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The root-symbiotic *Rhizoscyphus ericae* aggregate and *Hyaloscypha* (*Leotiomycetes*) are congeneric: Phylogenetic and experimental evidence

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Abstract: Data mining for a phylogenetic study including the prominent ericoid mycorrhizal fungus *Rhizoscyphus ericae* revealed nearly identical ITS sequences of the bryophilous *Hyaloscypha hepaticicola* suggesting they are conspecific. Additional genetic markers and a broader taxonomic sampling furthermore suggested that the sexual *Hyaloscypha* and the asexual *Meliniomyces* may be congeneric. In order to further elucidate these issues, type strains of all species traditionally treated as members of the *Rhizoscyphus ericae* aggregate (REA) and related taxa were subjected to phylogenetic analyses based on ITS, nrLSU, mtSSU, and *rpb2* markers to produce comparable datasets while an *in vitro* re-synthesis experiment was conducted to examine the root-symbiotic potential of *H. hepaticicola* in the *Ericaceae*. Phylogenetic evidence demonstrates that sterile root-associated *Meliniomyces*, sexual *Hyaloscypha* and *Rhizoscyphus*, based on *R. ericae*, are indeed congeneric. To this monophylum also belongs the phialidic dematiaceous hyphomycetes *Cadophora finlandica* and *Chloridium paucisporum*. We provide a taxonomic revision of the REA; *Meliniomyces* and *Rhizoscyphus* are reduced to synonymy under *Hyaloscypha*. *Pseudaegerita*, typified by *P. corticalis*, an asexual morph of *H. spiralis* which is a core member of *Hyaloscypha*, is also transferred to the synonymy of the latter genus. *Hyaloscypha melinii* is introduced as a new root-symbiotic species from Central Europe. *Cadophora finlandica* and *C. paucisporum* are confirmed conspecific, and four new combinations in *Hyaloscypha* are proposed. Based on phylogenetic analyses, some sexually reproducing species can be attributed to their asexual counterparts for the first time whereas the majority is so far known only in the sexual or asexual state. *Hyaloscypha bicolor* sporulating *in vitro* is reported for the first time. Surprisingly, the mycological and mycorrhizal sides of the same coin have never been formally associated, mainly because the sexual and asexual morphs of these fungi have been studied in isolation by different research communities. Evaluating all these aspects allowed us to stabilize the taxonomy of a widespread and ecologically well-studied group of root-associated fungi and to link their various life-styles including saprobes, bryophilous fungi, root endophytes as well as fungi forming ericoid mycorrhizae and ectomycorrhizae.

Key words: Ectomycorrhiza, Ericoid mycorrhiza, *Hyaloscypha hepaticicola*, *Hymenoscyphus ericae*, *Meliniomyces*, Molecular systematics, Mycorrhizal synthesis, *Pezoloma ericae*, *Pseudaegerita*, Sexual-asexual connection.
Taxonomic novelties: New species: *Hyaloscypha melinii* Vohník, Fehrer & Réblová.
New combinations: *Hyaloscypha bicolor* (Hambl. & Sigler) Vohník, Fehrer & Réblová, *Hyaloscypha finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová, *Hyaloscypha variabilis* (Hambl. & Sigler) Vohník, Fehrer & Réblová, *Hyaloscypha vraolstadiae* (Hambl. & Sigler) Vohník, Fehrer & Réblová.

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INTRODUCTION

The *Rhizoscyphus ericae* aggregate (= *Hymenoscyphus ericae* aggregate) (REA) is an ecologically important species complex that includes fungi living in symbiotic relationships with plant roots as either endophytes or ericoid mycorrhizal symbionts of the *Ericaceae* and ectomycorrhizal partners of the *Betulaceae*, *Fagaceae*, *Pinaceae* and *Salicaceae*. It is placed in the *Leotiomycetes incertae sedis* by molecular methods (Hambleton & Currah 1997, Vrålstad *et al.* 2000, 2002, Hambleton & Sigler 2005, Grelet *et al.* 2010, Vohník *et al.* 2013). The aggregate is named after the typical ericoid mycorrhizal (ErM) fungus which inhabits *Ericaceae* hair roots worldwide (Bruzone *et al.* 2017, Midgley *et al.* 2017).

A substantial part of the REA consists of strains which do not form any kind of sexual or asexual reproductive structures. These sterile fungi have been assigned to the REA based on comparison of DNA sequences and eventually accommodated in the genus *Meliniomyces* (Hambleton & Sigler 2005). REA members also include the phialidic dematiaceous hyphomycete *Cadophora finlandica* (Wang & Wilcox 1985, Harrington & McNew 2003) confirmed to form ectomycorrhizae with conifers and also ericoid mycorrhizae (Wang & Wilcox 1985, Vrålstad *et al.* 2002). However, the most prominent REA member is the inoperculate discomycete *R. ericae*, a taxon with a long history of taxonomic treatments. It was originally isolated from *Calluna vulgaris* (*Ericaceae*) hair roots in the United Kingdom, experimentally verified to form ericoid mycorrhizae with various ericaceous plants *in vitro* (Pearson & Read 1973) and subsequently, upon production of apothecia, described as *Pezizella ericae* (Read 1974). Later, the species was transferred to *Hymenoscyphus* (Kernan & Finocchio 1983) with some hesitation considering the thin and delicate nature of the excipular tissue that is absent in other members of *Hymenoscyphus*, and it was compared to morphologically similar *H. monotropae* associated with roots of *Monotropa uniflora*. Eventually, Zhang & Zhuang (2004) excluded *H. ericae* and *H. monotropae* from that genus and introduced *Rhizoscyphus* based on phylogenetic evidence from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) gene and different ecology

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(plant-associated biotrophic lifestyle). Apart from molecular sequencing and ecology, *Rhizoscyphus*, typified by *R. ericae*, is delimited from *Hymenoscyphus* by discoid apothecial ascomata with or without short hyphal protrusions on the receptacle surface, filiform paraphyses, inoperculate 8-spored asci with an amyloid apical ring and usually ellipsoidal to fusoid, hyaline, aseptate ascospores. However, this treatment did not last long when Baral & Krieglsteiner (2006) proposed a combination of *R. ericae* in *Pezoloma* (Clements 1909), a heterogeneous and broadly circumscribed genus of inoperculate discomycetes, based on similarities in the ascoma and ascus morphology and putative mycorrhizal life-style of *Pezoloma griseum* (Clements 1911), the type species.

The sexual-asexual connection of *R. ericae* with *Scytalidium vaccinii* (Dalpé *et al.* 1989) was first suggested by Egger & Sigler (1993) based on comparison of nrDNA genotypes of their ex-type strains. Subsequently, Hambleton *et al.* (1999) and Hambleton & Sigler (2005) experimentally confirmed that these species represent sexual and asexual morphs of the same organism. In their study, Hambleton & Sigler (2005) addressed the systematic placement of *R. ericae* and its close relationship with *C. finlandica* and *Meliniomyces* based on ITS and the nuclear ribosomal small subunit (nrSSU) sequences.

When compiling ITS sequences of the REA for a phylogenetic analysis of root mycobionts of *Gaultheria* (*Ericaceae*) from Argentine NW Patagonia (Bruzone *et al.* 2017), BLAST searches revealed sequences of *Hyaloscypha hepaticicola* that were nearly identical with *R. ericae* sequences. Furthermore, homologous sequences of *Hyaloscypha vitreola* (Baral *et al.* 2009), the lectotype species of the genus, and additional *Hyaloscypha* spp. retrieved from GenBank nested together with REA sequences in phylogenetic analyses. *Hyaloscypha* is an inoperculate discomycete (Boudier 1885, Huhtinen 1990) encompassing predominantly lignicolous fungi on bulky wood substrates, but some species can also fruit on herbaceous or arboreal litter and some occur on bryophytes. Their asexual morphs are largely unknown and have been experimentally proven for only a handful of species, and comprise hyphomycete genera such as *Cheiromycella*, *Clathrosphaerina*, *Monodictys*, *Pseudaegerita* and *Phialophora*-like fungi (Descals & Webster 1976, Abdullah & Webster 1983, Huhtinen 1990, Hosoya & Huhtinen 2002). *Hyaloscypha* is accommodated in the monotypic family *Hyaloscyphaceae s. str.* recently re-defined based on molecular DNA data (Han *et al.* 2014).

Hyaloscypha hepaticicola was described as *Trichopeziza hepaticicola* [as "*hepaticola*"], a mycobiont of the liverwort *Cephaloziella byssacea* from France (Grélet 1925) and was recently re-described based on numerous collections originating from Central and Northern Europe (Baral *et al.* 2009). It indeed mostly occurs in living parts of liverworts inhabiting moist places such as raw humus or decaying wood, which often share ecological niches with the *Ericaceae*. Its asexual morph is unknown. Based on the morphology of ascomata, asci and ascospores, *H. hepaticicola* is well comparable with *R. ericae* which was re-described by Hambleton *et al.* (1999) based on a Canadian collection. Although *R. ericae* is primarily connected with ericoid mycorrhiza and *H. hepaticicola* is mainly associated with bryophytes, their hosts often co-occur, and already Duckett & Read (1995) indicated that they can share fungal symbionts, namely the typical ErM fungus *R. ericae*. In fact, *R. ericae* was repeatedly isolated from rhizoids of the leafy liverwort *Cephaloziella* (Chambers *et al.* 1999, Upson *et al.* 2007, Kowal *et al.* 2016). However, despite their striking morphological similarities, shared ecological niches and the widely debated taxonomic status of *R. ericae*, to our knowledge these species have never been directly compared except for a note in Jaklitsch *et al.* (2015) who listed *Rhizoscyphus* as a synonym of *Hyaloscypha* and *Meliniomyces* among asexual morphs of the latter genus but without any justification, apparently based on unpublished or GenBank data, and one intriguing note in the CBS database (accessed 20/02/2018) regarding *H. hepaticicola* CBS 126283, which reads "close to *Rhizoscyphus ericae*".

During a survey of root mycobionts of montane plants in the Bohemian Forest National Park in the Czech Republic, two fungal strains were isolated from ectomycorrhizae of a *Picea abies* seedling. They formed sterile mycelium *in vitro* and their identical ITS sequences suggested relationship with *Meliniomyces* spp., but formed an isolated and well-supported lineage indicative of a new species (Vohník *et al.* 2013). Additionally, during the preparation of this study, a culture of *Meliniomyces bicolor* stored over a prolonged period at 6 °C started to form hyaline conidia on phialides on a penicillate conidiophore; sporulation has never been observed before for any species of *Meliniomyces*.

In order to confirm and further elaborate the preliminary results based on ITS phylogeny, we sequenced additional, commonly used markers, *i.e.* the nuclear ribosomal large subunit (nrLSU), the mitochondrial ribosomal short subunit (mtSSU) and the DNA-directed RNA polymerase II core subunit RPB2 (regions 5–7 and 7–11) for the type species and other members of the REA, and subjected them to phylogenetic analyses with homologous sequences of *Hyaloscypha* spp. Conversely, we generated ITS sequences of additional strains of *Hyaloscypha* spp. obtained from public collections. Furthermore, strains of *H. hepaticicola*, *R. ericae* and one isolate of the unknown sterile fungus from the Czech Republic were tested for their ErM potential in an ericaceous host.

Despite the turbulent taxonomic history of *R. ericae*, the use of "*Rhizoscyphus ericae* aggregate" (cf. Hambleton & Sigler 2005) and the related abbreviation "REA" is retained throughout the paper to avoid confusion regarding its several names.

