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

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Abstract: Resiniculous 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-resiniculous 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 *Leotiomyces*. 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 *Leotiomyces*, 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 *Leotiomyces* is similarly unsupported. Based on our data, *Sarea* forms an isolated and highly supported phylogenetic lineage within the "*Leotiomyces*". From the results of our multilocus phylogenetic analyses we propose here a new class, order, and family, *Sareomyces*, *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). Resiniculous fungi represent a polyphyletic assemblage of ascomycetes which grow exclusively on tree resins (Tuovila 2013, Rikkinen *et al.* 2016).

Resiniculous fungi occur scattered throughout many classes within the *Ascomycota*. Most resinicolous fungi described to date are ascomycetes within the order *Mycocaliciales* (*Eurotiomyces*) (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 *Eurotiomyces* 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. *Dothideomyces* contains at least six resinicolous species: *Helicoma resinae*, *Mytilinidion resinae*, *M. resinicola*, *Strigopodia batistae*, *S. resinae*, and *Torula resinicola*. *Leotiomyces* 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 resiniae 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, Connors 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, Löhmus *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 resiniae* 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. resiniae* 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
<i>Sarea difformis</i> s. l.	CB093	resin, <i>Picea abies</i>	Göttingen, Lower Saxony, Germany	University of Helsinki (H), Helsinki
<i>Sarea difformis</i> s. l.	JR6451	resin, <i>Picea abies</i>	Finland	University of Helsinki (H), Helsinki
<i>Sarea resiniae</i> s. l.	CB094	resin, <i>Picea abies</i>	Göttingen, Lower Saxony, Germany	University of Helsinki (H), Helsinki
<i>Sarea resiniae</i> s. l.	JR6450	resin, <i>Picea abies</i>	Finland	University of Helsinki (H), Helsinki
<i>Sarea resiniae</i> s. l.	PDD117345	resin, <i>Pseudotsuga menziesii</i>	Dunedin, Otago, New Zealand	New Zealand Fungarium (PDD) Collection, Auckland
<i>Sarea resiniae</i> s. l.	PDD117343	resin, <i>Abies</i> 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. resiniae*. **A.** Ascomata of *S. difformis* and **B.** *S. resiniae*; **C.** Young ascoma of *S. resiniae* arising on a fresh resin flow; **D.** Cross-section of *S. resiniae* 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. resiniae*; **H.** Young ascus of *S. resiniae*. Scale bars: 1 mm (A, B), 500 μ m (C, D), 10 μ m (E, G), 5 μ m (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 resiniae* 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. resiniae* were removed from the resinous substrate and transferred to double cavity glass slides containing a drop of sterile 0.9 % NaCl₂ 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 % NaCl₂ 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. resiniae* 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. resiniae* 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 (Invitex, 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. resiniae* 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/phyloids/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. resiniae* (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 “*Leotiomyces*” 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 to the clade including *Dothideomycetes-Arthoniomycetes* and *Leotiomyces-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 *Sareomyces*. 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*, *Leotiomyces-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*: *Sareomyces*, *Sareales* and *Sareaceae cl., ord. et fam. nov.*

Sareomyces 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.

