Picea abies*	Finland	University of Helsinki (H), Helsinki
Sarea resinae s. l.	PDD117345	resin, *Pseudotsuga menziesii*	Dunedin, Otago, New Zealand	New Zealand Fungarium (PDD) Collection, Auckland
Sarea resinae s. l.	PDD117343	resin, *Abies* sp.	Manapouri, Southland, New Zealand	New Zealand Fungarium (PDD) Collection, Auckland

Light microscopy

Fungal specimens were studied and imaged under a Carl Zeiss StereoDiscovery V8 dissection microscope and a Carl Zeiss AxioScope A1 compound microscope equipped with Canon EOS 5D digital cameras. All images (Fig. 1) represent digitally stacked photomicrographs obtained from up to 50 focal layers merged with the software package HeliconFocus v. 6.33 Pro (Helicon Soft Limited, Kharkiv, Ukraine). For Fig. 1D, incident and transmitted light were used simultaneously. To study hyphal growth inside

Fig. 1. Light micrographs of *Sarea difformis* and *S. resinae*. **A.** Ascomata of *S. difformis* and **B.** *S. resinae*; **C.** Young ascoma of *S. resinae* arising on a fresh resin flow; **D.** Cross-section of *S. resinae* showing hyphal growth into the liquid resin; **E.** Ascus and paraphyses of *S. difformis*; **F.** Young ascus of *S. difformis*; **G.** Asci and paraphyses of *S. resinae*; **H.** Young ascus of *S. resinae*. Scale bars: 1 mm (A, B), 500 µm (C, D), 10 µm (E, G), 5 µm (F, H).

the resin bodies, samples were embedded in epoxy resin Epo-Tek 301-2 (Epoxy Technology, Inc; Massachusetts) and ground using gradually fine-grained emery paper. Ascomatal details of *Sarea resinae* and *S. difformis* (Fig. 1E–H) were studied under 40× to 100× magnification using 100× oil-immersion objective, sometimes with an additional 1.6-fold magnification (Fig. 1H).

Cultivation

Ascospore germination was performed on solid malt yeast extract agar (MYA; 20 g malt extract, 2 g yeast extract, 20 g agar on 1 000 mL distilled water, pH = 6.5–7), malt extract agar (MEA; 20 g malt extract, 1 g peptone, 20 g glucose, 20 g agar in 1 000 mL distilled water, pH = 5–5.5) and potato dextrose agar (PDA; pre-formulated media, Carl Roth, Germany, pH = 5.6 ± 0.2) treated with 50 mg / mL penicillin G and streptomycin to prevent bacterial growth. For spore isolation, ascomata of *Sarea difformis* and *S. resinae* were removed from the resinous substrate and transferred to double cavity glass slides containing a drop of sterile 0.9 % $NaCl_2$ solution. Contaminations were removed under a Carl Zeiss Stemi 2000-C stereomicroscope and the ascomata were transferred to the edge of the second cavity and gently crushed with a flamed needle to liberate the spores. The spores were further diluted in 200–300 µL sterile 0.9 % $NaCl_2$ solution, transferred on the fungal media and incubated at 25–30 °C for up to 24 mo in the dark.

DNA isolation, amplification and sequencing

For DNA extraction, ascomata of *Sarea difformis* and *S. resinae* from environmental samples were cleaned of macroscopical contaminations under a Carl Zeiss Stemi 2000-C stereomicroscope, shock frozen with liquid nitrogen and crushed using a glass micromortar and pestle. Cultures of both species isolated from *Picea abies* were freeze dried (Christ, Alpha 1–4 LDplus, Osterode, Germany) and subsequently pulverized in Eppendorf tubes using plastic pestles. DNA was isolated from the fungal material using the Invisorb Spin Plant Mini Kit (Stratec, Berlin, Germany) by following the manufacturer's protocol, but modified by incubating the samples over night at 52 °C to ensure the lysis of the fungal cell walls. For phylogenetic analysis, we amplified parts of four protein coding and four ribosomal DNA regions. The protein coding genes represent the RNA polymerase II largest (*rpb1*) and second largest subunit (*rpb2*), the *tsr1* gene, a gene required for rRNA accumulation during biogenesis of the ribosome (Gelperin *et al.* 2001, Schmitt *et al.* 2009) and the *mcm7* gene, a DNA replication licensing factor required for DNA replication initiation and cell proliferation (Moir *et al.* 1982, Kearsey & Labib 1998). Ribosomal DNA regions include the small and large nuclear ribosomal subunit (18S rDNA and 28S rDNA respectively), the mitochondrial small ribosomal subunit (mtSSU) as well as the nuclear internal transcribed spacer region (ITS). DNA regions were isolated and amplified from *in vitro* cultures of *Sarea difformis* and *S. resinae* in order to exclude the amplification of DNA from potential contaminates of environmental samples. The nuclear ITS regions of the cultures and environmental samples were compared to make sure that the cultures correspond to the correct environmental sample.

Polymerase chain reaction (PCR) was conducted using *Taq* DNA polymerase (Promega, Madison, WI) by following the manufacturer's recommendations. Fungal specific primers and PCR conditions used to amplify the gene regions for phylogenetic analysis of this study are provided in Table S2. PCR products were purified using MSB® Spin PCRapace (Invitek, Berlin, Germany) and sequenced in both directions with a MegaBACE 1000 automated sequencing machine and DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Little Chalfont, UK). Sequences were assembled and edited with BioEdit v. 5.0.9 (Hall 1999).

Reference data sets

We combined the new ribosomal and protein coding sequences with data from the National Center for Biotechnology Information (NCBI). In total, seven marker sequences were used for the phylogenetic analyses. Since few *tsr1* sequences are available in GenBank we excluded the new, high quality *tsr1* sequences from our phylogenetic analyses in order to avoid a high percentage of missing data in any of the included gene/DNA regions. Accession numbers for all sequences used for the molecular analyses are provided in Table S1.

Three different taxon samplings were assembled:

1. *Trapeliales*/*Helotiales*: To assess whether or not the morphological similarities of *Sarea* and *Trapeliales* can be substantiated with molecular data we assembled a data set including members of the *Trapeliales* (*Lecanoromycetes*) and *Helotiales* (*Leotiomycetes*). Additionally, we included representatives of the recently proposed classes *Xylonomycetes* and *Candelariomycetes* because in our preliminary analyses (data not shown) included representatives of these two classes often grouped with *Sarea* when additional *Pezizomycotina* classes were included in the phylogenetic analyses. The operculate ascomycetes *Peziza arvernensis* and *P. varia* were used as outgroup. The representative dataset consists of 66 taxa with a total 1 295 base pairs of which 449 represent variable sites from the ITS region and 846 sites from the nucLSU. In addition to the sequences of *Sarea difformis* and *S. resinae* that we generated in this study, we incorporated some available ITS and nucLSU sequences from GenBank.
2. *Lecanoromycetes*: To assess whether or not the current (morphological) classification of *Sarea* in *Lecanoromycetes* can be confirmed with molecular data we assembled a taxon sampling which broadly corresponds to the well-balanced dataset by Prieto *et al.* (2013). The dataset comprises 96 taxa and includes 3 862 variable sites from four ribosomal (ITS, nucSSU, mtSSU, nucLSU) and two protein coding (*mcm7*, *rpb1*) sequences.
3. *Pezizomycotina*: To place *Sarea* within *Pezizomycotina* we assembled a taxon sampling including representatives of all major ascomycete classes except *Laboulbeniomycetes*, *Xylobotryomycetes* and *Coniocybomycetes* because preliminary analyses (data not shown) have shown that these classes are unlikely to be closely related to *Sarea*. Many of the implemented genes were compiled in a previous study by James *et al.* (2006). The dataset consists of 103 taxa including 160 base pairs of the ITS region, 916 sites from the small ribosomal subunit (nucSSU), 1 057 sites from the large ribosomal subunit (nucLSU), and 900 sites from the *rpb2* gene. All reference data sets are available via Treebase http://purl.org/phylo/treebase/phylows/study/TB2:S25817.