MATERIALS AND METHODS

Fungal strains and herbarium material

Herbarium material of *H. hepaticicola* and *H. vitreola*, and living cultures of *Meliniomyces bicolor* and *Meliniomyces* sp. were examined with an Olympus SZX12 dissecting microscope (Olympus America, Inc., Melville, USA). Ascomata were rehydrated with water; asci, ascospores and paraphyses, conidiophores and conidia from living cultures were mounted in water, 90 % lactic acid, Melzer's reagent or Lugol's iodine. All measurements were made in Melzer's reagent. Means ± standard deviation (SD) based on 20–25 measurements are given for dimensions of conidiogenous cells and conidia. Microscopic structures were examined using an Olympus BX51 compound microscope with differential interference contrast (DIC) and phase contrast (PC) illumination. Images of microscopic structures and macroscopic images of colonies were captured with an Olympus DP70 camera operated by Imaging Software CellD (Olympus) and QuickPhoto Micro 2.3 software (Promicra Ltd., Czech Republic). All images were

processed with Adobe Photoshop CS6 (Adobe Systems, San Jose, USA).

Cultures were maintained on Modified Leonian's agar (MLA) (Malloch 1981), Modified Melin-Norkrans agar (MMN) (Marx 1969) and MMN2 (MMN without malt extract with 1/2 glucose concentration; Vohník unpubl.). For comparative purposes, strains were grown on MLA, malt-extract agar (MEA, Oxoid), potato-carrot agar (PCA) (Gams *et al.* 1998) and potato-dextrose agar (PDA, Oxoid). Descriptions of colonies are based on 28-d-old cultures. Grown, ca. 6-wk-old cultures on MMN or MMN2 were kept in a low temperature incubator (6 °C). Ex-type and other cultures are maintained at the Westerdijk Institute (CBS), Utrecht, the Netherlands, the Culture Collection of Fungi (CCF) at the Department of Botany, Charles University, Prague, Czech Republic, and University of Alberta Microfungus Herbarium and Culture Collection (UAMH), Edmonton, Canada. Type and other herbarium material are deposited in the Herbarium of the Institute of Botany (PRA), Průhonice, Czech Republic.

Re-synthesis experiment

The ErM potential of *H. hepaticicola* CBS 126291, *H. bicolor* CBS 144009, *H. melinii* CBS 143705 and *R. ericae* UAMH 6735 was tested in an *in vitro* re-synthesis with *Vaccinium myrtillus* (*Ericaceae*) seedlings. The experimental setup followed the soil agar re-synthesis described in Vohník *et al.* (2012). In brief, the fungi were pre-cultivated on MMN at room temperature in the dark for two months. *Vaccinium* seeds of local origin were extracted from dried fruits, surface sterilized with 10 % SAVO (common household bleach, Unilever ČR Ltd., Czech Republic; 100 % SAVO contains 47 g/kg, *i.e.* 4.7 % NaClO) for 60 s and then 3-times washed in sterile de-ionized water. Subsequently, they were placed in 25-compartment square plastic Petri dishes on the surface of solidified autoclaved MMN adjusted as follows: no maltose, 1 g/L glucose, 50 µg/L Novobiocin added to suppress possible bacterial growth. The dishes were sealed with air-permeable foil and incubated in a growth chamber under a 21/15 °C – 16/8 h day/night regime for 85 d.

The cultivation substrate consisted of peat (AGRO CS Corp., Czech Republic) + perlite (Perlit Ltd., Czech Republic) mixed 1 : 1 (v/v), passed through a 3.15 mm sieve, moistened with tap water and autoclaved 2-times after 24 h (60 min at 121 °C). The autoclaved substrate was confirmed sterile by plating on maltose extract agar. Approximately 6 g of the substrate (dry weight) were placed in the lower part of each square 12 × 12 cm plastic Petri dish and 16 ml of molten 0.8 % water agar amended with 0.1 % activated charcoal were pipetted over its surface. Mycelial plugs (ca. 5 mm in diam) from the fungal cultures (see above) were placed on the surface of the solidified agar/cultivation substrate (three plugs per dish) and two seedlings were transferred to each dish so that their roots were in contact with the plugs; non-inoculated control dishes contained plugs without mycelium. Roots of the seedlings were then covered with a thin layer of the substrate and a piece of moistened filter paper (Whatman International Ltd., UK) autoclaved as above. The dishes were sealed with air-permeable foil, inserted in open transparent plastic sacs and incubated in the growth chamber under the same regime as described above. There were three Petri dishes (*i.e.* altogether six plants) per each inoculation variant + control.

The seedlings were harvested after 3.5 mo and treated as in Vohník *et al.* (2016), *i.e.* the roots were separated from shoots, gently washed with running tap water, subsequently cleared in 10 % KOH at 121 °C for 15 min, rinsed in water, acidified for 20 s in 3 % HCl, rinsed in water and placed on glass slides in 0.05 % trypan blue solution in lactoglycerol (lactic acid : glycerol : de-ionized water in a mixing ratio of 1 : 1 : 3). The slides were observed using an Olympus BX60 upright compound microscope equipped with DIC at 400× and 1 000× magnification. Photographs of fungal colonisation were taken using an Olympus DP70 camera, modified for clarity as needed in Paint.NET 4.0.13 (dotPDN LLC, Rick Brewster and contributors) and assembled in Adobe Photoshop CS6.

Selection of molecular markers, dataset completion, and new material

Initially, we assessed the availability of sequence data for the ITS region, which is commonly used for fungal identification at species level and represents the standard molecular marker for phylogenetic analyses of the REA. A large amount of sequence data was available for both the REA and *Hyaloscypha* spp. For the latter, additional cultures were obtained from CBS, and the ITS region was sequenced for some further species, especially those not yet represented in GenBank, *i.e. H. epiporia*, *H. alniseda* (as *H. fuckelii* var. *alniseda*), and *H. herbarum*.

In order to place the REA into the *Leotiomycetes* classification and phylogenetic context, we investigated sequence availability for markers commonly used in fungal systematics, namely nrLSU, nrSSU, mtSSU, *rpb*2, and beta-tubulin. Beta-tubulin data were available for only five *Hyaloscypha* spp. in GenBank, and none for the REA; the marker was therefore dismissed. For nrSSU, several sequences of both *Hyaloscypha* and REA spp. were available, but the sequence variation was too low to resolve relationships (<1 % *p*-distance) or even to ascertain correct species identification; this marker was therefore dismissed as well. For nrLSU, a few REA sequences and many sequences of *Hyaloscypha* spp. were available in GenBank. For mtSSU and *rpb*2, only *Hyaloscypha* spp., but no representatives of the REA were available. Species availability and sequence variation among *Hyaloscypha* spp. for nrLSU, mtSSU and *rpb*2 were appropriate for phylogenetic analysis; these markers were therefore used further. The majority of these data are from Baral *et al.* (2009), the first molecular phylogenetic study focused on genus *Hyaloscypha*, and from Han *et al.* (2014), a phylogenetic study including a broad sampling of the *Hyaloscyphaceae s. lat*. These author teams used a largely overlapping set of molecular markers, but different regions of the *rpb*2 gene. In order to include *Hyaloscypha* taxa from both studies for this gene and to supplement the datasets, we obtained samples of the type material of members of the REA from UAMH and CBS and generated sequences of both *rpb*2 regions (5–7 and 7–11) as well as for nrLSU and mtSSU. Novel nrLSU, mtSSU and *rpb*2 sequence data of the unknown sterile fungus from the Czech Republic and all markers of the sporulating *M. bicolor* were also generated and added to the phylogenetic analyses.

DNA isolation, PCR and sequencing for nrLSU, mtSSU and *rpb*2

For the REA type material, the unknown sterile isolate and the sporulating culture of *M. bicolor*, DNA was isolated according to a sorbitol extraction protocol (Štorchová *et al.* 2000) except that

fungal cultures were used as starting material. PCRs were done using the Combi PPP Master Mix with hot start polymerase (Top-Bio, Vestec, Czech Republic) in reaction volumes of 25 µL that contained 13.5 µL of Combi PPP Master Mix, 5–10 ng of DNA template and 0.5 µM of each primer. All cycling conditions consisted of 35 cycles with 95 °C for 5 min for predenaturation and 72 °C for 10 min for final extension and were done on a Mastercycler gradient (Eppendorf Czech & Slovakia, Říčany u Prahy, Czech Republic). The nrLSU region was amplified using primers LR0R (Cubeta *et al.* 1991) and LR7 (Vilgalys & Hester 1990) at an annealing temperature of 52 °C. For amplification of the mtSSU region, primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) at an annealing temperature of 50 °C were used. Cycling conditions for both markers were 95 °C for 1 min denaturation, 1 min for annealing, and 72 °C for 2 min extension. Two non-overlapping parts of the *rpb2* gene were amplified: Region 5–7 used by Han *et al.* (2014) was amplified with primers fRPB2-5F (Liu *et al.* 1999) and fRPB2-P7R (Hansen *et al.* 2005), and region 7–11 used by Baral *et al.* (2009) was amplified with primers fRPB2-7cF and fRPB2-11aR (Liu *et al.* 1999). Cycling conditions for both *rpb2* regions were 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min. Amplification products were checked on 1 % agarose gels, purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced in both directions with the PCR primers (GATC Biotech, Konstanz, Germany). Forward and reverse sequence reads were edited manually in Chromas v. 1.45 (McCarthy 1996–1998) and aligned in Bioedit v. 7.1.8 (Hall 1999).

General sequence data treatment and phylogenetic analyses

Sequences from GenBank were compiled as outlined below for the individual datasets; alignments were done using Bioedit (Hall 1999) with manual improvement of indels, especially for ITS and mtSSU. Only those parts of the molecular markers showing a reasonably large overlap of sequences generated by different authors were used. Sequences with poor reads at beginnings or ends according to the alignment (*e.g.* N's, single base indels, unlikely substitution patterns in coding regions) that most probably represent artefacts were trimmed to retain only supposedly reliable parts of the sequence or were entirely omitted. Each dataset was analysed separately to identify the most appropriate data treatment and outgroup combination. Besides, trees produced with different markers were compared to identify potentially wrongly assigned names or other pitfalls that might lead to problems in combined data analyses. Based on these tests, unreliable or erroneous sequences were excluded; these are indicated below for the particular markers. GenBank accession numbers for ITS, nrLSU, mtSSU, and *rpb2* sequences (MH018926–MH018960) generated during this study and homologous sequences of representatives of *Hyaloscypha* and other members of the *Leotiomycetes* retrieved from GenBank are listed in Table 1. The final alignments used for phylogenetic analyses and the Bayesian trees on which Figs 1–5 are based were submitted to TreeBASE (TB2:S22490).

For all datasets, Bayesian analysis (BA), Maximum Parsimony (MP) and Maximum Likelihood (ML) approaches were used for phylogenetic tree construction using MrBayes v. 3.1.2 or v. 3.2.2 (Ronquist & Huelsenbeck 2003), PAUP v. 4.0b10 (Swofford 2002) and MEGA v. 6.06 (Tamura *et al.* 2013), respectively. For each dataset, at first, the model of molecular evolution best fitting the data was determined using Modeltest v. 3.5 (Posada & Crandall 1998). The basic model parameters, *i.e.* the distribution of rates among sites and the number of different substitution rates, were used as priors for BA; apart from that, the default settings were used. Chains were computed for several million generations (depending on dataset, see below), sampling every 1 000th tree, until all indicators suggested that convergence between the different runs was achieved. The first 25 % of the trees per run were discarded as burn-in and the remaining trees were summarized. MP analyses were done as heuristic searches with 100 random addition sequence replicates and TBR branch swapping, saving no more than 100 trees with length $\geq$ 1 per replicate, automatically increasing the maximum number of trees saved. Bootstrapping was performed using the same settings and 1 000 replicates, but without branch swapping. ML analyses were done using the substitution model found by the Akaike Information Criterion in Modeltest. All models found optimal for particular datasets suggested gamma distribution of rates among sites with a proportion of invariant sites; six discrete gamma categories were specified for ML analyses. In MEGA, all sites, extensive subtree-pruning-regrafting and very strong branch swap filter were used; branch support was assessed with 1 000 bootstrap replicates. Details for particular datasets are given below.