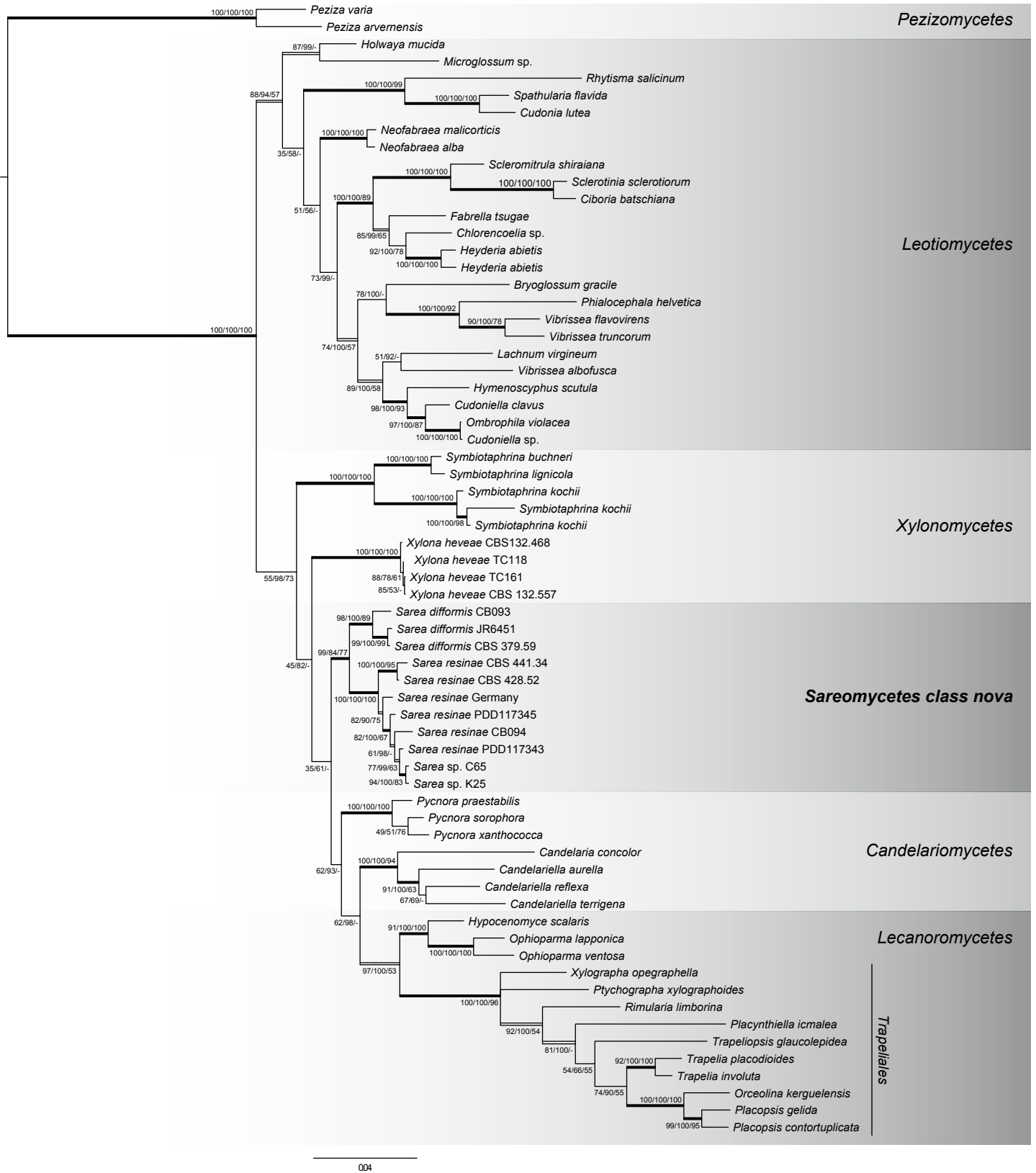
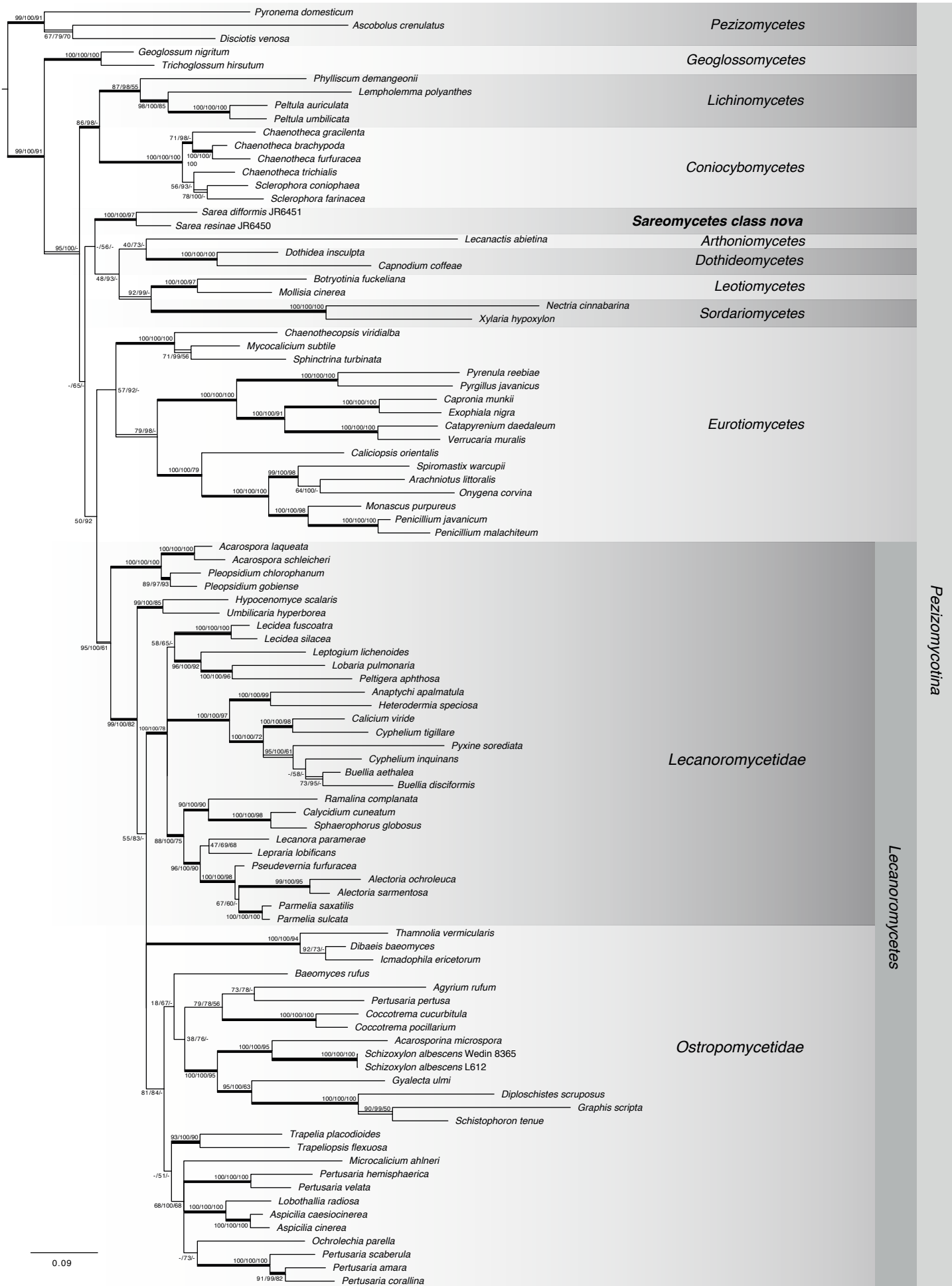


Fig. 2. Phylogenetic relationships of *Sarea*, *Trapeliales* and *Helotiales* based on two ribosomal genes (ITS, nuLSU) obtained from Bayesian, Maximum Likelihood and Maximum Parsimony (MP) analysis. Posterior Probabilities (PP), ML- and MP-Bootstraps are represented by the first, second and third numbers 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, nuLSU, *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.