Phylogenetic analyses

Phylogenetic hypotheses were calculated with the three most current approaches: Bayesian Inference, Maximum Likelihood and Maximum Parsimony. All analyses were performed on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010). For each dataset, included genes were aligned separately by using MAFFT v. 6 (Katoh & Toh 2008) sometimes with subsequent manual adjustment to minimize the number of possible false homologies using BioEdit v. 5.0.9. (Hall 1999) and SeaView v. 4 (Gouy *et al.* 2010). Unalignable regions and introns were excluded by using the mask function in BioEdit v. 5.0.9. For each dataset, genes were combined in a super matrix using BioEdit v. 5.0.9.

Maximum Likelihood search for the most likely tree was accomplished using RAxML VI-HPC (Stamatakis 2006, Stamatakis *et al.* 2008) by applying a GTR model of molecular evolution, 1 000 ML bootstrap replicates and the Gamma model of rate heterogeneity by letting RAxML optimize individual α-shape parameters and base frequencies for 6 separate gene partitions.

Maximum parsimony (MP) was performed using PAUP v. 4.0b10 (Swofford 1991, 2002) by treating gaps as missing characters, and by applying 1 000 random addition sequences (RAS), TBR (tree bisection reconnection) branch-swapping and MULTREES option. To assess statistical support of the clades, non-parametric bootstrapping (Felsenstein 1985) was performed with heuristic searches.

Bayesian analyses were performed using Markov Chain Monte Carlo (MCMC) in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Best fitting substitution models for each gene were chosen separately from seven substitution schemes included in the software package jModeltest v. 2.1.1 (Darriba *et al.* 2012), and models were chosen according to the Bayesian information criterion (BIC, Schwarz 1978).

Analyses were run using four chains for 5–10 M generations each, sampling parameters every 500 to 1 000 generations. Average standard deviations of split frequency (ASDSF) lower than 0.01 were interpreted as indicative of independent MCMC convergence.

RESULTS

Phylogenetic analyses

The phylogenetic tree obtained from the *Trapeliales*/*Helotiales* data (Fig. 2) displays well-supported clades of *Sarea*, *Trapeliales*, *Helotiales*, *Candelariomycetes* and *Xylonomycetes* from the Bayesian, Maximum Likelihood and Maximum Parsimony analyses. *Xylobotryomycetes* were placed as a sister clade to the remaining classes included in this taxon set (data not shown), which means that a relationship with *Sarea* is not likely. We therefore excluded *Xylobotryomycetes* in our further analysis. Both Bayesian and Maximum Likelihood approaches place *Sarea* as second order sister group to *Lecanoromycetes* with low node support (35 ML-BS and 61 PP). In each of the three applied methods *Sarea* species clustered in a well-supported clade (84 ML-BS, 99 PP, 77 MP-BS) and *S. difformis* (89 ML-BS, 100 PP, 89 MP-BS) and *S. resinae* (100 ML-BS, 100 PP, 100 MP-BS) build well-supported groups in this clade.

The phylogenetic hypothesis resulting from the six-gene *Lecanoromycetes* dataset is shown in Fig. 3. The topology of the resulting phylogeny is generally congruent with the analysis of Prieto *et al.* (2013) and members of currently defined *Pezizomycotina* classes group in well-supported clades. With three methods (Bayesian, MP and MB) *Sarea* was placed outside the *Lecanoromycetes*, but was placed inside the ''*Leotiomyceta*'' with unanimous support (99 ML-BS, 100 PP, 91 MP-BS). Maximum Parsimony analysis did not resolve relationships between the classes of *Pezizomycotina* and relationships between members of *Lecanoromycetes* were only partly resolved. Bayesian analysis grouped *Sarea* as sister group o to the clade including *Dothideomycetes-Arthoniomycetes* and *Leotiomycetes-Sordariomycetes* with low support (56 PP), but Maximum Likelihood analysis grouped *Sarea* as sister group of the *Coniocybomycetes-Lichinomycetes* clade with only very low node support (15 ML-BS).

The phylogenetic hypothesis obtained from our four-gene dataset of *Pezizomycotina* is shown in Fig. 4. With some exceptions, the topology of the phylogenetic tree broadly corresponds to other large-scale phylogenies of *Ascomycota* (*e.g*. Reeb *et al.* 2004, James *et al.* 2006, Schoch *et al.* 2009a, b, Beimforde *et al.* 2014). In our analysis *Xylonomycetes* forms two separate groups with *Symbiotaphrina* placed in the clade also including *Candelariomycetes* and the here-proposed new class *Sareomycetes*. However, these results are not congruent with the phylogenomic study of Gazis *et al.* (2016) which indicate that *Symbiotaphrinales* represent the sister clade to *Xylonomycetales*. Otherwise, members of currently defined *Pezizomycotina* classes group in well-supported clades and show relationships between the major classes of ascomycetes that have been described in other studies, such as *Arthoniomycetes-Dothideomycetes*, *Leotiomycetes-Sordariomycetes* and *Lecanoromycetes-Eurotiomycetes*. Maximum Parsimony did not resolve the relationships between the *Pezizomycotina* classes, but both Bayesian Inference and Maximum Likelihood placed *Sarea* in a clade also including *Geoglossomycetes*, *Candelariomycetes* and *Xylonomycetes*. This group, however, is only indicated by low node support (26 ML-BS, 89 PP).

Taxonomy

Justified by the distinct phylogenetic position of *Sarea* from other ascomycetes in our multilocus gene calculations and by the unique combination of ecological and morphological characteristics of the fungal group, we here propose a novel class, order, and family in the *Ascomycota* to accommodate the genus *Sarea*: *Sareomycetes*, *Sareales* and *Sareaceae cl., ord. et fam. nov.*

Sareomycetes Beimforde, A.R. Schmidt, Rikkinen & J.K. Mitch., ***cl. nov.*** MycoBank MB831369.

Type order: *Sareales* Beimforde, A.R. Schmidt, Rikkinen & J.K. Mitch., ***ord. nov.*** MycoBank MB831372.

Type family: *Sareaceae* Beimforde, A.R. Schmidt, Rikkinen & J.K. Mitch., ***fam. nov.*** MycoBank MB831373.

Type genus: *Sarea* Fr., *Systema Orbis Vegetabilis* **1**: 86. 1825.