Compilation of the ITS dataset

A representative selection of sequences of the REA from Bruzone *et al.* (2017) was used as a starting point and supplemented by additional sequences retrieved from GenBank (Table 1). Sequences of the type material for all described REA species were included. Of the three identical sequences of the type strain of *R. ericae* present in GenBank under genera *Rhizoscyphus* (AY762620), *Hymenoscyphus* (AJ319078) and *Pezoloma* (NR_111110), only one was used for tree construction. For comparison with previously published phylogenies of the aggregate, we included sequences of subclades 1–5 from Vrålstad *et al.* (2002) and *Meliniomyces* sp. 1–3 from Hambleton & Sigler (2005); the sequence of *Meliniomyces* sp. 4 (AJ430176), which we identified as a chimera between *R. ericae* (ITS1) and *Cadophora luteoolivacea* (ITS2), was excluded. Two sequences of epacrid root endophytes forming a sister clade to *R. ericae* in Hambleton & Sigler (2005) were also included, one of them (AY279181) suggested to be a new species in that paper.

Subsequently, ITS sequences of *Hyaloscypha* spp. were retrieved from GenBank and added manually to the alignment of the *R. ericae* aggregate. Additionally, we obtained cultures of all named *Hyaloscypha* spp. available in public collections (CBS and UAMH) and sequenced the ITS region for species not yet present in GenBank and also for the sporulating culture of *M. bicolor* as described in Vohník *et al.* (2013). The ITS sequence from the type material of *Scytalidium vaccinii* (Egger & Sigler 1993), the asexual morph of *R. ericae*, was also included. Furthermore, ITS sequences of *Hyaloscypha* spp. were subjected to BLAST searches to identify highly similar sequences of unidentified fungi isolated from roots for which information about the host plant was available. Sequences with close matches to *Hyaloscypha* spp. were also included in phylogenetic tree construction.

Table 1. Taxa and GenBank accession numbers used in this study. Original *Hyaloscypha* species are listed alphabetically; REA taxa of known species are listed according to their placement in the ITS tree (Fig. 1), followed by unidentified REA strains not assignable to known species; outgroup taxa are given at the end. Ex-type strains are indicated by asterisks (*). References (if from more than one study) are given in the same order as the accession numbers they refer to. If a sequence is used in several papers, all references are given. For accession numbers of unpublished studies, the name of the submitter and the year are indicated; the reference is indicated as unpublished in the table, but not listed in the References of the main text. A reference for the sequence of *H. vitreola* (JX981495) is incorrectly cited in GenBank (as Pawlowska *et al.* 2014).

Taxon	Source/type	ITS	nrLSU	mtSSU	*RPB2* (5–7)	*RPB2* (7–11)	Reference
Hyaloscypha albohyalina	TNS-F17137	JN033431	JN086734	JN086799	JN086874		Han *et al.* (2014)
	TNS-F11213	JN033437	JN086738	JN086807	JN086882		Han *et al.* (2014)
	TNS-F17333	AB546939	AB546938				Hosoya *et al.* (2011)
H. alniseda	CBS 123.91	MH018930					This study
H. aureliella	KUS-F52070	JN033394	JN086697	JN086771	JN086848		Han *et al.* (2014)
	TNS-F11209	AB546942	AB546943	JN086804	JN086879		Hosoya *et al.* (2011), Han *et al.* (2014)
	CBS 126298 (as M234)	MH018926	EU940152	EU940292		EU940361	This study, Stenroos *et al.* (2010)
	M235	JN943610	EU940153	EU940293		EU940362	Schoch *et al.* (2012), Stenroos *et al.* (2010)
	olrim148	AY354244					Lygis *et al.* (2004)
H. cf. bulbopilosa	TNS-F18073	JN033451	JN086751	JN086822	JN086897		Han *et al.* (2014)
	KUS-F52573	JN033423	JN086726	JN086793	JN086867		Han *et al.* (2014)
H. daedaleae	CBS 120.91	MH018927					This study
	CBS 121.91	MH018928					This study
	ZW-Geo138-Clark	AY789416	AY789415				Wang *et al.* (2005)
H. epiporia	CBS 125.91	MH018929					This study
H. fuckelii	CBS 126292 (as M233)	EU940230	EU940154	EU940294		EU940363	Baral *et al.* (2009), Stenroos *et al.* (2010)
H. hepaticicola	CBS 126283 (as M171)	EU940194	EU940118	EU940266		EU940330	Baral *et al.* (2009), Stenroos *et al.* (2010)
	CBS 126291 (as M339)	EU940226	EU940150	EU940290		EU940359	Baral *et al.* (2009), Stenroos *et al.* (2010)
H. herbarum	CBS 126.91	MH018931					This study
H. minuta	G.M. 2015-04-06.2		KY769526				Marson (2017), unpublished
H. monodictys	TNS-F5013	JN033456	JN086756	JN086832	JN086906		Han *et al.* (2014)
H. spiralis	TNS-F31133	AB546941	AB546940				Hosoya *et al.* (2011)
	KUS-F52652	JN033426	JN086729	JN086795	JN086870		Han *et al.* (2014)
	TNS-F17909	JN033440	JN086741	JN086810	JN086885		Han *et al.* (2014)
H. vitreola	CBS 127.91	JN033378	JN086681	JN086758	JN086834		Han *et al.* (2014)
	M220	FJ477059	FJ477058				Baral *et al.* (2009)
	CBS 126276 (as M39)	EU940231	EU940155	EU940295		EU940364	Baral *et al.* (2009), Stenroos *et al.* (2010)
	CBS 126275 (as M236)	EU940232	EU940156	EU940296			Baral *et al.* (2009), Stenroos *et al.* (2010)
	WA0000019123	JX981495					Pawlowska *et al.* (2014)
Hyaloscypha sp.	2-13c	KC790474					Long *et al.* (2013)
	TNS-F17694	JN033450	JN086750	JN086821	JN086896		Han *et al.* (2014)
	TNS-F17350	JN033434	JN086737	JN086803	JN086878		Han *et al.* (2014)
	TNS-F31287	JN033454	JN086754	JN086825	JN086900		Han *et al.* (2014)
	TNS-F17335	JN033432	JN086735	JN086801	JN086876		Han *et al.* (2014)
	M288	JN943609	EU940144	EU940284		EU940354	Schoch *et al.* (2012), Stenroos *et al.* (2010)

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Table 1. (Continued).

Taxon	Source/type	ITS	nrLSU	mtSSU	*RPB2* (5–7)	*RPB2* (7–11)	Reference
	M20	JN943608	EU940093	EU940245		EU940309	Schoch *et al.* (2012), Stenroos *et al.* (2010)
	M25	JN943607	EU940096	EU940248			Schoch *et al.* (2012), Baral *et al.* (2009), Stenroos *et al.* (2010)
	M19	JN943606	EU940092	EU940244		EU940308	Schoch *et al.* (2012), Baral *et al.* (2009), Stenroos *et al.* (2010)
Cadophora finlandica	CBS 444.86 Isotype*	NR_121279	MH018941	MH018934	MH018948	MH018954	Grünig *et al.* (2002), this study
	PRF15	DQ485204					Gorfer *et al.* (2009)
	B54J12	EF093155					Vohník *et al.* (2013)
	FAG 15	AF011327					Saenz & Taylor (1999)
	ARON 2948.S	AJ292202					Vrålstad *et al.* (2000, 2002)
	IFM 50530		AB190423				Fukushima *et al.* (2004), unpublished
Meliniomyces bicolor	CBS 116122, UAMH 10107 Type*	AJ430147	MH018942	MH018935	MH018949	MH018955	Vrålstad (2001), Vrålstad *et al.* (2002), this study
	CBS 144009	MH018932	MH018943	MH018936		MH018956	This study
	ARSL 180907.22	HQ157926					Kernaghan & Patriquin (2011)
	CBS 116123	AJ292203					Vrålstad *et al.* (2000, 2002)
	ARON 2810.S	AJ308340					Vrålstad (2001), Vrålstad *et al.* (2002)
	C51.7	KX611538					Bruzone *et al.* (2017)
	ARON2965.S	AJ430122					Vrålstad (2001), Vrålstad *et al.* (2002)
	MBI-1	EF093180					Vohník *et al.* (2013)
	NY077	KM216335					Prihatini *et al.* (2016)
	LVR4069	AY579413					Villarreal-Ruiz *et al.* (2004)
M. variabilis	UAMH 8861 Type*	AY762619	MH018944	MH018937	MH018950	MH018957	Hambleton & Sigler (2005), this study
	MV-S-4	EF093166					Vohník *et al.* (2013)
	ARON 2879.S	AJ292201					Vrålstad *et al.* (2002)
	LF1GA16D9	JQ272355					Baird *et al.* (2014)
M. vraolstadiae	CBS 116126, UAMH 10111 Type*	AJ292199	MH018945	MH018938	MH018951	MH018958	Vrålstad *et al.* (2002), this study
	UAMH 11203	MH018933					This study
	CBS 116127, ARON2917.S	AJ292200					Vrålstad *et al.* (2002)
	ARSL 070907.12	HQ157928					Kernaghan & Patriquin (2011)
	ARSL 230507.46	HQ157836					Kernaghan & Patriquin (2011)
	FG34P1	FN678887					Grelet *et al.* (2010)
H. melinii sp. nov.	SM7-2, CBS 143705 Type*	EF093175	MH018946	MH018939	MH018952	MH018959	Vohník *et al.* (2013), this study
	SM7-1	EF093174					Vohník *et al.* (2013)
Meliniomyces sp.	ECRU075	KM678388					Bizabani (2015)
	GMU_LL_03_G4	KC180693					Bruzone *et al.* (2015)
Rhizoscyphus ericae	UAMH 6735 Type*	NR111110	MH018947	MH018940	MH018953	MH018960	Vrålstad *et al.* (2002), this study
	ARON 3024.S	AJ430126					Vrålstad (2001)
	ARON 2888.S	AJ308337					Vrålstad (2001), Vrålstad *et al.* (2002)
	Isolate 21	AF069439					Chambers *et al.* (1999)
	UAMH 8680	AY762622					Hambleton & Sigler (2005)
	C43.4	KX611525					Bruzone *et al.* (2017)

Table 1. (Continued).