Pezizomycotina

Lecanoromycetes

0.09

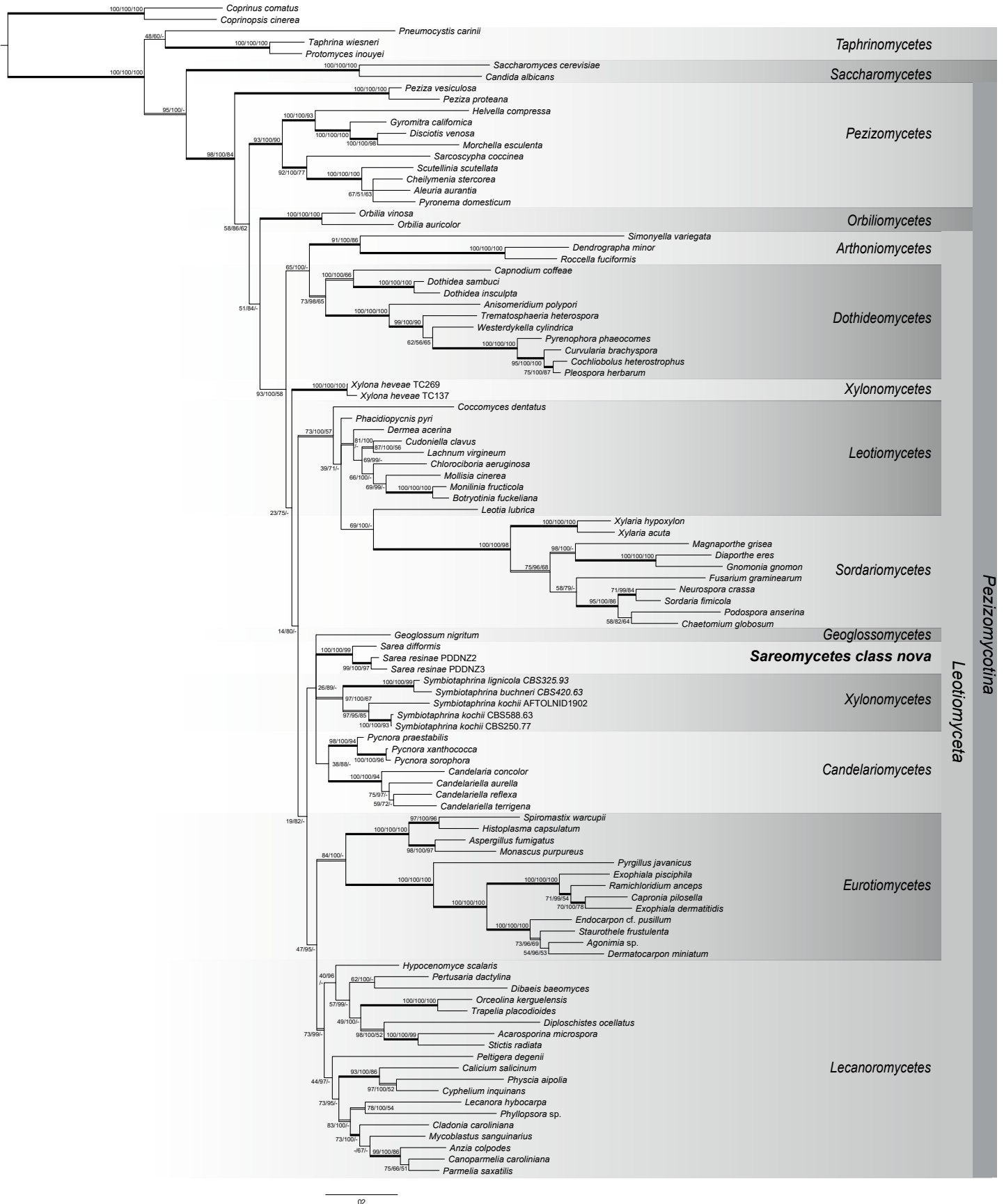


Fig. 4. Phylogenetic relationship of Pezizomycotina based on four genes (ITS, nucSSU, nucLSU, *rbp2*) obtained from Bayesian, Maximum Likelihood and Maximum Parsimony (MP) analysis. Posterior Probabilities (PP), ML- and MP-Bootstraps are represented by the first, second and third numbers associated with internodes. Branches in bold indicate PP \geq 95 %, and both ML and MP bootstrap values \geq 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 epithelial 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 collarete 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 resiniae* 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 resiniae* (*Peziza resiniae*), 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 resiniae* CB094 (H), *Sarea resiniae* JR6450 (H), *Sarea resiniae* PDD117343, *Sarea resiniae* 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 resiniae*. 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. resiniae*. 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 (nuLSU) 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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Table S1. GenBank accession numbers and voucher information.

Table S2. PCR primers and PCR conditions used in this study.

Table S1

List of taxa used in this study with GenBank accession numbers and voucher information