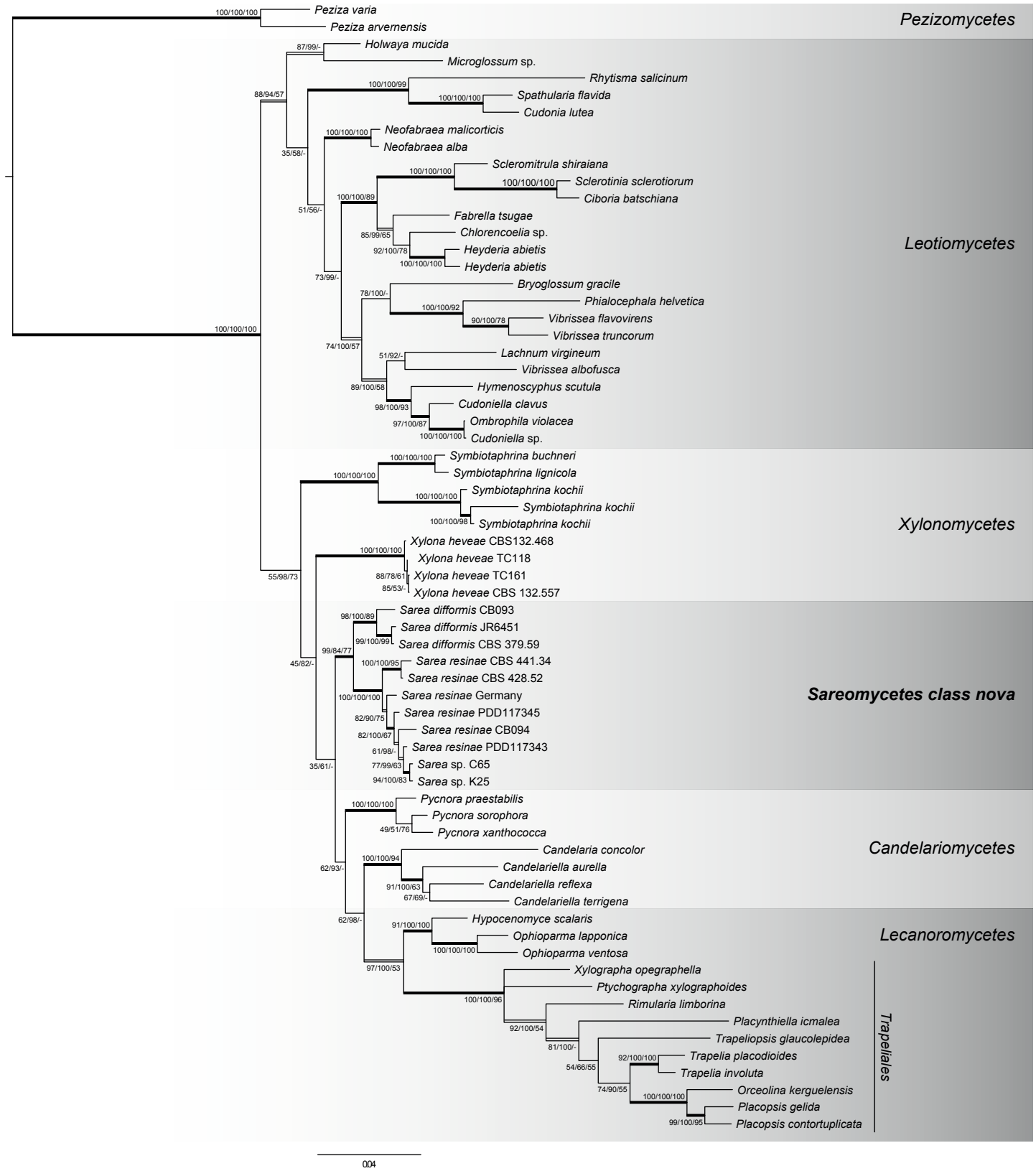
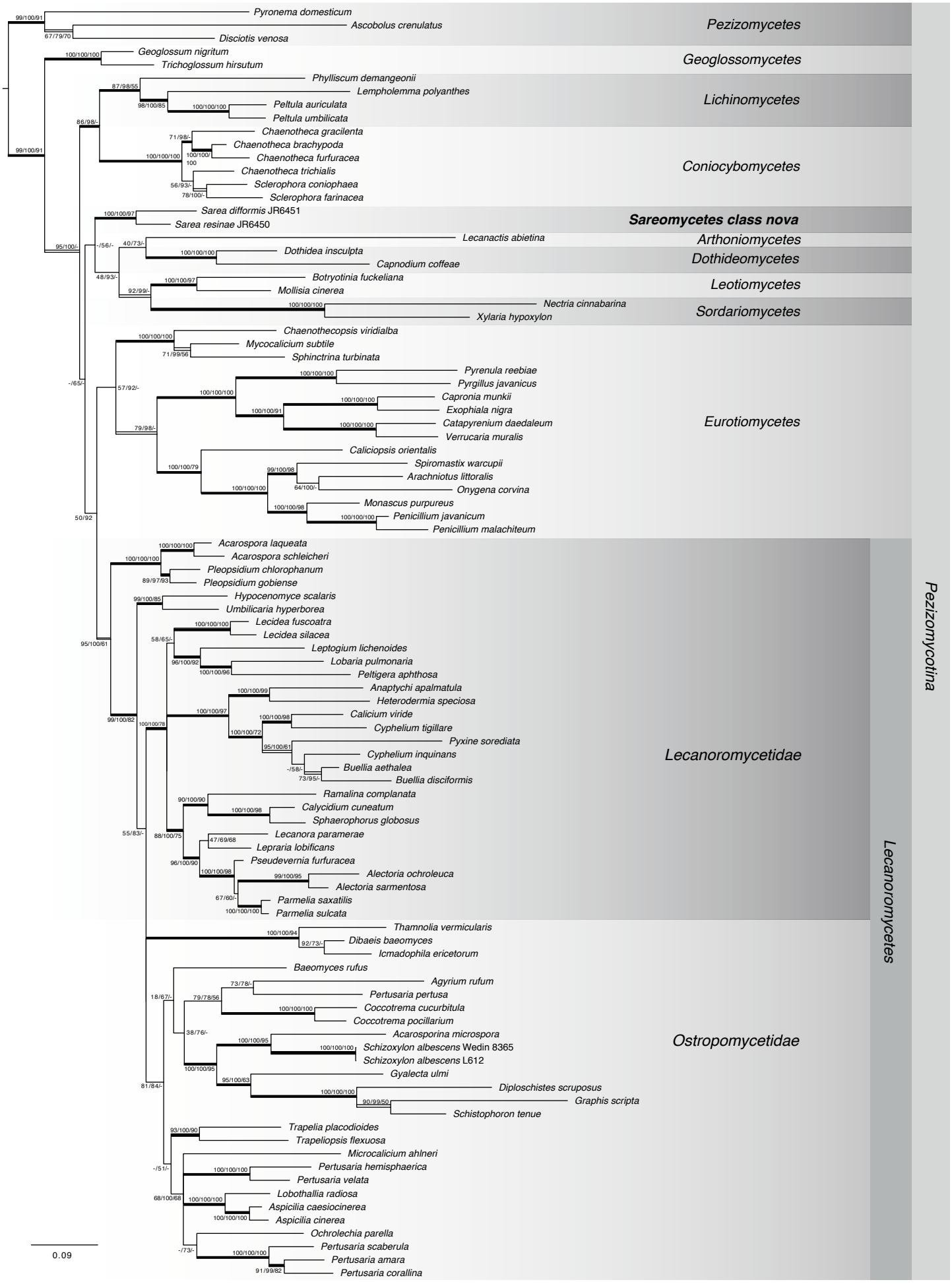


Fig. 2. Phylogenetic relationships of *Sarea*, *Trapeliales* and *Helotiales* based on two ribosomal genes (ITS, nucLSU) obtained from Bayesian, Maximum Likelihood and Maximum Parsimony (MP) analysis. Posterior Probabilities (PP), ML- and MP-Bootstraps are represented by the first, second and third numbers associated with internodes. Branches in bold indicate PP ≥ 95 %, and both ML and MP bootstrap values ≥ 70 %. Double lined branches indicate significant support obtained by two out of the three analyses. Scale = number of substitutions per site.

Fig. 3. Phylogenetic relationship of *Sarea* and *Lecanoromycetes* based on six genes (ITS, mtSSU, nucSSU, nucLSU, *mcm7, rpb1*) obtained from Bayesian, Maximum Likelihood and Maximum Parsimony (MP) analysis. Taxon sampling broadly corresponds to the data set by Prieto *et al.* (2013). Posterior Probabilities (PP), ML- and MP-Bootstraps are represented by the first, second and third numbers associated with internodes. Branches in bold indicate PP ≥ 95 %, and both ML and MP bootstrap values ≥ 70 %. Double lined branches indicate significant support obtained by two out of the three analyses. Scale = number of substitutions per site.