Taxon	Source/type	ITS	nrLSU	mtSSU	*RPB2* (5–7)	*RPB2* (7–11)	Reference
	D. J. Read 100	AF151089					McLean *et al.* (1999)
	pkc29		AY394907				Lim et al. (2003), unpublished
	strain 111		AM887699				Turnau *et al.* (2007)
	UBCM8	AF081435					Monreal *et al.* (1999)
Scytalidium vaccinii	UAMH 5828 Type*	AF081439					Monreal *et al.* (1999)
Ericoid endophyte	GU32	AF252837					Sharples et al. (2000), unpublished
Fungal sp.	3.44.4J	KJ649999					Sarjala et al. (2014), unpublished
Calluna vulgaris root associated	Fungus agrKH180	FM172867					Pietrowski et al. (2008), unpublished
Epacris microphylla root associated	Fungus 13	AY268197					Williams *et al.* (2004)
Epacrid root endophyte	RK1-11	AY279179					Williams *et al.* (2004)
Epacrid root endophyte	RK2.4	AY279181					Williams *et al.* (2004)
cf. *H. ericae* agg.	ARON 3014.S	AJ430121					Vrålstad (2001), Vrålstad *et al.* (2002)
Salal root associated fungus	UBCtra264	AF149070					Millar et al. (1999), unpublished
Amicodisca castaneae	KUS-F51377	JN033389	JN086692	JN086766	JN086843		Han *et al.* (2014)
Arachnopeziza aurata	KUS-F52038		JN086696	JN086770	JN086847		Han *et al.* (2014)
A. aurelia	TNS-F11211		AB546937	JN086805	JN086880		Hosoya *et al.* (2011), Han *et al.* (2014)
A. delicatula	TNS-F12770		JN086736	JN086802	JN086877		Han *et al.* (2014)
A. obtusipila	TNS-F12769		JN086747	JN086816	JN086891		Han *et al.* (2014)
Ascocoryne cylichnium	KUS-F52351		JN086709	JN086782			Han *et al.* (2014)
A. sarcoides	AFTOL-ID 1834		FJ176886		FJ238369		Schoch *et al.* (2009)
Chloridium paucisporum	CBS 445.86 Type*	EU938675					Alberton *et al.* (2010)
Coleophoma cylindrospora	CBS 592.70	KU728487					Crous & Groenewald (2016)
C. cylindrospora	CBS 591.70	KU728486					Crous & Groenewald (2016)
Cudoniella clavus	AFTOL-ID 166	DQ491502	DQ470944	FJ713604	DQ470888	DQ470888	Spatafora *et al.* (2006), Schoch *et al.* (2009)
Cyathicula microspora	M267	EU940165	EU940088	EU940240		EU940304	Baral *et al.* (2009), Stenroos *et al.* (2010)
Dematioscypha delicata	TNS-F17834	JN033438	JN086739	JN086808	JN086883		Han *et al.* (2014)
Hyalopeziza leuconica	KUS-F52474		JN086719				Han *et al.* (2014)
H. nectrioidea	CBS 597.77		JN086684	JN086761	JN086836		Han *et al.* (2014)
Hyalopeziza sp.	TNS-F17879		JN086740	JN086809	JN086884		Han *et al.* (2014)
"H." aff. paludosa	M229		EU940138	EU940281		EU940350	Stenroos *et al.* (2010)
"H." aff. paludosa	M228		EU940137	EU940280			Stenroos *et al.* (2010)
"H." aff. paludosa	M178		EU940121	EU940269		EU940333	Stenroos *et al.* (2010)
"H." aff. paludosa	M132		EU940103	EU940255		EU940319	Stenroos *et al.* (2010)
Hymenoscyphus caudatus	KUS-F52291	JN033402	JN086705	JN086778	JN086856		Han *et al.* (2014)
H. fructigenus	M159		EU940157				Baral *et al.* (2009), Stenroos *et al.* (2010)

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Table 1. (Continued).

Taxon	Source/type	ITS	nrLSU	mtSSU	*RPB2* (5–7)	*RPB2* (7–11)	Reference
H. monotropae	CC 19-47	KF359569					Baird *et al.* (2014)
H. monotropae	S8	KJ817283					Yang & Yan (2014), unpublished
H. monotropae	ATCC 52305	AF169309					Bills *et al.* (1999)
H. monotropae	PP_S1_1_270_1	JX630593					Timling *et al.* (2012)
Hyphodiscus hymeniophilus	MUCL 40275		DQ227258				Untereiner *et al.* (2006)
H. otanii	TNS-F7099	AB546949	AB546947	JN086827	JN086902		Hosoya *et al.* (2011), Han *et al.* (2014)
H. theiodeus	TNS-F32000, TNS-F31803	AB546953	AB546952	JN086828	JN086903		Hosoya *et al.* (2011), Han *et al.* (2014)
Mollisia cinerea	AFTOL-ID 76				DQ470883	DQ470883	Spatafora *et al.* (2006)
Proliferodiscus sp.	TNS-F17436		JN086752	JN086823	JN086898		Han *et al.* (2014)
Proliferodiscus sp.	KUS-F52660		JN086730	JN086796	JN086871		Han *et al.* (2014)
Pseudaegerita viridis	ICMP 15542	EF029235					Cooper et al., unpublished
P. viridis	GMU_LL_03_A3	KC180694					Bruzone *et al.* (2015)
P. corticalis	ICMP 15324	EF029224					Cooper et al., unpublished
P. corticalis	ICMP 15046	EF029214					Cooper et al., unpublished
P. corticalis	ICMP 14614	EF029194					Cooper et al., unpublished
P. corticalis	ICMP 14387	EF029188					Cooper et al., unpublished
P. corticalis	NBRC 102375		AB646520				Yamagushi *et al.* (2012)
P. corticalis	NBRC 108037		AB646521				Yamagushi *et al.* (2012)

In addition, we included four available sequences of *Pseudaegerita corticalis* (Cooper *et al.*, unpubl.), an asexual morph of *Hyaloscypha spiralis* (Abdullah & Webster 1983) and two sequences of *Pseudaegerita viridis* (Cooper *et al.*, unpubl., Bruzone *et al.* 2017) along with highly similar sequences of *Coleophoma cylindrospora* (Crous *et al.* 2014). Four sequences of *Hymenoscyphus monotropae* from different studies were added because of the morphological similarity to *R. ericae*. A sequence of the type strain of *Chloridium paucisporum* (Alberton *et al.* 2010) morphologically similar to *Cadophora finlandica* was included for comparison. Other taxa considered to be members of *Hyaloscypha* were either not available in GenBank or their ITS sequences were too divergent to be alignable.

As outgroup, at first, a broad selection of the *Hyaloscyphaceae s. lat.*, for which ITS sequences were available, was chosen based on Han *et al.* (2014) and Baral *et al.* (2009). Genera that were too divergent or could only be aligned with considerable ambiguity in some parts of ITS1 (*e.g. Proliferodiscus*, *Arachnopeziza*, *Hyalopeziza*, *Bryoglossum* etc.) were subsequently discarded. After preliminary analyses in which we tested various outgroup combinations and their effect on ingroup topology and stability, a selection of seven species representing six genera (*Amicodisca*, *Cudoniella*, *Cyathicula*, *Dematioscypha*, *Hymenoscyphus* and *Hyphodiscus*) was found most appropriate and used for phylogenetic analysis of this marker.

Fine-tuning of the sampling and phylogenetic analysis of the ITS dataset

In preliminary analyses, one sequence (KJ663835) of *Hyaloscypha* sp. (CBS 109453) that clustered with other species of the genus revealed an unusually long branch in ML analyses, and parallel runs of BA did not converge after a reasonable number of generations. This sample was labelled *incertae sedis* by its authors (Crous *et al.* 2014) and omitted from further analysis, also for the nrLSU dataset (KJ663875). Another "*Hyaloscypha* sp." sequence from an unpublished study (KC790474) clustered among outgroup taxa; its exclusion or inclusion did not affect tree construction, and the sample was therefore maintained although it appeared to be misidentified. Two accessions of *H. aureliella* (M234, M235) contained an intron in the 3′ part of the 18S rDNA gene; the intron was deleted. One sequence (EU940227) of "*H. albohyalina* var. *spiralis*" (M259) was very different from those of other accessions of the same species. Han *et al.* (2014) revised this taxon, which comprises two genetically distant lineages, as either *H. albohyalina* or *H. spiralis*. Of both species, several highly similar accessions were available; the questionable sequence did not correspond to any of these and was excluded as a likely misidentification. Finally, the sequence (AY354244) of "*Hymenoscyphus*" sp. (olrim148) (Lygis *et al.* 2004) is a reverse complement of the ITS region; it was included in the right orientation with the addition "rc".

The resulting ITS dataset consisted of 103 taxa and 630 aligned characters; of these, 61 variable characters were uninformative, and 203 characters were parsimony informative. Preliminary tests showed that indels contained additional phylogenetic signal and generally resulted in increased branch support. Therefore, indel coding was performed for this dataset using FastGap v. 1.2 (Borchsenius 2009) based on the simple method of Simmons & Ochoterena (2000). The matrix consisted of 126 additional characters so that the final dataset including the matrix comprised 756 characters, of which 94 variable ones were

uninformative and 296 characters were parsimony informative. The model of molecular evolution most appropriate for the ITS dataset (excluding coded gaps) was a General Time Reversible (GTR) model with six substitution rates. For Bayesian analysis, 10 M generations were needed to reach convergence.

Compilation and phylogenetic analysis of the nrLSU dataset

The nrLSU dataset comprised the second largest selection of sequence data. In addition to material from Baral *et al.* (2009) and Han *et al.* (2014), three additional accessions and two additional species of *Hyaloscypha* were available for nrLSU (Table 1). Also, several accessions of *Hyaloscypha* aff. *paludosa* from Baral *et al.* (2009) for which no ITS data were available, were included in this and most further datasets. Two sequences of *Pseudaegerita corticalis* were available (Yamaguchi *et al.* 2012) and added as well. For the REA, one sequence of *C. finlandica* (AB190423) and two sequences of *R. ericae* (AM887699, AY394907), partly from unpublished studies, were included, because they clustered with the type material in preliminary analyses. Several sequences were excluded in order to avoid confusion, *i.e.* one strain attributed to *M. bicolor* (UAMH 10356) whose ITS region (AY394885, Lim *et al.,* unpubl.) fell into the range of variation of *C. finlandica*, one *Meliniomyces* sp. isolate (Me10_10MI10, KJ425314, Welc *et al.*, unpubl.), which probably also represents *C. finlandica* according to sequence similarity with the type, and one sample labelled *R. ericae* (EF658765, Upson *et al.* 2007), but with a sequence identical to that of the type material of *M. vraolstadiae*. New sequences of the REA type material, the sporulating culture of *M. bicolor* and the unknown sterile fungus were included.

A broad range of outgroup taxa were tested. In addition to those used for ITS, we also included several species of *Proliferodiscus*, *Arachnopeziza*, *Ascocoryne* and *Hyalopeziza*. One sample of *Arachnopeziza variepilosa* (EU940086, M337) from Baral *et al.* (2009) is probably misidentified, because its nrLSU sequence is identical to that of *Pezoloma cilifera* (see also Stenroos *et al.* 2010) and divergent from four other species of *Arachnopeziza*. Further outgroup taxa were tested, but eventually excluded, among them are: *Bryoglossum gracile* and nearly identical *Roseodiscus formosus*, which were too divergent according to preliminary analyses; *Mollisia cinerea*, which was very divergent and produced an unusually long branch that caused problems in the analyses, besides, the sequence produced unusual indels in the alignment and may contain mistakes; and one sequence of *Ascocoryne sarcoides* (AJ406399), which contained many polymorphisms.

The alignment was unambiguous, also for the outgroup. Several sequences contained a group I intron: one accession of *Hyaloscypha aureliella* (JN086697), one accession of *Ascocoryne sarcoides* (FJ176886), *Hyphodiscus hymeniophilus* (DQ227258) as well as the sporulating strain of *M. bicolor*. The introns were deleted, and the ongoing part of the sequence was used, if available. Further ingroup and outgroup species may also contain the intron, because in several samples that were included in the final alignment, the sequences ended at or near the insertion point.

The final dataset used for phylogenetic analyses consisted of 66 taxa and 1303 aligned characters. After alignment position 555, sequences of only 45 taxa continued; after position 840, sequences of only 31 taxa remained; missing ends were specified as missing data. The alignment contained very few indels, most of them 1 bp long; a single deletion of 3 bp was observed. Indels were not coded. Altogether, 55 variable characters were uninformative, and 125 characters were parsimony informative. A Tamura-Nei (TrN) model with six substitution rates was found most appropriate for the nrLSU dataset. For BA, 5 M generations were needed to reach convergence.