	Collection /Strain number	GenBank accession number						
		ITS	mtSSU	nuLSU	nuSSU	RPB1	RPB2	MCM7
<i>Acarospora laqueata</i>	Westberg 10-170 (S)	DQ842014	DQ991757	AY640943	AY640984	DQ782860	—	JX000147
<i>Acarospora schleicheri</i>	Obermayer 2929 (UPS)	HQ650721	AY584694	AY640945	AY640986	DQ782859	—	JX000148
<i>Acarosporina microspora</i>		DQ782834	AY584612	AY584643	AY584667	DQ782818	AY584682	—
<i>Agonimia</i> sp.		—	—	DQ782913	DQ782885		DQ782874	
<i>Agyrium rufum</i>	Wedin 7931 (UPS)	JX000097	EF581823	EF581826	—	EF581822	—	GU980988
<i>Alectoria ochroleuca</i>		HQ650597	DQ986785	DQ986801	DQ983483	DQ986857	—	—
<i>Alectoria sarmentosa</i>		DQ979998	—	DQ899291	AF140233	DQ899290	—	JN009675
<i>Anaptychia palmatula</i>		HQ650702	DQ912286	DQ883801	DQ883792	DQ883744	—	—
<i>Anisomeridium polyperi</i>		DQ782838	—	DQ782906	DQ782877	—	DQ782864	—
<i>Anzia colpodes</i>		DQ980000	—	DQ923651	DQ923622	—	—	—
<i>Arachniotus littoralis</i>		AB566293	FJ225784	FJ358272	FJ358340	FJ358404	—	—
<i>Ascobolus crenulatus</i>	F9477 (S)	DQ491504	—	AY544678	AY544721	DQ471132	—	JX000149
<i>Aspergillus fumigatus</i>		FJ878717	—	AY660917	AB008401	—	XM741647	
<i>Aspicilia caesiocinerea</i>		HQ650636	DQ780271	DQ780303	DQ986736	DQ870931	—	GQ272390
<i>Aspicilia cinerea</i>		HQ650637	DQ780272	DQ780304	DQ986735	DQ870932	—	GQ272391
<i>Aleuria aurantia</i>		MH225453		AY544654	AY544698		DQ247785	
<i>Baeomyces rufus</i>	F178482 (S)	AF448457	DQ871016	JX000080	AF113718	DQ870937	—	JX000150
<i>Botryotinia fuckeliana</i>		DQ491491	AY544732	AY544651	AY544695	DQ471116	DQ247786	XM_001556412
<i>Buellia aethalea</i>	F138222 (S)	JX000098	JX000115	JX000081	—	JX000133	—	JX000151
<i>Buellia disciformis</i>	Westberg 10-002 (S)	AY143392	JX000116	JX000082	AF241543	—	—	JX000152
<i>Bryoglossum gracile</i>		AY789421	—	AY789420	—	—	—	—
<i>Caliciopsis orientalis</i>		—	FJ190654	DQ470987	DQ471039	DQ471185	—	—
<i>Calicium viride</i>	M. Prieto 3015 (S)	HQ650703	AY584696	AY340538	—	—	—	JX000153
<i>Calicium salicinum</i>		AY453645		KF157982	KF157970	—	KF157998	
<i>Calycidium cuneatum</i>	Wedin 8034 (S)	JX000114	JX000117	JX000083	—	JX000134		JX000154
<i>Candelaria concolor</i>		AF182075	EF436460	DQ986791	—	—	DQ992419	—
<i>Candelariella aurella</i>	Hermansson 10056 (UPS)	EF535163	AY853313	AY853361	—	DQ915594		JX000155
<i>Candelariella reflexa</i>		EF535189	—	DQ912331 AFTOL-ID 1271	DQ912309	—	DQ912380	—
<i>Candelariella terrigena</i>		HQ650602	—	DQ986745 AFTOL-ID 227	DQ986730	—	DQ992427	—
<i>Candida albicans</i>		JN606311	AACQ01000290 REGION: 9209.. 10587	AACQ01000290 REGION: 7072.. 8646	—	—	XM713346	—
<i>Canoparmelia caroliniana</i>		KY929409	—	AY584634	AY584658	—	AY584683	—
<i>Capnodium coffeae</i>		DQ491515	FJ190609	GU214400	DQ247808	DQ471162	DQ247788	—
<i>Capronia munkii</i>		AF050250	FJ225723	EF413604	EF413603	EF413605		—
<i>Capronia pilosella</i>		DQ826737		DQ823099	DQ823106		DQ840561	
<i>Catapyrenium daedaleum</i>	M. Prieto 3051 (S)	JX000099	JX000118	EF643748	EF689830	EF689748	—	JX000156
<i>Curvularia brachyspora</i>		MG250426	—	AF279380	L36995.1	—	AF107803.1	—
<i>Chaenotheca brachypoda</i>	M. Prieto 3023 (S)	AF297963	JX000122	JX000086	—	JX000135	—	—
<i>Chaenotheca furfuracea</i>	Wedin 6366 (UPS)	JX000101	JX000121	JX000087	JX000068	JX000137	—	JX000158
<i>Chaenotheca gracilentia</i>	Wedin 7022 (S)	JX000100	JX000119	JX000084	JX000067	—	—	JX000157
<i>Chaenotheca trichialis</i>	M. Prieto 3028 (S)	JX000102	JX000120	JX000085	JX000069	JX000136	—	JX000159
<i>Chaenothecopsis viridalba</i>	Wedin 6728 (UPS)	JX000103	AY853317	AY853365	—	—	—	JX000160