Pyronema domesticum
Ascobolus crenulatus
Disciotis venosa
Pezizomycetes
Geoglossum nigritum
Trichoglossum hirsutum
Geoglossomycetes
Phylliscum demangeonii
Lempholemma polyanthes
Peltula auriculata
Peltula umbilicata
Lichinomycetes
Chaenotheca gracilenta
Chaenotheca brachypoda
Chaenotheca furfuracea
Chaenotheca trichialis
Sclerophora coniophaea
Sclerophora farinacea
Coniocybomycetes
Sarea difformis JR6451
Sarea resinae JR6450
Sareomycetes class nova
Lecanactis abietina
Arthoniomycetes
Dothidea insculpta
Capnodium coffeae
Dothideomycetes
Botryotinia fuckeliana
Mollisia cinerea
Leotiomycetes
Nectria cinnabarina
Xylaria hypoxylon
Sordariomycetes
Chaenothecopsis viridialba
Mycocalicium subtile
Sphinctrina turbinata
Pyrenula reebiae
Pyrgillus javanicus
Capronia munkii
Exophiala nigra
Catapyrenium daedaleum
Verrucaria muralis
Caliciopsis orientalis
Spiromastix warcupii
Arachniotus littoralis
Onygena corvina
Monascus purpureus
Penicillium javanicum
Penicillium malachiteum
Eurotiomycetes
Acarospora laqueata
Acarospora schleicheri
Pleopsidium chlorophanum
Pleopsidium gobiense
Hypocenomyce scalaris
Umbilicaria hyperborea
Lecidea fuscoatra
Lecidea silacea
Leptogium lichenoides
Lobaria pulmonaria
Peltigera aphthosa
Anaptychi apalmatula
Heterodermia speciosa
Calicium viride
Cyphelium tigillare
Pyxine sorediata
Cyphelium inquinans
Buellia aethalea
Buellia disciformis
Ramalina complanata
Calycidium cuneatum
Sphaerophorus globosus
Lecanora paramerae
Lepraria lobificans
Pseudevernia furfuracea
Alectoria ochroleuca
Alectoria sarmentosa
Parmelia saxatilis
Parmelia sulcata
Lecanoromycetidae
Thamnolia vermicularis
Dibaeis baeomyces
Icmadophila ericetorum
Baeomyces rufus
Agyrium rufum
Pertusaria pertusa
Coccotrema cucurbitula
Coccotrema pocillarium
Acarosporina microspora
Schizoxylon albescens Wedin 8365
Schizoxylon albescens L612
Gyalecta ulmi
Diploschistes scruposus
Graphis scripta
Schistophoron tenue
Ostropomycetidae
Trapelia placodioides
Trapeliopsis flexuosa
Microcalicium ahlneri
Pertusaria hemisphaerica
Pertusaria velata
Lobothallia radiosa
Aspicilia caesiocinerea
Aspicilia cinerea
Ochrolechia parella
Pertusaria scaberula
Pertusaria amara
Pertusaria corallina
Lecanoromycetes
Pezizomycotina
0.09

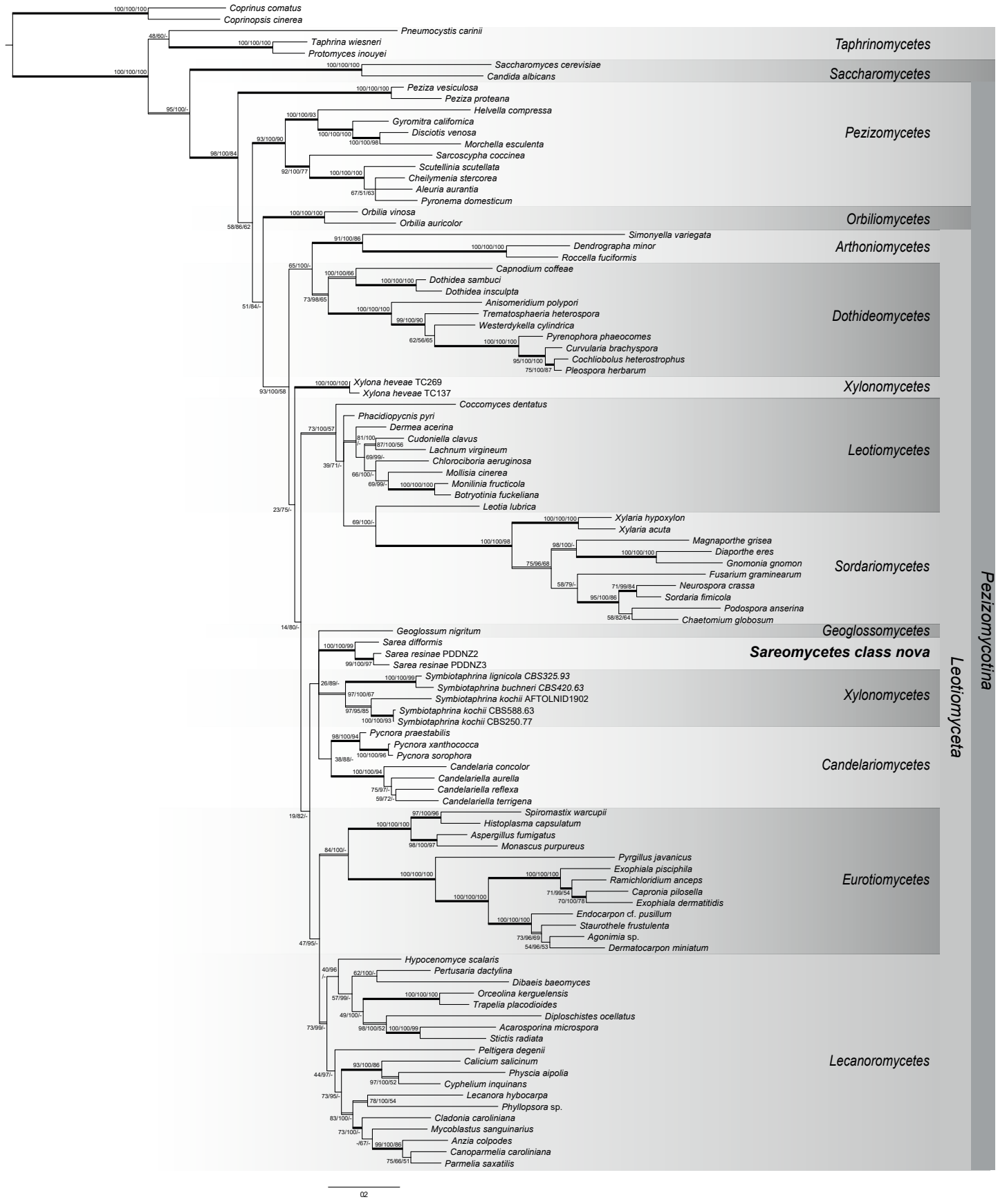


Fig. 4. Phylogenetic relationship of Pezizomycotina based on four genes (ITS, nucSSU, nucLSU, *rpb2*) obtained from Bayesian, Maximum Likelihood and Maximum Parsimony (MP) analysis. Posterior Probabilities (PP), ML- and MP-Bootstraps are represented by the first, second and third numbers associated with internodes. Branches in bold indicate PP ≥ 95 %, and both ML and MP bootstrap values ≥ 70 %. Double lined branches indicate significant support obtained by two out of the three analyses. Scale = number of substitutions per site.

Type species: *Sarea difformis* (Fr.) Fr., *Elenchus Fungorum* **2**: 14. 1828. (lectotype)

Sanctioned name: *Peziza difformis* Fr., *Systema Mycologicum* **2**: 151. 1822.

Type specimen: Rehm's Ascomyceten No. 577, Royal Botanical Garden, Kew, England UK. (neotype)
Etymology: The name of the class, order, and family are derived from the generic name of the type genus, *Sarea* Fr., *Systema Orbis Vegetabilis* **1**: 86 (1825).