Compilation and analysis of the mtSSU dataset

The mtSSU dataset consisted almost exclusively of sequences from Baral *et al.* (2009) and Han *et al.* (2014). The only species additionally included in the final alignment was *Cudoniella clavus*. No REA sequences except the newly generated ones were available for mtSSU. The same ten genera as for nrLSU were used as outgroup. Further genera were initially tested as outgroup, but eventually dismissed: *Mollisia cinerea* (DQ976372) and *Lachnum* (AY544744, AY544745) were too divergent and caused many ambiguities in the alignment. One strain (TNS-F-17333) attributed to *Hyaloscypha albohyalina* clustered with other strains of that species with ITS and nrLSU (sequences from Hosoya *et al.* 2011, used also in Han *et al.* 2014), but was almost identical with *Hyaloscypha* sp. TNS-F-17335 for mtSSU (sequences only from Han *et al.* 2014). Both taxa are genetically very divergent from each other, and the sequence for mtSSU (JN086800) was dismissed to avoid artefacts in analyses of the combined dataset.

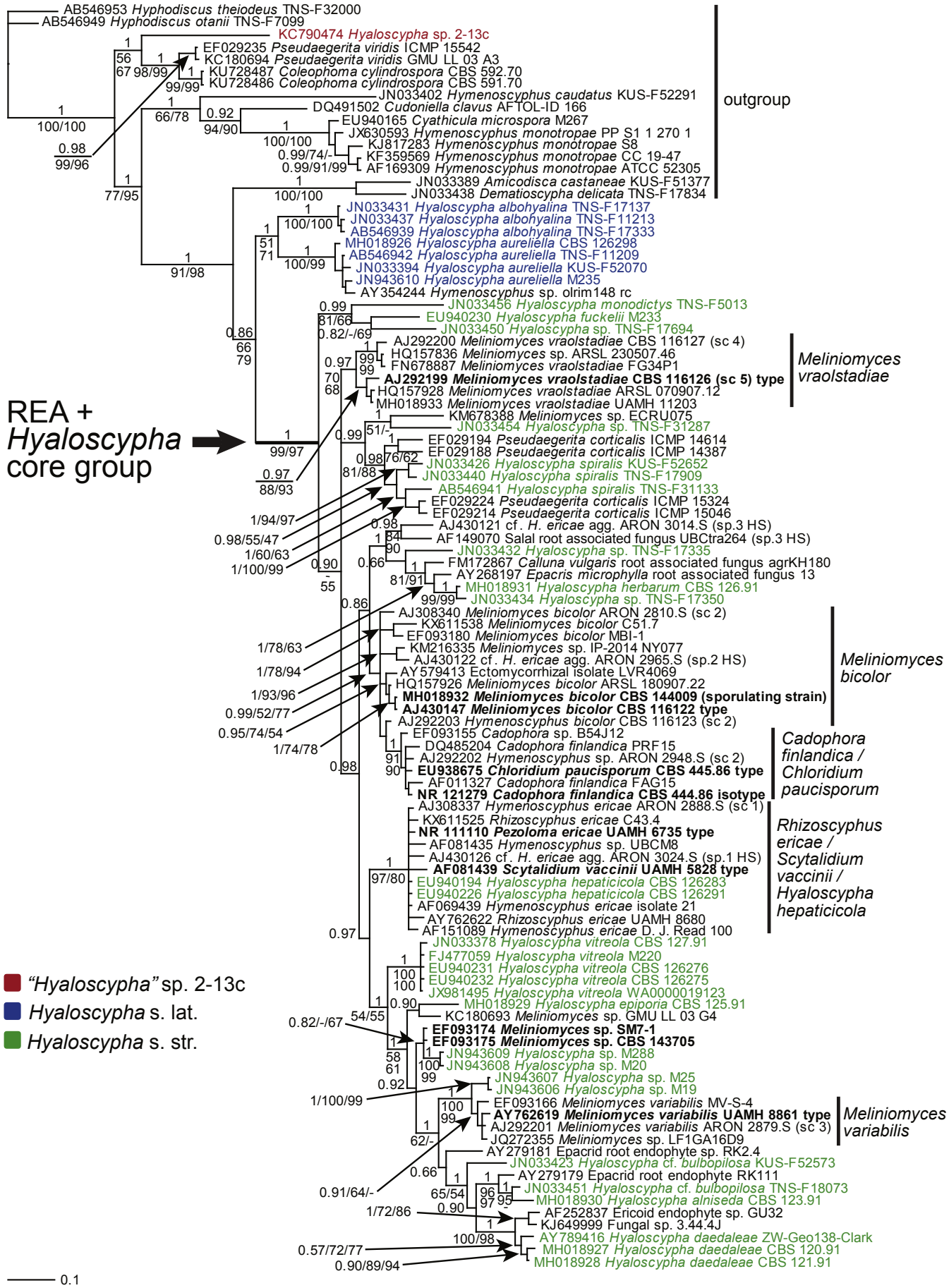
Generally, mtSSU sequences were difficult to align because of several long indel regions and relatively high variation. In order to be able to align any outgroup taxa at all, only the 3′ part of the amplified region could be used. Some outgroup samples that were fairly well alignable throughout this region were still too divergent at its beginning or end so that some unalignable sequence parts were deleted and treated as missing data. A unique insert or intron of *ca*. 160 bp in the sequence of *Hymenoscyphus caudatus* (JN086778) was also deleted. Some ambiguity remained in two indel regions, but it concerned only relationships among outgroup taxa and was considered tolerable; no indel coding was done for mtSSU in order not to amplify ambiguity.

The final dataset comprised 52 taxa and 826 aligned characters; 59 variable characters were uninformative, and 171 characters were parsimony informative. A transversion model (TVM) with six substitution rates was determined for the mtSSU dataset. As MEGA does not offer this model, for ML analyses, the model was replaced by a similar one (GTR). Bayesian analyses needed 2 M generations to converge.

Compilation and analysis of *rpb2*, regions 5–7

For regions 5–7 of the *rpb2* dataset, almost exclusively sequences from Han *et al.* (2014) were available (all *Hyaloscypha* spp., most outgroup taxa). The sequence of *H. albohyalina* strain TNS-F-17333 was again identical with TNS-F-17335 (like above for mtSSU), but not with *H. albohyalina* as with ITS and nrLSU; the *rpb2* sequence (JN086875) was dismissed although it is unclear if the strain was confused for ITS/nrLSU or for the other two markers. No sequences of the REA were available for this dataset; from the set of newly sequenced samples, the sporulating culture of *M. bicolor* did not yield a PCR product. As

ITS


AB546953 Hyphodiscus theiodeus TNS-F32000
AB546949 Hyphodiscus otanii TNS-F7099
KC790474 Hyaloscypha sp. 2-13c
EF029235 Pseudaegerita viridis ICMP 15542
KC180694 Pseudaegerita viridis GMU LL 03 A3
KU728487 Coleophoma cylindrospora CBS 592.70
KU728486 Coleophoma cylindrospora CBS 591.70
JN033402 Hymenoscyphus caudatus KUS-F52291
DQ491502 Cudoniella clavus AFTOL-ID 166
EU940165 Cyathicula microspora M267
JX630593 Hymenoscyphus monotropae PP S1 1 270 1
KJ817283 Hymenoscyphus monotropae S8
KF359569 Hymenoscyphus monotropae CC 19-47
AF169309 Hymenoscyphus monotropae ATCC 52305
JN033389 Amicodisca castaneae KUS-F51377
JN033438 Dematioscypha delicata TNS-F17834
outgroup
JN033431 Hyaloscypha albohyalina TNS-F17137
JN033437 Hyaloscypha albohyalina TNS-F11213
AB546939 Hyaloscypha albohyalina TNS-F17333
MH018926 Hyaloscypha aureliella CBS 126298
AB546942 Hyaloscypha aureliella TNS-F11209
JN033394 Hyaloscypha aureliella KUS-F52070
JN943610 Hyaloscypha aureliella M235
AY354244 Hymenoscyphus sp. olrim148 rc
REA + Hyaloscypha core group
JN033456 Hyaloscypha monodictys TNS-F5013
EU940230 Hyaloscypha fuckelii M233
JN033450 Hyaloscypha sp. TNS-F17694
AJ292200 Meliniomyces vraolstadiae CBS 116127 (sc 4)
HQ157836 Meliniomyces sp. ARSL 230507.46
FN678887 Meliniomyces vraolstadiae FG34P1
AJ292199 Meliniomyces vraolstadiae CBS 116126 (sc 5) type
HQ157928 Meliniomyces vraolstadiae ARSL 070907.12
MH018933 Meliniomyces vraolstadiae UAMH 11203
Meliniomyces vraolstadiae
KM678388 Meliniomyces sp. ECRU075
JN033454 Hyaloscypha sp. TNS-F31287
EF029194 Pseudaegerita corticalis ICMP 14614
EF029188 Pseudaegerita corticalis ICMP 14387
JN033426 Hyaloscypha spiralis KUS-F52652
JN033440 Hyaloscypha spiralis TNS-F17909
AB546941 Hyaloscypha spiralis TNS-F31133
EF029224 Pseudaegerita corticalis ICMP 15324
EF029214 Pseudaegerita corticalis ICMP 15046
AJ430121 cf. H. ericae agg. ARON 3014.S (sp.3 HS)
AF149070 Salal root associated fungus UBCtra264 (sp.3 HS)
JN033432 Hyaloscypha sp. TNS-F17335
FM172867 Calluna vulgaris root associated fungus agrKH180
AY268197 Epacris microphylla root associated fungus 13
MH018931 Hyaloscypha herbarum CBS 126.91
JN033434 Hyaloscypha sp. TNS-F17350
AJ308340 Meliniomyces bicolor ARON 2810.S (sc 2)
KX611538 Meliniomyces bicolor C51.7
EF093180 Meliniomyces bicolor MBI-1
KM216335 Meliniomyces sp. IP-2014 NY077
AJ430122 cf. H. ericae agg. ARON 2965.S (sp.2 HS)
AY579413 Ectomycorrhizal isolate LVR4069
HQ157926 Meliniomyces bicolor ARSL 180907.22
MH018932 Meliniomyces bicolor CBS 144009 (sporulating strain)
AJ430147 Meliniomyces bicolor CBS 116122 type
Meliniomyces bicolor
AJ292203 Hymenoscyphus bicolor CBS 116123 (sc 2)
EF093155 Cadophora sp. B54J12
DQ485204 Cadophora finlandica PRF15
AJ292202 Hymenoscyphus sp. ARON 2948.S (sc 2)
EU938675 Chloridium paucisporum CBS 445.86 type
AF011327 Cadophora finlandica FAG15
NR 121279 Cadophora finlandica CBS 444.86 isotype
Cadophora finlandica / Chloridium paucisporum
AJ308337 Hymenoscyphus ericae ARON 2888.S (sc 1)
KX611525 Rhizoscyphus ericae C43.4
NR 111110 Pezoloma ericae UAMH 6735 type
AF081435 Hymenoscyphus sp. UBCM8
AJ430126 cf. H. ericae agg. ARON 3024.S (sp.1 HS)
AF081439 Scytalidium vaccinii UAMH 5828 type
EU940194 Hyaloscypha hepaticicola CBS 126283
EU940226 Hyaloscypha hepaticicola CBS 126291
AF069439 Hymenoscyphus ericae isolate 21
AY762622 Rhizoscyphus ericae UAMH 8680
AF151089 Hymenoscyphus ericae D. J. Read 100
Rhizoscyphus ericae / Scytalidium vaccinii / Hyaloscypha hepaticicola
JN033378 Hyaloscypha vitreola CBS 127.91
FJ477059 Hyaloscypha vitreola M220
EU940231 Hyaloscypha vitreola CBS 126276
EU940232 Hyaloscypha vitreola CBS 126275
JX981495 Hyaloscypha vitreola WA0000019123
MH018929 Hyaloscypha epiporia CBS 125.91
KC180693 Meliniomyces sp. GMU LL 03 G4
EF093174 Meliniomyces sp. SM7-1
EF093175 Meliniomyces sp. CBS 143705
JN943609 Hyaloscypha sp. M288
JN943608 Hyaloscypha sp. M20
JN943607 Hyaloscypha sp. M25
JN943606 Hyaloscypha sp. M19
EF093166 Meliniomyces variabilis MV-S-4
AY762619 Meliniomyces variabilis UAMH 8861 type
AJ292201 Meliniomyces variabilis ARON 2879.S (sc 3)
JQ272355 Meliniomyces sp. LF1GA16D9
Meliniomyces variabilis
AY279181 Epacrid root endophyte sp. RK2.4
JN033423 Hyaloscypha cf. bulbopilosa KUS-F52573
AY279179 Epacrid root endophyte RK111
JN033451 Hyaloscypha cf. bulbopilosa TNS-F18073
MH018930 Hyaloscypha alniseda CBS 123.91
AF252837 Ericoid endophyte sp. GU32
KJ649999 Fungal sp. 3.44.4J
AY789416 Hyaloscypha daedaleae ZW-Geo138-Clark
MH018927 Hyaloscypha daedaleae CBS 120.91
MH018928 Hyaloscypha daedaleae CBS 121.91
"Hyaloscypha" sp. 2-13c
Hyaloscypha s. lat.
Hyaloscypha s. str.
0.1