<i>Chaetomium globosum</i>		AY429056	—	AY545729	AY545725	—	AAFU01001128 REGION: 238 .. 2979	—
<i>Cheilymenia stercorea</i>		MH930238	—	AY544661	AY544705	—	AY544733	—
<i>Chlorenchocleia</i> sp.	ZW-Geo55-Clark	AY789352	—	AY789351	—	—	—	—
<i>Chromocleista malachitea</i>		—	FJ225777	FJ358281	FJ358346	FJ358409	—	—
<i>Ciboria batschiana</i>		AY526234	—	AY789322	—	—	—	—
<i>Cladonia caroliniana</i>		MK179649	—	AY584640	AY584664	—	AY584684	—
<i>Coccomyces dentatus</i>		KF797433	—	AY544657	AY544701	—	DQ247789	—
<i>Coccotrema cucurbitula</i>		AF329162	AF329161	AF274092	AF274114	DQ870939	—	GU980990
<i>Coccotrema pocillarium</i>		AF329167	AF329166	AF274093	AF274113	DQ870940	—	GU980992
<i>Cochliobolus heterostrophus</i>		—	—	AY544645	AY544727	—	DQ247790	—
<i>Coprinus comatus</i>		JQ901444	—	AY635772	AY665772	—	AY780934	—
<i>Coprinopsis cinerea</i>		FJ904826	—	AF041494	M92991	—	AACS01000026	—
<i>Chromocleista malachitea</i>		—	FJ225777	FJ358281	FJ358346	FJ358409	—	—
<i>Cudonia lutea</i>	WZ164	AF433149	—	KC833187	—	—	—	—
<i>Cudoniella clavus</i>		AY789374	—	AY789373	—	—	—	—
<i>Cudoniella</i> sp.	ZW 0068	AY789342	—	AY789341	—	—	—	—
<i>Cudoniella clavus</i>		JQ256415/ AY789374	—	DQ470944	DQ470992	—	DQ470888	—
<i>Cyphelium inquinans</i>	M. Prieto 3008 (S)	AY450583/AY1433 95	AY143404	AY453639	U86695	—	—	JX000161
<i>Cyphelium tigillare</i>	M. Prieto 3038 (S)	JX000104	JX000123	JX000088	AF241545	—	—	JX000162
<i>Dendrographa minor</i>		DQ842015	—	AF279382	AF279381	—	AY641034	—
<i>Dermatocarpon minutum</i>		MF521951	—	AY584644	AY584668	—	DQ782863	—
<i>Dermea acerina</i>		MH855942	—	DQ247801	DQ247809	—	DQ247791	—
<i>Diploschistes ocellatus</i>		AF228316	—	AY605077	AF038877	—	DQ366253	—
<i>Diaporthe eres</i>		MK024710	—	AF408350	DQ471015	—	DQ470919	—
<i>Dibaeis baemyces</i>		DQ782844	AY300883	AF279385	—	DQ842011	AY641037	—
<i>Diploschistes scruposus</i>	F178255 (S)	HQ650716	AY584692	AF279389	AF279388	DQ870943	—	JX000163
<i>Disciotis</i> sp.		AJ544207	—	AY544667	AY544711	—	DQ470892	—
<i>Disciotis venosa</i>	F12784 (S)	DQ491503	JX000124	AY544667	AY544711	DQ471131	—	—
<i>Dothidea insculpta</i>		AF027764	FJ190602	DQ247802	DQ247810	DQ471154	DQ247792	—
<i>Dothidea sambuci</i>		AY883094	—	AY544681	AY544722	—	DQ522854	—
<i>Eupenicillium javanicum</i>		GU981614	FJ225778	EF413621	EF413620	—	—	—
<i>Endocarpon pallidulum</i>		HM237334	—	DQ823097	DQ823104	—	DQ840559	—
<i>Exophiala dermatitidis</i>		KU664383	—	DQ823100	DQ823107	—	DQ840562	—
<i>Exophiala nigra</i>		EF551550	FJ225742	FJ358244	FJ358312	FJ358375	—	—
<i>Exophiala pisciphila</i>		KC354799	—	DQ823101	DQ823108	—	DQ840563	—
<i>Fabrella tsugae</i>		—	—	AF356694	—	—	—	—
<i>Fusarium graminearum</i>		JX162395	—	AY188924	—	—	AACM01000132 REGION: 154552.. 157390	v
<i>Geoglossum nigratum</i>		DQ491490	AY544740	AY544650	AY544694	DQ471115	DQ470879	—
<i>Gnomonia gnomon</i>		AY818957	—	AF408361	DQ471019	—	DQ470922	—
<i>Graphis scripta</i>	Wedin 6476 (UPS)	AF229195	AY853322	AY853370	AF038878	DQ870947	—	JX000164
<i>Gyalecta ulmi</i>	L67816 (S)	HQ650713	AY300888	AF465463	AF088237	JX000138	—	JX000165
<i>Gyromitra californica</i>		EU837204	—	AY544673	AY544717	—	DQ470891	—
<i>Helvella compressa</i>		KU739801	—	AY544655	AY544699	—	DQ497613	—
<i>Heterodermia speciosa</i>	Wetmore 88030 (S)	JX000105	JX000125	JX000089	—	—	—	JX000166
<i>Heyderia abietis</i>	OSC60392	AY789290	—	AY789289	—	—	—	—
<i>Heyderia abietis</i>	HMAS71954	AY789297	—	AY789296	—	—	—	—