The class, order, and family are based on the same description below:
Multispored, non-lichenized ascomycetes with resinicolous ecology, *ascomata* apothecial, scattered, formed exclusively on conifer resin, ascohymenial, sessile to short stipitate, pale to deep orange or black, the pigment localized at least in granules in the epithecial layer and marginal extracellular material as well as in oily inclusions in the interior tissues or in patches in the extracellular matrix, fleshy and gelatinous when fresh, becoming coriaceous when dry; excipulum paraplectenchymatous, composed of radiating hyphae immersed in a gel; subhymenium gelatinous, of interwoven hyphae forming a *textura intricata*, hyaline to brownish or coloured by intracellular pigments. *Hymenial elements* sometimes lightly bluing in KOH. Paraphyses numerous, often containing numerous oily inclusions, pigmented or not, filiform; septate, mainly unbranched but sometimes anastomosing and often becoming forked near the apices; apices slightly swollen and embedded in gel to form an epithecium-like layer. *Asci* with croziers, multispored, clavate with thick multilayered walls, not fully functionally bitunicate, the outermost layer amorphous and gelatinous, turning blue in IKI and Melzer's reagent with or without pretreatment in KOH, but staining more intensely after pretreatment, the innermost layer forming a thick apical cap pierced by a central pore, lacking a reaction in IKI and Meltzer's with or without KOH pretreatment. *Ascospores* numerous, spherical, minute, hyaline, smooth-walled, thin- to thick-walled, aseptate. *Asexual morphs* pycnidial, arising singly or in small groups, on conifer resin, superficial or immersed, subglobose, more or less concolourous with their sexual morph, walls composed of interwoven plectenchymatous hyphae forming a *textura intricata*, hyphae gelatinized or not, walls sometimes convoluted and appearing multilocular in section; ostiolate and papillate when young and expanding with age due to extrusion of conidia or opening by breakdown or tearing of the upper wall to form an irregular hole. *Conidiophores* lining the cavity of the pycnidium, hyaline, short, branched or not and septate at the base, bearing one to three conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, not proliferating or sometimes with one to four short proliferations, lageniform to cylindrical, tapering towards the apex, hyaline, smooth-walled, with a minute collarette and channel but marked periclinal thickening. *Conidia* abundantly produced, slimy or forming slimy masses, subglobose when mature but somewhat pyriform when young, sometimes slightly angular due to mutual compression, aseptate, hyaline to pale brown, more or less smooth-walled, thin- or thick-walled, more or less isodiametric with the ascospores of the sexual morph, usually containing a single minute guttule not disappearing in KOH.

Notes: The diagnosis above was modified from the generic description of *Sarea* and the specific descriptions for *Sarea resinae* and *S. difformis* published in Hawksworth & Sherwood (1981). Hawksworth and Sherwood (1981) also discussed the nomenclatural situation of *Sarea* in extraordinary detail. As no type species was designated for *Sarea* by Fries (1822, 1825, 1828), Kuntze (1898) lectotypified *Sarea* by *Peziza difformis*. Neither Kuntze (1898) nor Fries (1822, 1828) mentioned any locality of the described specimens and no original material is known to exist, and therefore Hawksworth & Sherwood (1981) selected a neotype for the name *Peziza difformis*, which is stored in the Royal Botanical Garden, Kew, England UK. Hawksworth & Sherwood (1981) also designated a lectotype for *Sarea resinae* (*Peziza resinae*), which is stored in the Acharius Herbarium in the University of Helsinki Herbarium in Helsinki.
Specimens examined: *Sarea difformis* CB093 (H), *Sarea difformis* JR6451 (H), *Sarea resinae* CB094 (H), *Sarea resinae* JR6450 (H), *Sarea resinae* PDD117343, *Sarea resinae* PDD117345. Information of the substrate, collection locality, voucher number and collection where the specimens are deposited is listed in Table 1.

DISCUSSION

Phylogeny

According to our phylogenetic results (Figs 2–4) *Sarea* does not belong in *Trapeliales* (*Lecanoromycetes*) — as the current taxonomic classification suggests (Lumbsch & Huhndorf 2010, Hodkinson & Lendemer 2011) — and cannot be classified within *Lecanoromycetes*. All of our analyses placed *Sarea* in the clade of inoperculate euascomycetes which corresponds to the rankless ''*Leotiomyceta*'' (Eriksson & Winka 1997) with unanimous support, but none satisfactorily assigned it to any of the existing classes in "*Leotiomyceta*".

Based on morphological similarities, previous studies placed the two *Sarea* species in various genera of *Lecanoromycetes*, for instance *Biatorella* within *Acarosporaceae*, *Biatora* in *Ramalinaceae*, or *Lecidea* within *Lecideaceae*. Nannfeldt (1932) placed both as species of *Tromera* within *Lecanorales* due to their thick ascus walls and the presence of an epithecium and amyloid reaction in the hymenium. Hawksworth & Sherwood (1981) also assigned *Sarea* to *Lecanoromycetes* because it resembles *Agyrium rufum* (*Agyriaceae*) in ascus structure, pigmentation and excipular structure.

Like *Sarea*, most genera in which *Sarea* was previously classified also include species with polyspored asci. True polyspory (= meiosis followed by several mitoses generating more than 100 spores, Gueidan *et al.* 2015) occurs in many other species in *Lecanoromycetes*. In the past, *Acarosporaceae* was characterized by its true polyspory (Gueidan *et al.* 2015), but molecular studies revealed that lichenized polysporous species do not form a monophyletic group and that polysporous asci evolved several times within lichenized species (Reeb *et al.* 2004, Aptroot & Schumm 2012). However, true polyspory has also evolved in non-lichenized genera such as *Deltopyxis* (Baral & Marson 2012), *Podospora* (Mirza & Cain 1969), *Thelebolus* (de Hoog *et al.* 2005) and *Tromeropsis*. The last was shown to be congeneric to *Symbiotaphrina* in *Xylonomycetes* (Baral *et al.* 2018). It is not known if the polyspory is linked to ecological environmental conditions, but it is noticeable that many

polyspored species occur in xeric habitats (Sherwood 1981).

The polyspored asci, apothecial ascomata and the non-lichenized resinicolous ecology are fundamental characters of all *Sarea* species. *Claussenomyces olivaceus* also possesses polyspored asci while occurring on resin. However, in contrast to *Sarea*, its ascospores (ascoconidia) arise from septate primary ascospores (Medardi 2007).

Another feature that Hawksworth & Sherwood (1981) did not mention is the distribution of pigments in *Sarea resinae*. The pigment may be located in the excipulum, subhymenium, hymenium, and apothecial surface, and can vary in intensity to the point of being absent in some structures between clades of *S. resinae*. Additionally, the excipular cells may vary in tightness between *Sarea* clades and differences in stipe length, presence and amount of granular material at the margins of the cups appear, depth of hymenium or thickness of epithecium seem to be other variable features between *Sarea* clades. However, these features are variable also based on environmental conditions and developmental stages.

Previous classifications of *Ascomycota* emphasized the morphology and development of the ascoma, and especially similar ascus structures and the mechanisms of spore release. Since then, molecular methods have revolutionized phylogenetic systematics of fungi (*e.g.* Lutzoni *et al.* 2004, Hibbett *et al.* 2007, Schoch *et al.* 2009a, Miadlikowska *et al.* 2006, Prieto *et al.* 2013). Lumbsch *et al.* (2007) pointed out that the ascus types in *Trapeliaceae* and *Agyriaceae* are phylogenetically misleading, since the ascus type of *Agyrium* agrees with those of *Trapeliaceae*, but the morphological similarities are inconsistent with molecular analyses. They excluded *Sarea* from their phylogenetic study since molecular data rather suggested a placement outside *Ostropomycetidae*.

In molecular approaches, potential sources of error include undetected (*e.g.* homoplasy, Goloboff *et al.* 2008) or wrongly inferred substitutions (*e.g.* long branch attraction, Bergsten 2005), polymorphism and gene specific evolution. Because most species have not been sequenced and/or even discovered to date (Blackwell 2011), taxon sampling biases also have to be considered (*e.g.* Cusimano *et al.* 2012). Often new gene sequences, such as the *tsr1* genes of *Sarea* generated in this study, are difficult to include in phylogenetic analyses, because they are underrepresented in GenBank. However, in the future more use could be made from genome extractions provided that the quality of the genes can be guaranteed. In any case, morphological and physiological traits provide additional diagnostic and biological information and should not be disregarded in current classifications (*e.g.* Hibbett *et al.* 2007).