outgroup, 15 species of nine genera (according to availability) used in previous datasets were employed and, additionally, a sequence of *Mollisia cinerea*, which was too divergent or too difficult to align for other datasets. Aligning the sequences of this protein coding gene was straightforward. *Mollisia* and *Hymenoscyphus caudatus* produced different indels (1–2 bp in close vicinity, maintaining the reading frame), and all species of *Arachnopeziza* were missing one triplet.

The dataset of regions 5–7 of the *rpb*2 gene consisted of 36 taxa and 694 aligned characters of which 44 variable ones were uninformative and 293 characters were parsimony informative. A GTR model with six substitution rates was found most appropriate for this gene region. For BA, 1.5 M generations were needed to reach convergence.

Compilation and analysis of *rpb*2, regions 7–11

For this dataset, the smallest number of taxa was available; compared to regions 5–7 they represented a largely non-overlapping set of *Hyaloscypha* spp. and included *H.* aff. *paludosa*. Most data were from Baral *et al.* (2009) and Stenroos *et al.* (2010). As outgroup, only *Mollisia*, *Cyathicula* and *Cudoniella* were available and usable. As further potential outgroups, only a partial sequence of *Lachnum virgineum* (DQ470877, AFTOL-ID 49), which was used in Baral *et al.* (2009) was available. It contained many N's, a reading frame shift and poor ends and was dismissed as unreliable. Sequences of *Hymenoscyphus fructigenus* (EU940365, M159) and *Hyaloscypha* sp. (EU940312, M25) contained many polymorphisms at positions differing between taxa or whole groups of taxa; they were also excluded from phylogenetic analyses. For the strain of "*H. albohyalina* var. *spiralis*" (M259, Baral *et al.* 2009) that represents a wrongly identified sample according to ITS (see above), also a *rpb*2 sequence was available (EU940360). Similar sequences for comparison are missing in this dataset, and the sequence was excluded as a potential artefact based on the ITS results.

The *rpb*2 dataset (regions 7–11) consisted of 22 taxa and 940 aligned characters; of these, 50 variable characters were uninformative and 284 were parsimony informative. The same model as for regions 5–7 was found; only 0.5 M generations were needed for BA to converge for this small dataset.

Combined dataset

All strains for which nrLSU, mtSSU and at least one of the *rpb*2 datasets was available were concatenated for combined phylogenetic analysis. As outgroup, 18 taxa representing 10 genera were used. The mtSSU dataset was shortened by 96 characters of the two most variable indel regions to a total of 730 aligned characters in order to reduce ambiguity of outgroup relationships.

The concatenated dataset consisted of 48 taxa and a total of 3 556 aligned characters of which 159 variable ones were uninformative, and 822 were parsimony informative. A GTR model was most appropriate; BA was run for 1 M generations (convergence was already reached after 800 000 generations).

RESULTS

ITS phylogeny

All species of the REA are nested among species of *Hyaloscypha* (Fig. 1) confirming that the root-symbiotic fungi actually belong to this genus. The tree reveals a core group consisting of taxa that form a well-supported monophyletic clade together with REA species; we refer to them as *Hyaloscypha s. str.* Two species of *Hyaloscypha* (*H. albohyalina* and *H. aureliella*) fall outside this group; we treat them here as *Hyaloscypha s. lat.* One sample named *Hyaloscypha* sp. 2-13c (Long *et al.* 2013) appears among the outgroup and is most likely misidentified.

Usually, multiple accessions of the same species of *Hyaloscypha* formed well-supported branches (*e.g. H. aureliella*, *H. albohyalina*, *H. spiralis*, *H. vitreola*) with very little intraspecific variation, but there are a few exceptions. Two accessions (JN033423, JN033451) of *Hyaloscypha* cf. *bulbopilosa* (as *H. leuconica* var. *bulbopilosa*, strains KUS-F52573, TNS-F18073, Han *et al.* 2014) may represent different species as their sequences are fairly divergent and not monophyletic; one of them (JN033451) seems to be conspecific with *H. alniseda* (CBS 123.91), one of the strains newly sequenced for this study. The latter strain was originally named *H. fuckelii* var. *alniseda*, but its large genetic distance to *H. fuckelii* M233 suggests they represent different species. *Hyaloscypha spiralis* and *Pseudaegerita corticalis* form a well-supported monophyletic group which is in keeping with their previously described sexual-asexual association. Sequence variation of *P. corticalis* is, however, relatively high compared to other examples.

Concerning named REA species, accessions of *Meliniomyces vraolstadiae* form a well-supported group, which is split into two lineages corresponding to subclades 4 and 5 according to Vrålstad *et al.* (2002). The ex-type strain belongs to subclade 5. *Cadophora finlandica* constitutes a subclade of *Meliniomyces bicolor*, rendering *M. bicolor* paraphyletic. The genetic variation within *M. bicolor* is relatively high so that a distinction of the two species based solely on ITS sequence similarity may be impossible or at least unreliable. Importantly, the ex-type strain of *M. bicolor* is most similar to the sporulating culture which enables us for the first time to perform a morphological comparison with *C. finlandica* (see below). Sequences of the type strains of *C. finlandica* and *Chloridium paucisporum* group together and are nearly identical showing these taxa to be conspecific. Samples of *R. ericae*, *H. hepaticicola* and *Scytalidium vaccinii*

Fig. 1. Phylogenetic analysis based on the ITS region. The Bayesian consensus tree is shown with posterior probabilities (pp) above branches. Below branches, bootstrap support (bs) for MP and ML analyses is given unless <50 %. Depending on space, support for some very short intraspecific branches is omitted, or values for BA, MP and ML are separated by slashes. REA subclades of Vrålstad *et al.* (2002) are indicated by sc 1–5 in brackets after species names; sp. 1–3 HS are REA species from Hambleton & Sigler (2005). Sequences based on type cultures as well as those of the new root-symbiotic species (SM7-1, CBS 143705) and the sporulating strain of *M. bicolor* are indicated in boldface. Taxon names are maintained as in GenBank (accession numbers included), followed by the isolate. Exceptions are *Hyaloscypha spiralis* and *H. monodictys*, for which we adopted the revised taxonomic treatment according to Han *et al.* (2014); *Dematioscypha delicata* is in GenBank as *Haplographium delicatum* and under the wrong name *Dematioscypha dematiicola* in Han *et al.* (2014); accessions of *H. leuconica* var. *bulbopilosa* are given as *H.* cf. *bulbopilosa*; the erroneous spelling of "*H. hepaticola*" is corrected. Strain identifiers are replaced by their CBS numbers in some cases. REA species are outlined based on clades containing the type strain (in case of *R. ericae* as *Pezoloma ericae*). The *R. ericae* clade also includes the type of its asexual state *Scytalidium vaccinii*, and the *Cadophora finlandica* clade includes the type strain of *Chloridium paucisporum*. *Hyaloscypha* spp. forming a well-supported core group (bold branch) that also comprises the REA species are distinguished by colour from two *Hyaloscypha s. lat.* species and from a "*Hyaloscypha*" sample that nests among outgroup genera.

Fig. 2. Phylogenetic analysis based on nrLSU. The Bayesian consensus tree is shown with pp above branches. Below branches, bs for MP and ML analyses is given if above 50 %. "*Hyaloscypha*" aff. *paludosa* clusters with *Arachnopeziza* and is distinguished from *Hyaloscypha s. lat.* and *s. str.*; the latter are labelled in the same colours as in other trees for better comparison. REA sequences based on type cultures as well as those of the new root-symbiotic species (CBS 143705) and the sporulating strain of *M. bicolor* are indicated in boldface.

form together a well-supported clade with a relatively long branch proving that these three taxa are conspecific as well. Our data support the segregation of *Rhizoscyphus* from *Hymenoscyphus*, represented by *H. caudatus* which falls into the outgroup and are in agreement with Zhang & Zhuang (2004). However, *Rhizoscyphus monotropae*, represented by four sequences from different studies (as *Hymenoscyphus monotropae*) appears to be conspecific with *Cyathicula microspora* or at least congeneric