<i>Histoplasma capsulatum</i>		AF 129547	—	—	—	—	—	—
<i>Holwaya mucida</i>		DQ257357	—	DQ257356	—	—	—	—
<i>Hymenoscyphus scutula</i>		AY789432	—	AY789431	—	—	—	—
<i>Hypocnemomyce scalaris</i>	Wedin 7008 (UPS)	HQ650632	AY853325	AY853373/ DQ986748	DQ782886	DQ915596	DQ782875	JX000167
<i>Icmadophila ericetorum</i>		—	DQ986897	DQ883694	DQ883704	DQ883723	—	—
<i>Lachnum virgineum</i>		MH857308	—	AY544646	AY544688	—	DQ470877	—
<i>Lecanactis abietina</i>		AY548804	AY548813	AY548812	AY548805	GU561850	—	—
<i>Lecanora paramerae</i>		EF 105413	EF 105418	EF 105422	—	DQ870950	—	—
<i>Lecidea fuscoatra</i>		HQ650707	DQ912275	DQ912332	DQ912310	DQ912355	—	—
<i>Lecidea silaceae</i>		HQ650629	DQ986878	AY756340	DQ986723	DQ986820	—	—
<i>Lempholemma polyanthes</i>	M. Prieto 3052 (S)	JX000106	—	JX000090	AF356690	—	—	JX000168
<i>Leotia lubrica</i>		AY144561	—	AY544644	AY544687	—	DQ470876	—
<i>Lepraria lobificans</i>		HQ650623	DQ986887	DQ986768	DQ986733	DQ986837	—	—
<i>Leptogium lichenoides</i>		HQ650672	DQ923120	DQ917412	DQ917413	DQ917414	—	—
<i>Lobaria pulmonaria</i>	Wedin 6167 (UPS)	HM448799	AY340504	AY340548	AF 183935	DQ915591	—	JX000169
<i>Lobothallia radiosa</i>		JF 703124	DQ780274	DQ780306	—	DQ870954	—	GQ272397
<i>Magnaporthe grisea</i>		KM484885	—	AB026819	AB026819	—	XM362269	—
<i>Microcalicium ahneri</i>	Wedin 12/6 2011 (S)	JX000108	JX000126	—	JX000070	JX000139	—	JX000170
<i>Microglossum</i> sp.	PDD70355	DQ257363	—	—	—	—	—	—
<i>Mollisia cinerea</i>	M. Prieto 3055 (S)	DQ491498	DQ976372	DQ470942	DQ470990	FJ238440	DQ470883	JX000172
<i>Monascus purpureus</i>		DQ782847	FJ225780	DQ782908	DQ782881	DQ842012	—	—
<i>Morchella aff. esculenta</i>		AB509785	—	AY544664	AY544708	—	DQ470880	—
<i>Monilia fruticicola</i>		KY038837	—	AY544670	AY544714	—	DQ470889	—
<i>Mycoblastus sanguinarius</i>		JF744960	—	DQ782915	DQ782879	—	DQ782867	—
<i>Mycocalicium subtile</i>	Wedin 8492 (S)	AF225445	AY853330	AY853379	JX000072	JX000141	—	JX000173
<i>Nectria cinnabarina</i>	F118002 (S)	HM484710	FJ713622	AF 193237	JX000073	GQ506027	—	JX000174
<i>Neofabraea alba</i>		AY359236	—	AY064705	—	—	—	—
<i>Neofabraea malicorticis</i>		AF281386	—	AY544662	—	—	—	—
<i>Neurospora crassa</i>		KF040479	—	AF286411	X04971	—	XM952013	—
<i>Orbilina vinosa</i>		DQ491511	—	DQ470952	DQ471000	—	—	—
<i>Orbilina auricolor</i>		DQ656611	—	DQ470953	DQ471001	—	DQ470903	—
<i>Ochrolechia parella</i>		AF332123	GU980977	AF274097	AF274109	DQ870959	—	GQ272421
<i>Ombrophila violacea</i>		AY789366	—	AY789365	—	—	—	—
<i>Onygena corvina</i>		—	FJ225792	FJ358287	FJ358352	FJ358414	—	—
<i>Ophioparma lapponica</i>		KF360414	—	DQ973028	—	—	—	—
<i>Ophioparma ventosa</i>		KF360415	—	KF360474/ KJ766611	—	—	—	—
<i>Orceolina kerguelensis</i> <i>Orceolina kerguelensis</i>		AY212814	—	AY212830	DQ366257	—	DQ3662560	—
<i>Parmelia saxatilis</i>	Wedin 7091 (UPS)	AF058037	AY340514	AY300849	AF 117985	DQ923695	—	JX000175
<i>Parmelia sulcata</i>		GU994574	GU994669	GU994621	—	EF092135	—	—
<i>Peltigera aphthosa</i>	Wedin 6164 (UPS)	AF158645	AY340515	AF286759	AY424225	DQ915598	—	JX000176
<i>Peltigera degenii</i>		MH758420	—	AY584657	AY584681	—	AY584688	—
<i>Peltula auriculata</i>		DQ832329	DQ922953	DQ832330	DQ832332	DQ782856	—	—
<i>Peltula umbilicata</i>		DQ832333	DQ922954	DQ832334	DQ782887	DQ782855	—	—
<i>Pertusaria amara</i>		HQ650677	AY300900	AF274101	AF356682	DQ870965	—	GQ272423
<i>Pertusaria corallina</i>	F178261 (S)	FR799261	AY300901	AY300850	JX000074	DQ870967	—	GU980997
<i>Pertusaria dactylina</i>		DQ782843	—	DQ782907	DQ782880	—	DQ782868	—
<i>Pertusaria hemisphaerica</i>		HQ650676	DQ973000	AF381556	DQ902340	DQ902341	—	GU980998
<i>Pertusaria pertusa</i>		AF332127	AF381565	AF279300	AY779282	DQ870978	—	—
<i>Pertusaria scaberula</i>		—	AF431959	AF274099	AF274105	DQ870980	—	GU981003
<i>Pertusaria velata</i>	F76497 (S)	JX000109	GU980981	AY300855	JX000075	DQ870982	—	GU981005