We provide the first phylogenetic study of *Sarea* that includes molecular data from protein coding and ribosomal gene regions. Our results are consistent with previous molecular studies in that *Sarea* was placed within the clade of inoperculate euascomycetes, but could not be assigned to any of the currently defined classes in *Ascomycota*. Giraldo *et al.* (2014) reported affiliations of *Sarea* with *Lecanoromycetes*, but this was only based on data from a single gene (nucLSU) and the placement had no statistical support. Only a few other studies (Lutzoni *et al.* 2004, Reeb *et al.* 2004, Wang *et al.* 2006, 2009, Miadlikowska *et al.* 2014) supported the placement of *Sarea* outside *Lecanoromycetes* and an affiliation of *Sarea* with the *Leotiomycetes* was found by Reeb *et al.* (2004) and Wang *et al.* (2006). Here we cannot confirm an affiliation of *Sarea* with the *Leotiomycetes* (Figs 2–4), nor can we suggest a well-supported affiliation to any other class within "*Leotiomyceta*". However, in previous phylogenetic studies (Reeb *et al.* 2004, Wang *et al.* 2006) as well as our own, relationships between *Sarea* and other *Pezizomycotina* classes were indicated by only low node support and we therefore cannot assume a closer relationship of these taxon groups. It is rather the case that taxon groups of uncertain affiliations (including *Sarea*) in the assembled taxon sets cluster together (long branch attraction, Bergsten 2005, 1978) and it is likely that the placement of *Sarea* as sister taxon to *Leotiomycetes* in previous studies is just coincidence.

Our phylogenetic results (Figs 2–4) show that *Sarea* does not belong to *Lecanoromycetes* as currently assigned. Based on the information from the seven DNA regions, *Sarea* cannot be assigned to any of the classes of *Pezizomycotina*, but forms an isolated and highly supported lineage within "*Leotiomyceta*". We therefore propose to recognize this group formally as the new class, order, and family *Sareomycetes*, *Sareales* and *Sareaceae*.

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REFERENCES

Adams AS, Aylward FO, Adams SM, *et al.* (2013). Mountain pine beetles colonizing historical and naïve host trees are associated with a bacterial community highly enriched in genes contributing to terpene metabolism. *Applied and Environmental Microbiology* **79**: 3468–3475.

Aptroot A, Schumm F (2012). The genus *Melanophloea*, an example of convergent evolution towards polyspory. *The Lichenologist* **44**: 501–509.

Ayers TT (1941). *Biatorella resinae*: The Perfect Stage of *Zythia resinae*. *Mycologia* **33**: 130–135.

Baral HO, Marson G (2012). *Deltopyxis triangulispora gen. et sp. nov.*, a polysporous *Tromeropsis*-like discomycete of unclear relationship. *Andrias* **19**: 175–183.

Baral HO, Weber E, Marson G, *et al.* (2018). A new connection between wood saprobism and beetle endosymbiosis: the rarely reported saprobic discomycete *Tromeropsis* is congeneric with the symbiotic yeast *Symbiotaphrina* (*Symbiotaphrinales*, *Xylonomycetes*) and two asexual morphs misplaced in *Hyphozyma*. *Mycological Progress* **17**: 215–254.

Baranyay JA (1966). Fungi from dwarf mistletoe infections in western hemlock. *Canadian Journal of Botany* **44**: 597–604.

Bartkowiak ME, Bennett JP (2015). Floristic study of lichens in Portage County, Wisconsin. *Evansia* **32**: 176–188.

Bazhina EV, Aminev PI (2012). Effect of Biatorella canker on pollen viability and variation of shoot characters in Scots pine. *Russian Journal of Ecology* **43**: 101–106.

Bednarek P, Osbourn A (2009). Plant-microbe interactions: chemical

diversity in plant defense. *Science* **324**: 746–747.
Beimforde C, Feldberg K, Nylinder S, *et al.* (2014). Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. *Molecular Phylogenetics and Evolution* **77**: 307–319.
Berbee ML, Taylor JW (1992). Detecting morphological convergence in true fungi using 18S rRNA gene sequence. *BioSystems* **28**: 117–125.
Bergsten J (2005). A review of long-branch attraction. *Cladistics* **21**: 163–193.
Blackwell M (2011). The Fungi: 1, 2, 3 … 5.1 Million Species? *American Journal of Botany* **98**: 426–438.
Cappelletti C (1924). Studi su la vegetazione resinicola. *Annali di Botanica* **16**: 253–297.
Conners IL (1967). *An annotated index of plant diseases in Canada, and fungi recorded on plants in Alaska, Canada and Greenland*. Queen's Printers, Ottawa.
Crous PW, Wingfield MJ, Lombard L, *et al*. (2019). Fungal Planet description sheets: 951–1041. *Persoonia* **43**: 223–425.
Cusimano N, Stadler T, Renner SS (2012). A new method for handling missing species in diversification analysis applicable to randomly or non-randomly sampled phylogenies. *Systematic Biology* **61**: 785–792.
Czyżewska K, Motiejūnaitė J, Cieśliński S (2005). New and noteworthy species of lichens and allied fungi from north-eastern Poland. *Acta Mycologica* **40**: 277–291.
Darriba D, Taboada GL, Doallo R, *et al.* (2012). JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
de Hoog GS, Göttlich E, Platas G, *et al.* (2005). Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Studies in Mycology* **51**: 33–76.
Eriksson OE, Winka K (1997). Supraordinal taxa of the Ascomycota. *Myconet* **1**: 1–16.
Etayo J (1996). Contribution to the lichen flora of the Canary Islands. II. Epiphytic lichens from La Palma. *Österreichische Zeitschrift für Pilzkunde* **5**: 149–159.
Farrell BD, Dussourd DE, Mitter C (1991). Escalation of plant defense: Do latex/resin canals spur plant diversification? *American Naturalist* **138**: 881–900.
Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
Fink B (1935). *The lichen flora of the United States*. University of Michigan Press, Ann Arbor.
Fries EM (1822). *Systema Mycologicum, Sistens Fungorum Ordines, Genera et Species, Huc Usque Cognitas, Quas Ad Normam Methodi Naturalis Determinavit, Disposuit Atque Descripsit*. Voluminis II Sectio I. Ex Officina Berlingiana. Lundæ.
Fries EM (1825). *Systema Orbis Vegetabilis: Primas Lineas Novæ Constructionis Periclitatur*. Pars I. Plantæ Homonemeæ. E Typographia Academica. Lundæ.
Fries EM (1828). *Elenchus fungorum, sistens commentarium in Systema mycologicum*. Vol.1. E. Mauritius, Greifswald.
Funk A (1981). *Parasitic microfungi of western trees.* Information Report BC-X-222. Environment Canada, Canadian Forestry Service. Pacific Forest Research Centre, Victoria, BC.
Gadgil PD, Dick M (1999). Fungi Silvicolae Novaezelandiae: 1. *New Zealand Journal of Forestry Science* **29**: 428–439.
Gazis R, Kuo A, Riley R, *et al.* (2016). The genome of *Xylona heveae* provides a window into fungal endophytism. *Fungal Biology* **120**: 26–42.
Garrido-Benavent I (2015). Contribución al conocimiento del género *Sarea* Fr. (*Ascomycota*, *Incertae sedis*) en la Península Ibérica. *Sociedad Micológica Errotari* **12**: 42–51.
Gelperin D, Horton L, Beckman J, *et al.* (2001). Bms1p, a novel GTP-binding protein, and the related Tsr1p are required for distinct steps of 40S ribosome biogenesis in yeast. *Rna-a Publication of the Rna Society* **7**: 1268–1283.
Gershenzon J, Dudareva N (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology* **3**: 408–414.
Giordano L, Gonthier P, Varese G, *et al.* (2009). Mycobiota inhabiting sapwood of healthy and declining Scots pine (*Pinus sylvestris* L.) trees in the Alps. *Fungal Diversity* **38**: 69–83.
Giraldo A, Gené J, Sutton DA, *et al.* (2014). Phylogenetic circumscription of *Arthrographis* (*Eremomycetaceae*, *Dothideomycetes*). *Persoonia* **32**: 102–114.
Goloboff PA, Carpenter JM, Salvador Ariasc J, *et al.* (2008). Weighting against homoplasy improves phylogenetic analysis of morphological data sets. *Cladistics* **24**: 758–773.
Gouy M, Guindon S, Gascuel O (2010). Seaview version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**: 221–224.
Gueidan C, Hill DJ, Miadlikowska J, *et al.* (2015). *Pezizomycotina: Lecanoromycetes*. In: *The Mycota.* Vol. 7B. 2nd edition. Systematics and Evolution (McLaughlin DJ, Spatafora JW, eds). Springer-Verlag, Berlin Heidelberg, Germany: 89–121.
Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
Hasse HE (1898). *Lichens of Southern California*. 2nd edn. B. R. Baumgardt & Co, Los Angeles.
Hasse HE (1908). Lichens collected in the Tehachepi Mountains, California, June, 1907. *The Bryologist* **11**: 55.
Hawksworth DL, Sherwood MA (1981). A reassessment of three widespread discomycetes. *Canadian Journal of Botany* **59**: 357–372.
Hibbett DS, Binder M, Bischoff JF, *et al.* (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509–547.
Himelbrant DE (2016). The lichens and allied fungi from the Leningrad Region and Saint Petersburg in the lichen herbarium of the University of Tartu. *Folia Cryptogamica Estonica* **53**: 35–42.
Hodkinson BP, Lendemer JC (2011). The orders of *Ostropomycetidae* (*Lecanoromycetes, Ascomycota*): recognition of *Sarrameanales* and *Trapeliales* with a request to retain *Pertusariales* over *Agyriales*. *Phytologia* **93**: 407–412.
Howe GA, Schaller A (2008). Direct defense in plants and their induction by wounding and insect herbivores. In: Schaller A. (ed), *Induced Plant Resistance to Herbivory*. Springer, Germany: 7–29.
James TY, Kauff F, Schoch CL, *et al.* (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**: 818–822.
Katoh K, Toh H (2008). Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **92**: 86–98.
Kearsey SE, Labib K (1998). MCM proteins: evolution, properties, and role in DNA replication. *Biochimica et Biophysica Acta* **1398**: 113–136.
Kobayashi T, Zhao JZ (1989). Notes on diseases of woody plants and their causal fungi in Heilongjiang province, China, 1. *Transactions of the Mycological Society of Japan* **30**: 277–293.
Koerber GW (1865). *Parerga lichenologica: Ergänzungen zum Systema lichenum Germaniae*. Verlag von Eduard Trewendt. Breslau.
Koukol O, Kolařík M, Kolářová Z, *et al.* (2012). Diversity of foliar endophytes in wind-fallen *Picea abies* trees. *Fungal Diversity* **54**: 69–77.
Kowalski T, Kehr RD (1992). Endophytic fungal colonization of branch