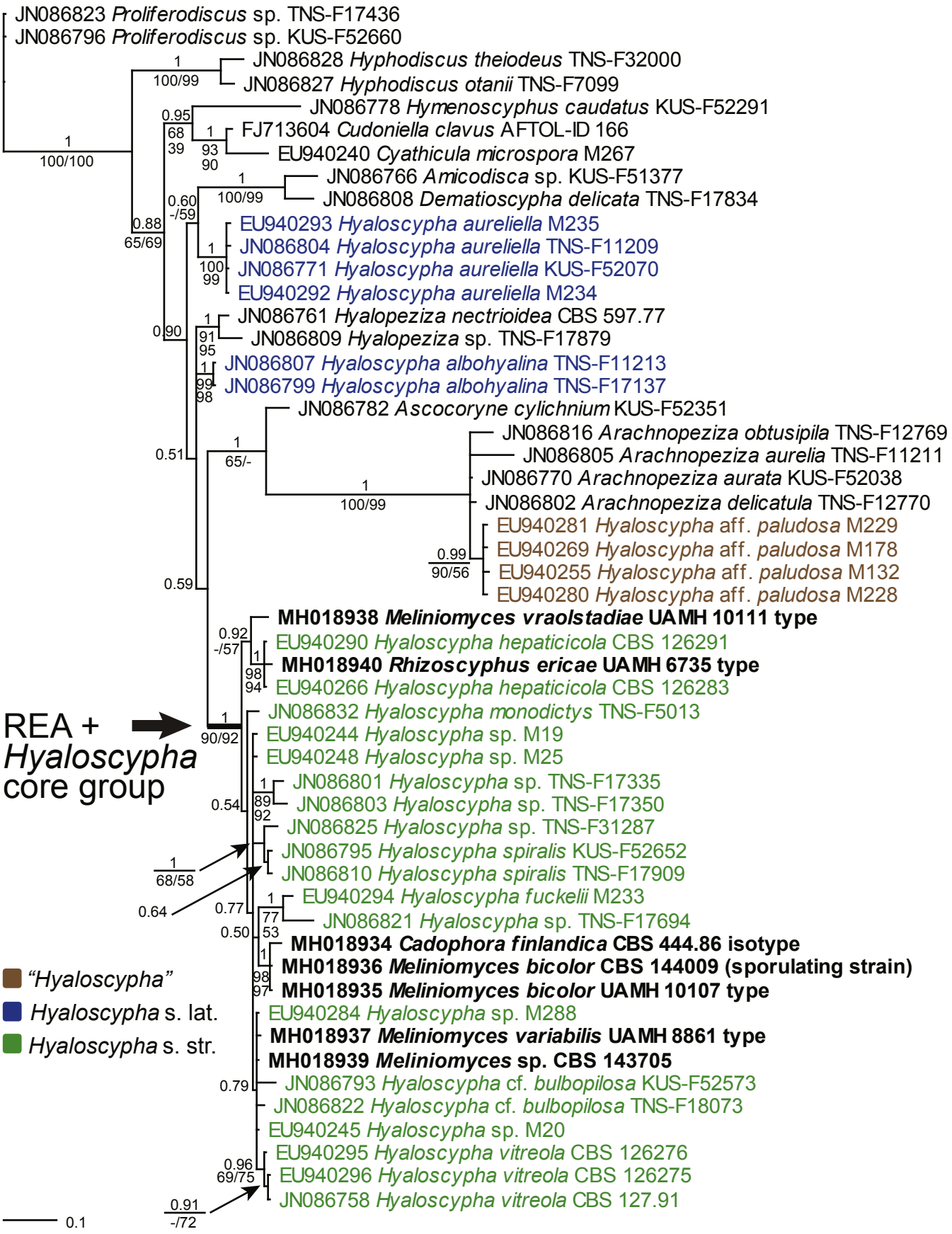
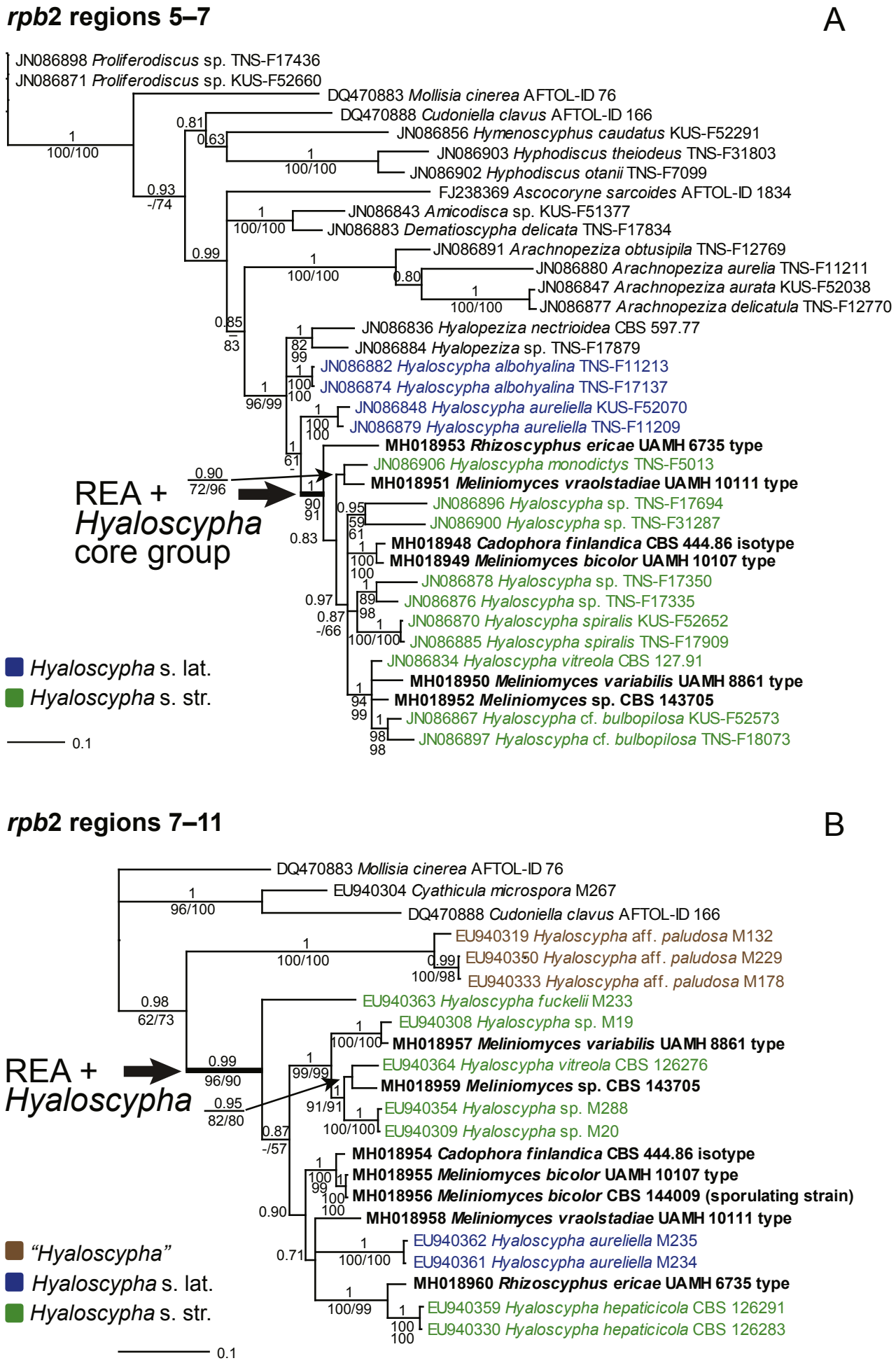


Fig. 3. Phylogenetic analysis based on mtSSU. The Bayesian consensus tree is shown with pp above branches. Below branches, bs for MP and ML analyses is given if above 50 %. "*Hyaloscypha*" aff. *paludosa* clusters with *Arachnopeziza* and is distinguished from *Hyaloscypha s. lat.* and *s. str.*; colours are the same as before for better comparison. REA sequences based on type cultures, the new root-symbiotic species (CBS 143705) and the sporulating strain of *M. bicolor* are in boldface.


rpb2 regions 5–7
A
JN086898 Proliferodiscus sp. TNS-F17436
JN086871 Proliferodiscus sp. KUS-F52660
DQ470883 Mollisia cinerea AFTOL-ID 76
DQ470888 Cudoniella clavus AFTOL-ID 166
JN086856 Hymenoscyphus caudatus KUS-F52291
JN086903 Hyphodiscus theiodeus TNS-F31803
JN086902 Hyphodiscus otanii TNS-F7099
FJ238369 Ascocoryne sarcoides AFTOL-ID 1834
JN086843 Amicodisca sp. KUS-F51377
JN086883 Dematioscypha delicata TNS-F17834
JN086891 Arachnopeziza obtusipila TNS-F12769
JN086880 Arachnopeziza aurelia TNS-F11211
JN086847 Arachnopeziza aurata KUS-F52038
JN086877 Arachnopeziza delicatula TNS-F12770
JN086836 Hyalopeziza nectrioidea CBS 597.77
JN086884 Hyalopeziza sp. TNS-F17879
JN086882 Hyaloscypha albohyalina TNS-F11213
JN086874 Hyaloscypha albohyalina TNS-F17137
JN086848 Hyaloscypha aureliella KUS-F52070
JN086879 Hyaloscypha aureliella TNS-F11209
MH018953 Rhizoscyphus ericae UAMH 6735 type
JN086906 Hyaloscypha monodictys TNS-F5013
MH018951 Meliniomyces vraolstadiae UAMH 10111 type
JN086896 Hyaloscypha sp. TNS-F17694
JN086900 Hyaloscypha sp. TNS-F31287
MH018948 Cadophora finlandica CBS 444.86 isotype
MH018949 Meliniomyces bicolor UAMH 10107 type
JN086878 Hyaloscypha sp. TNS-F17350
JN086876 Hyaloscypha sp. TNS-F17335
JN086870 Hyaloscypha spiralis KUS-F52652
JN086885 Hyaloscypha spiralis TNS-F17909
JN086834 Hyaloscypha vitreola CBS 127.91
MH018950 Meliniomyces variabilis UAMH 8861 type
MH018952 Meliniomyces sp. CBS 143705
JN086867 Hyaloscypha cf. bulbopilosa KUS-F52573
JN086897 Hyaloscypha cf. bulbopilosa TNS-F18073
REA + Hyaloscypha core group
Hyaloscypha s. lat.
Hyaloscypha s. str.
0.1
rpb2 regions 7–11
B
DQ470883 Mollisia cinerea AFTOL-ID 76
EU940304 Cyathicula microspora M267
DQ470888 Cudoniella clavus AFTOL-ID 166
EU940319 Hyaloscypha aff. paludosa M132
EU940350 Hyaloscypha aff. paludosa M229
EU940333 Hyaloscypha aff. paludosa M178
EU940363 Hyaloscypha fuckelii M233
EU940308 Hyaloscypha sp. M19
MH018957 Meliniomyces variabilis UAMH 8861 type
EU940364 Hyaloscypha vitreola CBS 126276
MH018959 Meliniomyces sp. CBS 143705
EU940354 Hyaloscypha sp. M288
EU940309 Hyaloscypha sp. M20
MH018954 Cadophora finlandica CBS 444.86 isotype
MH018955 Meliniomyces bicolor UAMH 10107 type
MH018956 Meliniomyces bicolor CBS 144009 (sporulating strain)
MH018958 Meliniomyces vraolstadiae UAMH 10111 type
EU940362 Hyaloscypha aureliella M235
EU940361 Hyaloscypha aureliella M234
MH018960 Rhizoscyphus ericae UAMH 6735 type
EU940359 Hyaloscypha hepaticicola CBS 126291
EU940330 Hyaloscypha hepaticicola CBS 126283
REA + Hyaloscypha
"Hyaloscypha"
Hyaloscypha s. lat.
Hyaloscypha s. str.
0.1