<i>Peziza proteana f. sparassoides</i>		JF908566	—	AY544659	AY544703	—	—	—
<i>Peziza arvernensis</i>		KP125489	—	AF133162	—	—	—	—
<i>Peziza varia</i>		JF908557	—	MG871335	—	—	—	—
<i>Peziza vesiculosa</i>		JF908568	—	DQ470948	DQ470995	—	DQ470898	—
<i>Pneumocystis carinii</i>		KY197742	—	AF047831	S83267.1	—	AY485631	—
<i>Phialocephala helvetica</i>		AY347413	—	—	—	—	—	—
<i>Phylliscum demangeonii</i>	Wedin 7241 (UPS)	JX000110	AY853333	AY853382	—	JX000142	—	JX000177
<i>Phyllopsora sp.</i>		MG926004	—	KF157990	KF157978	—	KF158005	—
<i>Physcia aipolia</i>		EU682185	—	DQ782904	DQ782876	—	DQ782862	—
<i>Placopsis contortuplicata</i>		DQ534479	—	EF489925	—	—	—	—
<i>Placopsis gelida</i>		AF274084	—	AY212836	—	—	—	—
<i>Placynthiella icmalea</i>		AF274082	—	EU940160	—	—	—	—
<i>Pleopsidium chlorophanum</i>	M. Prieto 3056 (S)	EU870691	DQ991756	DQ842017	AY316151	DQ782858	—	JX000178
<i>Pleopsidium gobiense</i>		HQ650723	DQ991755	DQ883698	DQ525552	DQ883746	—	—
<i>Pleospora herbarum</i>		AB938190	—	DQ247804	DQ247812	—	DQ247794	—
<i>Protomyces inouyei</i>		MK045398	—	AY548294	AY548295	—	AY548299	—
<i>Podospora anserina</i>		MF380264	—	—	—	—	—	—
<i>Potebniamyces pyri</i>		EU156058	—	DQ470949	DQ470997	—	DQ470900	—
<i>Pseudevernia furfuracea</i>		AY611112	AY611169	AY607826	AY548817	DQ870990	—	—
<i>Pycnora praestabilis</i>		KF360399	—	KJ766644	—	—	—	—
<i>Pycnora sorophora</i>		KF360406	—	AY853387	MH468790	—	MH468793	—
<i>Pycnora xanthococca</i>		KF360412	—	KF360472	—	—	—	—
<i>Pyrenula pseudobufonia</i>		DQ782845	AY584720	AY640962	AY641001	DQ840558	—	—
<i>Pyrgillus javanicus</i>		DQ826741	FJ225774	DQ823103	DQ823110	DQ842010	DQ842009	XM_001586126
<i>Pyronema domesticum</i>		MH758709	—	DQ247805	DQ247813	—	DQ247795	—
<i>Pyrenophora phaeocomes</i>		DQ491507	—	DQ499596	DQ499595	—	DQ497614	—
<i>Pyxine soredata</i>	Wetmore 91254 (S)	JX000111	JX000129	JX000093	—	—	—	JX000179
<i>Ramalina complanata</i>		HQ650720	DQ972986	DQ973038	DQ883784	DQ973059	—	—
<i>Rhinocladiella anceps</i>		AY163559	—	DQ823102	DQ823109	—	DQ840564	—
<i>Rhytisma salicimum</i>		AY465516	—	HM140566	—	—	—	—
<i>Rimularia limborina</i>		KJ462273	—	KJ462349	—	—	—	—
<i>Saccharomyces cerevisiae</i>		KC542799	—	U53879 REGION: 24144.. 25525	Z75578	—	Z75059	—
<i>Sarcoscypha coccinea</i>		KU973865	—	AY544647	AY544691	—	DQ497612	—
		ITS	mtSSU	nuLSU	nuSSU	RPB1	RPB2	MCM7
<i>Sarea difformis s.l.</i>	CB093 (H)	MN938392	—	MN938400	—	—	—	—
<i>Sarea difformis s.l.</i>	JR6451 (H)	MN938393	MN938398	MN938401	MT028543	—	MN938406	—
<i>Sarea difformis s.l.</i>	CBS 379.59	MH857896	—	MH867108	—	—	—	—
<i>Sarea resinae s.l.</i>	CB094 (H)	MN938395	—	MN938403	—	—	—	—
<i>Sarea resinae s.l.</i>	JR6450 (H)	MN938394	MN938399	MN938402	MT028544	—	MN938407	MN938409
<i>Sarea resinae s.l.</i>	CBS 428.52	MH857111	—	MH869439	—	—	—	—
<i>Sarea resinae s.l.</i>	CBS 441.34	MH855597	—	MH867108	—	—	—	—
<i>Sarea resinae s.l.</i>	PDD 117345	MN938397	—	MN938405	MT028545	—	—	—
<i>Sarea resinae s.l.</i>	PDD 117343 Abies	MN938396	—	MN938404	MT028546	—	MN938408	—
<i>Simonyella variegata</i>		DQ782835	—	AY584645	AY584669	—	DQ782861	—
<i>Schistophoron tenue</i>	L64691 (S)	JX000112	EU544933	EU544932	—	—	—	JX000181
<i>Schizoxylon albescens</i>	Wedin 8365 (S)	HQ287353	DQ401142	DQ401144	JX000077	JX000143	—	JX000182
<i>Schizoxylon albescens</i>		JX000077	—	—	—	—	—	—
<i>Scleromitrla shiraiana</i>		AY789408	—	AY789407	—	—	—	—
<i>Sclerophora farinacea</i>	Wedin 6414 (UPS)	JX000113	JX000130	JX000095	JX000078	JX000144	—	JX000183
<i>Sclerotinia sclerotiorum</i>		AF455526	—	AY789347	—	—	—	—

<i>Scutellinia scutellata</i>		FJ235141	—	DQ247806	DQ247814	—	DQ247796	
<i>Sphaerophorus globosus</i>	Stenroos 5539 (TUR)	HQ650622	DQ986866	DQ986767	AF117983	DQ986836	—	JX000185
<i>Spathularia flavida</i>	WZ 214	—	—	MH868630	—	—	—	—
<i>Sphinctrina turbinata</i>		AY795877	FJ71361	EF413632	EF413631	—	—	—
<i>Spiromastix warcupii</i>		DQ782848	FJ225794	DQ782909	DQ782882	EF413613	DQ782870	—
<i>Sordaria fimicola</i>		KY930619	—	AY545728	AY545724	—	—	—
<i>Staurothele frustulenta</i>		KC990385	—	DQ823098	DQ823105	—	DQ840560	—
<i>Stictis radiata</i>		MH578520	—	AF356663	U20610	—	AY641079	—
<i>Symbiotaphrina lignicola</i>	CBS 325.93	MH862405	—	NG_057675	—	—	—	—
<i>Symbiotaphrina buchneri</i>		KY105569	—	KY109806	AY227716	—	JCM9740	—
<i>Symbiotaphrina kochii</i>	AFTOL-ID 1902	—	—	—	FJ176833	—	FJ238443	—
<i>Symbiotaphrina kochii</i>	CBS 588.63/589.63	DQ248314	—	—	U26207	—	DQ248316	—
<i>Symbiotaphrina kochii</i>	CBS 250.77	MH861058	—	NG_057719	U26206	—	GU397369	—
<i>Symbiotaphrina kochii</i>	DC-1-15	KC215110	—	—	—	—	—	—
<i>Symbiotaphrina kochii</i>	DC-1-75	KC215113	—	—	—	—	—	—
<i>Taphrina wiesneri</i>		AB435051	—	AY548292	AY548293	—	AY548298	—
<i>Thamnotia vermicularis</i>		AY853345	AY853395	—	AF085472	—	—	—
<i>Trematosphaeria heterospora</i>		GQ203795	—	AY016369	AY016354	—	DQ497615	—
<i>Trapelia involuta</i>		—	—	AF274098	—	—	—	—
<i>Trapelia placodioides</i>		AF274081	AF431962	AF274103	AF119500	DQ366259	DQ366260	—
<i>Trapeliopsis flexuosa</i>		HQ650634	AY212875	AF274118	DQ986709	DQ871000	—	—
<i>Trapeliopsis glaucolepidea</i>		—	—	—	—	—	—	—
<i>Trapelia placodioides</i>		—	—	KU844623	—	—	—	—
<i>Trichoglossum hirsutum</i>	F39542 (S)	DQ491494	AY544758	AY544653	AY544697	DQ471119	—	JX000188
<i>Roccella fuciformis</i>		KF036010	—	AY584654	AY584678	—	DQ782866	—
<i>Umbilicaria hyperborea</i>	Wiklund 25 (UPS)	AF096216	AY853349	AY853399	—	DQ915600	—	JX000189
<i>Verrucaria muralis</i>		EU010261	FJ225708	EF689878	EF689878	EF689805	—	GQ272418
<i>Vibrissea albofusca</i>		AY789384	—	AY789383	—	—	—	—
<i>Vibrissea flavovirens</i>		AY789427	—	AY789426	—	—	—	—
<i>Vibrissea truncorum</i>		AY789403	—	AY789402	—	—	—	—
<i>Westerdykella cylindrica</i>		DQ491519	—	AY779322	AY016355	—	DQ470925	—
<i>Xylographa opegraphella</i>		—	—	KJ462364	—	—	—	—
<i>Xylona haveae</i>	CBS 132.468	MH866027	—	MH877475	—	—	—	—
<i>Xylona heveae</i>	CBS 132.557	NR_121539	—	MH878330	NG_061134	—	—	—
<i>Xylona heveae</i>	TC118	JQ838233	—	—	—	—	—	—
<i>Xylona heveae</i>	TC161	JQ838232	—	JQ838238	JQ838237	—	—	—
<i>Xylona heveae</i>	TC137	JQ838234	—	JQ838240	JQ838235	—	JQ838245	—
<i>Xylona heveae</i>	TC269	JQ838225	—	JQ838236	JQ838239	—	JQ838246	—
<i>Xylaria acuta</i>		JQ862676	—	AY544676	AY544719	—	DQ247797	—
<i>Xylaria hypoxylon</i>	F118002 (S)	DQ491487	AY544760	AY544648	AY544692	DQ471114	DQ470878	JX000190