bases in several forest tree species. *Sydowia* **44**: 137–168.
Kujala V (1950). Über die Kleinpilze der Koniferen in Finnland. Ascomycetes, Fungi Imperfecti, Uredinales. *Metsätieteellisen Tutkimuslaitoksen Julkaisuja* **38**: 1–121.
Kukwa M, Schiefelbein U, Czarnota P, *et al.* (2008). Notes on some noteworthy lichens and allied fungi found in the Białowieża Primeval Forest in Poland. *Bryonora* **41**: 1–11.
Kuntze O (1898). *Revisio Generum Plantarum*. Pars III(III). Arthur Felix. Leipzig.
Kuz'michev EP, Sokolova ES, Kulikova EG (2001). Common fungal diseases of Russian forests. General Technical Report NE-279. USDA Forest Service. Newtown Square, PA.
Kuznetsova ES, Kataeva OA, Himelbrant DE, *et al.* (2016). Lichens and allied fungi of the Ragusha River Protected Area (Leningrad Region, Russia). *Folia Cryptogamica Estonica* **53**: 71–80.
LoBuglio KF, Pfister DH (2010). Placement of *Medeolaria farlowii* in the *Leotiomycetes*, and comments on sampling within the class. *Mycological Progress* **9**: 361–368.
Lõhmus P, Leppik E, Motiejūnaitė J, *et al.* (2012). Old selectively cut forests can host rich lichen communities – lessons from an exhaustive field survey. *Nova Hedwigia* **95**: 493–515.
Łubek A., Jaroszewicz B (2012). New, rare and noteworthy species of lichens and lichenicolous fungi from Białowieża Forest. *Polish Journal of Natural Sciences* **27**: 275–287.
Lumbsch TH, Schmitt I, Mangold A, *et al.* (2007). Ascus types are phylogenetically misleading in *Trapeliaceae* and *Agyriaceae* (*Ostropomycetidae, Ascomycota*). *Mycological Research* **111**: 1133–1141.
Lumbsch HT, Huhndorf SM (2010). Myconet Volume 14. Part One. Outline of Ascomycota – 2009. Part Two. Notes on Ascomycete Systematics. Nos. 4751–5113. *Fieldiana Life Earth Sciences* **1**: 1–64.
Lutzoni F, Kauff F, McLaughlin D, *et al.* (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.
Malençon G (1979). Nouvelles contributions a la flore mycologique du Maroc II. *Bulletin Trimestriel de la Société Mycologique de France* **95**: 119–137.
McMullin RT, Lendemer JC (2016). Lichens and Allied Fungi of Awenda Provincial Park, Ontario: Diversity and Conservation Status. *The American Midland Naturalist* **176**: 1–19.
Medardi G (2007). Overview of the genus *Claussenomyces* and a description of Italian collections. *Czech Mycology* **59**: 101–109.
Miądlikowska J, Kauff F, Hofstetter V, *et al.* (2006). New insights into classification and evolution of the *Lecanoromycetes* (*Pezizomycotina*, *Ascomycota*) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98**: 1089–1102.
Miadlikowska J, Kauff F, Högnabba F, *et al.* (2014). A multigene phylogenetic synthesis for the class Lecanoromycetes (*Ascomycota*): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Molecular Phylogenetics and Evolution* **79**: 132–168.
Miller MA, Pfeiffer W, Schwartz T (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Institute of Electrical and Electronics Engeneers*. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA: 1–8.
Mirza JH, Cain RF (1969). Revision of the genus *Podospora*. *Canadian Journal of Botany* **47**: 1999–2048.
Moir D, Stewart SE, Osmond BC, *et al.* (1982). Cold-sensitive cell division-cycle mutants of yeast: Isolation, properties, and pseudoreversion studies. *Genetics* **100**: 547–563.
Motiejūnaitė J (2015). Lichens And Allied Fungi From The Čepkeliai State Nature Reserve (Southern Lithuania). *Botanica Lithuanica* **21**: 3–12.
Mudd W (1861). A manual of British lichens. Harrison Penney. Darlington.
Nannfeldt JA (1932). Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Societas Scientiarum Upsaliensis, Ser. IV* **8**: 1–368.
Nylander W (1866). Lichenes lapponiae orientalis. *Notiser ur Sällskapets por Fauna et Flora Fennica Förhandlingar* **8**: 101–192.
Ohlert A (1870). *Zusammenstellung der Lichenen der Provinz Preussen*. Druck der Universität-Buch- und Steindruckerei von E. J. Dalkowski, Königsberg.
Petrini O, Carroll G (1981). Endophytic fungi in foliage of some *Cupressaceae* in Oregon. *Canadian Journal of Botany* **59**: 629–636.
Petrini O, Fisher PJ (1988). A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. *Transactions of the British Mycological Society* **91**: 233–238.
Prieto M, Baloch E, Tehler A, *et al.* (2013). Mazaedium evolution in the Ascomycota (Fungi) and the classification of mazaediate groups of formerly unclear relationship. *Cladistics* **29**: 296–308.
Rautio M, Sipponen A, Lohi J, *et al.* (2011). *In vitro* fungistatic effects of natural coniferous resin from Norway spruce (*Picea abies*). *European Journal of Clinical Microbiology and Infectious Diseases* **31**: 1783–1789.
Reeb V, Lutzoni F, Roux C (2004). Contribution of rpb2 to multilocus phylogenetic studies of the euascomycetes (*Pezizomycotina*, Fungi) with special emphasis on the lichen-forming *Acarosporaceae* and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.
Rikkinen J (2003). New resinicolous ascomycetes from beaver scars in western North America. *Annales Botanici Fennici* **40**: 443–450.
Rikkinen J, Poinar G (2000). A new species of resinicolous *Chaenothecopsis* (*Mycocaliciaceae*, *Ascomycota*) from 20 million year old Bitterfeld amber, with remarks on the biology of resinicolous fungi. *Mycological Research* **104**: 7–15.
Rikkinen J, Tuovila H, Beimforde C, *et al.* (2014). *Chaenothecopsis neocaledonica sp. nov.*: The first resinicolous mycocalicioid fungus from *Araucariaceae*. *Phytotaxa* **173**: 49–60.
Rikkinen J, Beimforde C, Seyfullah LJ, *et al.* (2016). *Resinogalea humboldtensis gen. et sp. nov.*, a new resinicolous fungus from New Caledonia, placed in *Bruceomycetaceae fam. nova* (*Ascomycota*). *Annales Botanici Fennici* **53**: 205–215.
Rikkinen J, Schmidt AR (2018). Morphological convergence in forest microfungi provides a proxy for Eocene forest structure. In: *Transformative Palaeobotany*. Papers to commemorate the life and legacy of Thomas N. Taylor (Krings M, Harper CJ, Cúneo NR, *et al.*, eds). Academic Press, UK: 527–549.
Robertson J (2002). Pygmy Forest Fieldtrip, Mendocino Co., March 16, 2002 and list of Macrolichens of the Pygmy Forest. *Bulletin of the California Lichen Society* **9**: 8–12.
Ronquist F, Huelsenbeck JP (2003). Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
Safronova IE, Palnikova EN (2010). Correlation between Big Pine Weevil and *Biatorella* Canker in the Krasnoyarsk Priangarye Pine Saplings. *The Bulletin of the Krasnoyarsk State Agrarian University* **2010**: 74–79.
Safronova IE, Sorokin ND (2013). The pine undergrowth density influence on Biatorella pine canker prevalence. *The Bulletin of the Krasnoyarsk State Agrarian University* **2013**: 91–96.
Sanz-Ros AV, Müller MM, San Martín R, *et al.* (2015). Fungal endophytic communities on twigs of fast and slow growing Scots pine (*Pinus sylvestris* L.) in northern Spain. *Fungal Biology* **119**: 870–883.
Schmitt I, Mueller G, Lumbsch HT (2005). Ascoma morphology is homoplaseous and phylogenetically misleading in some pyrenocarpous lichens. *Mycologia* **97**: 362–374.