Table S2. Primers used for specific gene amplification of fungal DNA. Sequencing primers are identical to those used in PCR.

Primer name/Publication	Primer Sequence	PCR conditions
ITS1F/ Gardes & Bruns (1993)	5'-CTT GGT CAT TTA GAG GAA GTA A-3'	(1) 95 °C for 2 min (2) 35 cycles of 45 s at 95 °C, 45 s at 52 °C and 45 s at 72 °C (3) 72 °C for 10 min
ITS4/White <i>et al.</i> (1990)	5'-TCC TCC GCT TAT TGA TAT GC-3'	
LR0R/ Rehner & Samuels (1994)	5'-ACCCGCTGAACTTAAGC-3'	(1) 95 °C for 2 min (2) 35 cycles of 45 s at 95 °C, 45 s at 52 °C and 45 s at 72 °C (3) 72 °C for 10 min
LR5/Vilgalys & Hester (1990)	5'-TCCTGAGGGAACTTCG-3'	
LR7/Vilgalys & Hester (1990)	5'-TACTACCACCAAGAT CT-3'	(1) 95 °C for 2 min (2) 35 cycles of 45 s at 95 °C, 50 s at 52 °C and 60 s at 72 °C (3) 72 °C for 10 min
LR3R/ Moncalvo <i>et al.</i> (2000)	5'-GTCTTGAAACACGGA CC-3'	
Mcm7-709/ Schmitt <i>et al.</i> (2009)	5'-ACI MGI GTI TCV GAY GTH AAR CC-3'	
Mcm7-1348/ Schmitt <i>et al.</i> (2009)	5'-GAY TTD GCI ACI CCI GGR TCW CCC AT-3'	(1) 95 °C for 2 min (2) 35 cycles of 45 s at 95 °C, 50–60 s at 50–52 °C and 60 s at 72 °C (3) 72 °C for 10 min
NS1/ White <i>et al.</i> (1990)	5'- GTA GTC ATA TGC TTG TCT C-3'	(1) 95 °C for 2 min (2) 35 cycles of 45 s at 95 °C, 50–60 s at 50–52 °C and 60 s at 72 °C (3) 72 °C for 10 min
NS4/ White <i>et al.</i> (1990)	5'-CTTCCGTCAATTCCTTTAAG-3'	

RPB1-AFasc/Hofstetter <i>et al.</i> (2007)	5'-ADTG YCCYGGYCATT TYGGT-3'	(1) 95 °C for 2 min (2) 40 cycles of 50 s at 95 °C, 60 s at 52–55 °C and 60 s at 72 °C (3) 10 min at 72 °C.
RPB1-6R2asc/ Hofstetter <i>et al.</i> (2007)	5'-ATGACCCATCATRGAYTCCT-3'	
RPB1-DF2asc/ Hofstetter <i>et al.</i> (2007)	5'-CAYAAGGARTCYATGATGG-3'	
RPB1G1R/ Hofstetter <i>et al.</i> (2007)	5'-ACNCCNACCATYTCNCCNGG-3'	
fRPB2-5F/ Liu <i>et al.</i> (1999)	5'-GAYGAYMGWGATCAYTTYGG-3'	(1) 95 °C for 2 min (2) 40 cycles of 50 s at 95 °C, 60 s at 50–55 °C and 60 s at 72 °C (3) 10 min at 72 °C.
fRPB2-7cR/ Liu <i>et al.</i> (1999)	5'-CCCATRGCTTGYTTRCCCAT-3'	
TSR1453/ Schmitt <i>et al.</i> (2009)	5'-GAR TTC CCI GAY GAR ATY GAR CT-3'	(1) 95 °C for 2 min (2) 35–40 cycles of 45 s at 95 °C, 50 s at 52 °C and 60 s at 72 °C (3) 10 at 72 °C.
TSR2308/ Schmitt <i>et al.</i> (2009)	5'-CTT RAA RTA ICC RTG IGT ICC-3'	

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