Schmitt I, Crespo A, Divakar PK, *et al.* (2009). New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23**: 35–40.

Schoch CL, Sung GH, López-Giráldez F, *et al.* (2009a). The Ascomycota Tree of Life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58**: 224–239.

Schoch CL, Wang Z, Townsend JP, *et al.* (2009b). *Geoglossomycetes cl. nov.*, *Geoglossales ord. nov.* and taxa above class rank in the Ascomycota Tree of Life. *Persoonia* **22**: 129–138.

Schwarz G (1978). Estimating the dimension of a model. *Annals of Statistics* **6**: 461–464.

Seifert KA, Hughes SJ, Boulay H, *et al.* (2007). Taxonomy, nomenclature and phylogeny of three *Cladosporium*-like hyphomycetes, *Sorocybe resinae*, *Seifertia azaleae* and the *Hormoconis* anamorph of *Amorphotheca resinae*. *Studies in Mycology* **58**: 235–245.

Seyfullah LJ, Beimforde C, Dal Corso J, *et al.* (2018). Production and preservation of resins – past and present. *Biological Reviews* **93**: 1684–1714.

Sherwood MA (1981). Convergent evolution in discomycetes from bark and wood. *Botanical Journal of the Linnean Society* **82**: 15-34.

Sipponen A, Laitinen K (2011). Antimicrobial properties of natural coniferous rosin in the European Pharmacopoeia challenge test. *Acta Pathologica Microbiologica et Immunologica Scandinavia* **119**: 720–724.

Smerlis E (1973). Pathogenicity tests of some discomycetes occurring on conifers. *Canadian Journal of Forest Research* **3**: 7–16.

Stamatakis A (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stamatakis A, Hoover P, Rougemont J (2008). A fast bootstrapping algorithm for the RAxML Web-Servers. *Systematic Biology* **57**: 758–771.

Suto Y (2000). Etiological studies on the resinous stem canker of *Chamaecyparis obtusa*: A review of studies on the fungi relating to symptom development. *The Journal of the Japanese Forestry Society* **82**: 397–406.

Swofford DL (1991). *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.* Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford DL (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), v. 4.0b10*. Sinauer Associates, MA.

Szymczyk R, Kuwka M, Flakus A, *et al.* (2014). Lichens and allied non-lichenized fungi on the special area of conservation Natura 2000 "Swajnie" PLH 280046 (Northern Poland). *Polish Journal of Natural Sciences* **29**: 319–336.

Tuovila H (2013). *Sticky business – diversity and evolution of Mycocaliciales (Ascomycota) on plant exudates*. Publications from the Department of Botany, University of Helsinki, Helsinki.

Tuovila H, Cobbinah JR, Rikkinen J (2011a). *Chaenothecopsis khayensis*, a new resinicolous calicioid fungus on African mahogany. *Mycologia* **103**: 610–615.

Tuovila H, Larsson P, Rikkinen J (2011b). Three resinicolous North American species of *Mycocaliciales* in Europe with a re-evaluation of *Chaenothecopsis oregana* Rikkinen. *Karstenia* **51**: 37–49.

Tuovila H, Rikkinen J, Huhtinen S (2012). Nomenclatural corrections in calicioid fungi. *Karstenia* **52**: 73–4.

Tuovila H, Schmidt AR, Beimforde C, *et al.* (2013). Stuck in time – a new *Chaenothecopsis* species with proliferating ascomata from *Cunninghamia* resin and its fossil ancestors in European amber. *Fungal Diversity* **58**: 199-213.

Wang Z, Johnston PR, Takamatsu S, *et al.* (2006). Toward a phylogenetic classification of the *Leotiomycetes* based on rDNA data. *Mycologia* **98**: 1065–1075.

Wang Z, Johnston PR, Yang ZL, *et al.* (2009). Evolution of reproductive morphology in leaf endophytes. *PLOS ONE* **4**, e4246.

Watson W (1948). List of British fungi parasitic on lichens or which have been included as lichens (or vice versa), with some notes on their characters and distribution. *Transactions of the British Mycological Society* **31**: 305–339.

Wirth V (1995). *Die Flechten Baden-Württembergs*, 2nd ed. Verlag Eugen Ulmer, Stuttgart.

Yatsyna A (2015). Lichens from Manor Parks in Minsk Region (Belarus). *Botanica Lithuanica* **20**: 159–168.

Supplementary Material: http://fuse-journal.org/

Table S1. GenBank accession numbers and voucher information.
Table S2. PCR primers and PCR conditions used in this study.