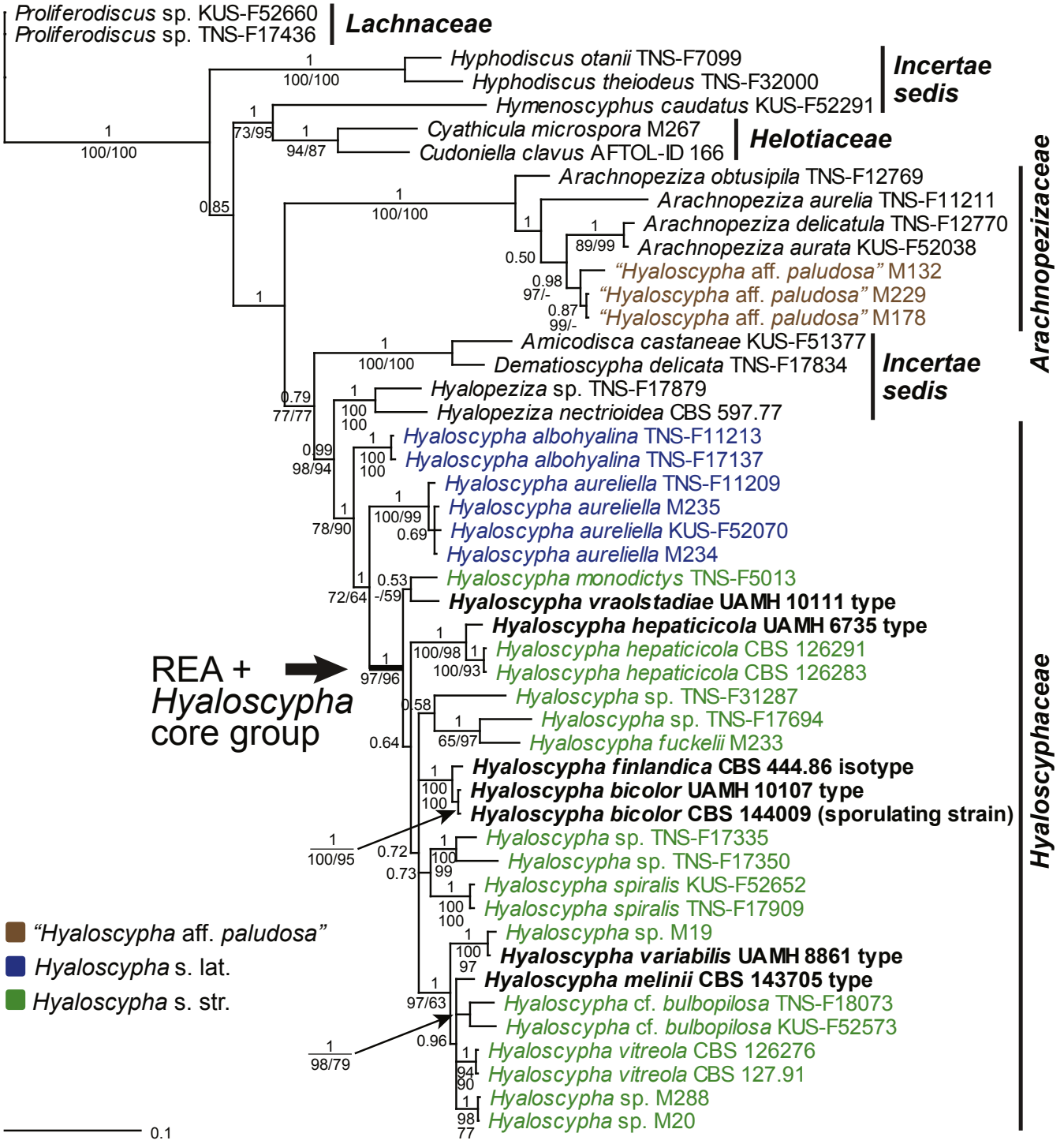


Fig. 5. Phylogenetic analysis of the combined dataset. The Bayesian consensus tree of the concatenated dataset (nrLSU, mtSSU and *rpb2* regions 5–7 and 7–11) is shown with pp above branches. Below branches, bs for MP and ML analyses is given. Colours of the original *Hyaloscypha* samples are maintained as in previous trees. REA species are given in bold black with their revised names (for *H. hepaticicola*, the type refers to *Pezizella* (*Rhizoscyphus*) *ericae*). Family names are provided for outgroup taxa.

with *Cyathicula* (Fig. 1). Similarly, other species described in *Pezoloma* and *Cadophora* have their generic names wrongly applied; these genera, based on their type species, are genetically very divergent from *Hyaloscypha*/REA.

Relationships among REA/*Hyaloscypha s. str.* species are generally not very well resolved, with some notable exceptions, some of which may be indicative of conspecific pairs in addition to the clear case of *R. ericae*/*H. hepaticicola* outlined above: (i)

Fig. 4. Phylogenetic analysis based on the *rpb2* gene. Bayesian consensus trees are shown with pp above branches. Below branches, bs for MP and ML analyses is given. *Hyaloscypha s. str.* and *Hyaloscypha s. lat.* are labelled in the same colours as in other trees. REA sequences from type cultures, the new root-symbiotic species (CBS 143705) and the sporulating strain of *M. bicolor* are in boldface. **A.** Regions 5–7 as in Han *et al.* (2014). **B.** Regions 7–11 as in Baral *et al.* (2009). "*Hyaloscypha* aff. *paludosa*" is only available for this region and indicated by different colour.

Meliniomyces variabilis falls into a strongly supported clade along with *Hyaloscypha* sp. (M19, M25); (ii) three accessions of *Hyaloscypha daedaleae* group with fungal sequences isolated from plant roots, one of them from the *Ericaceae*; (iii) one accession of *H.* cf. *bulbopilosa* and *H. alniseda* (see above) cluster with a fungal sequence isolated from orchid roots; closely related is one of the epacrid root endophytes forming a sister to *R. ericae* in Hambleton & Sigler (2005); (iv) *Hyaloscypha* sp. TNS-F17350 appears to be conspecific with *H. herbarum* which was newly sequenced for this study; this taxon nests with fungi isolated from roots of *Calluna vulgaris* and *Epacris microphylla* (both *Ericaceae*); (v) a sequence wrongly attributed to *Hymenoscyphus* sp. olrim148, isolated from live xylem of *Betula pendula*, evidently belongs to *Hyaloscypha aureliella* (*Hyaloscypha s. lat.*). Strain CBS 126298 of *H. aureliella* was re-sequenced; the previously published sequence (EU940228, as strain M234, Baral *et al.* 2009) contains one potential mistake, a unique 1 bp-insertion, and is 71 bp shorter than the new one (data not shown). There are other species of *Hyaloscypha* as well as members of the REA without particularly close relatives; among them is "species 3" from Hambleton & Sigler (2005) to which no candidate sexual counterpart was found yet. The same applies also to *M. vraolstadiae*, *M. bicolor* and *C. finlandica*. Conversely, *H. vitreola* is an example of a sexual species without close matches among root-associated fungi. By and large, sexual and asexual taxa are well intermingled in the phylogenetic tree, *i.e.* they are not displaying a particular evolutionary pattern.

nrLSU phylogeny

Similarly as with ITS, also the nrLSU region unequivocally confirms *Hyaloscypha* and REA to be congeneric (Fig. 2) except that two species of *Hyaloscypha* previously falling outside the core group emerge from a basal polytomy along with the other taxa, but they are characterised by relatively long branches compared to the majority of other species. In contrast, four *Hyaloscypha* aff. *paludosa* accessions group with *Arachnopeziza* and most likely belong to that genus. Like before, well-supported branches are formed by (i) *R. ericae* and *H. hepaticicola*, (ii) *Meliniomyces variabilis* and *Hyaloscypha* sp. (M19, M25), and (iii) *M. bicolor* and *C. finlandica*, whereas relationships among other species are mostly unsupported. Conspecificity of *Hyaloscypha spiralis* and *Pseudaegerita corticalis* is confirmed. In the same well-supported clade falls also a sequence of *H. minuta* (KY769526) from an unpublished study (the ITS part of that sequence clusters with the same species; data not shown); it is the only available sequence of *H. minuta*, and its identification may be erroneous. Results of nrLSU and ITS based phylogenies are expectedly similar because these markers are linked. Some differences in tree topology and resolution can be attributed to differential data availability (*e.g.* a host of data of asexual fungi for ITS, but no *Hyaloscypha* aff. *paludosa*) and the extent of sequence variation (*e.g.* alignability of nrLSU with many more outgroup taxa, but no clear distinction between *Hyaloscypha s. lat.* and *s. str.*).

mtSSU phylogeny

The tree based on the mtSSU reveals a core group of *Hyaloscypha s. str.* species with the REA accessions nesting among them (Fig. 3). The two previously identified *Hyaloscypha s. lat.* species are not sister to this group as with ITS, but their positions are among other closely related outgroup genera. *Hyaloscypha* aff. *paludosa* clusters with *Arachnopeziza* as in nrLSU; *R. ericae* groups with *H. hepaticicola*, and *M. bicolor* groups with *C. finlandica* as in nrLSU and ITS. Otherwise, relationships among most outgroup genera as well as within *Hyaloscypha s. str.* are largely unresolved with this marker. Although the genetic variation in the mtSSU is generally higher than with the nrLSU, most of the variation in the mtSSU concerns the outgroup whereas sequence similarity within the core group of *Hyaloscypha* is very high in contrast to both ITS and nrLSU.

rpb2 phylogenies

Both trees based on different regions of the *rpb*2 gene reveal the REA as congeneric with *Hyaloscypha* (Fig. 4); in case of regions 5–7, REA taxa are falling into the core group (Fig. 4A) whereas based on regions 7–11, the only *Hyaloscypha s. lat.* species available nests within *Hyaloscypha s. str.* (Fig. 4B). However, basal internal branches within the ingroup are not supported in the latter case (given that pp's < 0.95 are not significant) so that similar features of the trees are found for nrLSU (Fig. 2) and *rpb*2 regions 7–11 (Fig. 4B), namely unclear basal relationships among ingroup taxa and relatively long branches of *Hyaloscypha s. lat.* species (in *rpb*2 only *H. aureliella*).

For both regions of *rpb*2, largely non-overlapping sets of ingroup and outgroup taxa were available with the exception of the newly generated REA sequences. *Hyaloscypha* aff. *paludosa* and *Arachnopeziza* are available for only one of the datasets, respectively, but their position as sister to *Hyaloscypha s. lat.* (including *Hyalopeziza* in Fig. 4A) along with relatively long branches compared to other outgroup taxa suggests that these accessions may belong to *Arachnopeziza*. Both trees show the close relationship of *M. bicolor* and *C. finlandica*, and Fig. 4B shows the associations of *R. ericae*/*H. hepaticicola* and *M. variabilis*/*Hyaloscypha* sp. (M19) also revealed by other markers (data for the other *rpb*2 region are not available for these *Hyaloscypha* species).

Concerning ingroup relationships, *rpb*2 is the first marker revealing a subclade consisting of *M. variabilis*, the unknown sterile fungus from the Czech Republic (CBS 143705, see below), and *H. vitreola* that is well-supported in all three analyses. To these taxa can be added *H.* cf. *bulbopilosa*, *Hyaloscypha* sp. (M19) and *Hyaloscypha* sp. (M20, M288), which are available only for the one or other dataset (Fig. 4A,B). The same assemblage of taxa is also seen in the mtSSU tree, but without significant support. In nrLSU and ITS trees, this group of taxa also includes *H. daedaleae*, in ITS also *H. epiporia* and *H. alniseda*, however, the clade is supported only in BA. Thus, only *rpb*2 as the most variable marker is able to resolve relationships for a subset of ingroup taxa with significant support.

Phylogenetic analysis of the combined dataset

In the phylogenetic tree based on combined analyses of nrLSU, mtSSU and *rpb*2 (Fig. 5), *Hyaloscypha albohyalina* and *H. aureliella* (*Hyaloscypha s. lat.*) constitute separate branches that are sister to the core group. Most closely related to *Hyaloscypha s. lat.* are *Hyalopeziza* and the *Amicodisca*/*Dematioscypha* clade. "*Hyaloscypha* aff. *paludosa*" based on four specimens evidently belongs to *Arachnopeziza* according to its

position in the tree, but we refrain from formally proposing a new combination. Among outgroup genera, a group consisting of *Hymenoscyphus*, *Cyathicula* and *Cudoniella* was highly supported; it was found with all markers except *rpb*2. One subclade including *H. vitreola* and several other species was supported (see above), and a sister relationship of *H. fuckelii* and *Hyaloscypha* sp. TNS-F17694 was observed that also occurred in the mtSSU and (albeit poorly supported) in the ITS tree.

Re-synthesis experiment

The bryophyte-derived *H. hepaticicola* CBS 126291 formed the typical ErM structures in the host rhizodermis, *i.e.* dense intracellular hyphal coils (Fig. 6B–F). The same was true for the *Ericaceae*-derived *R. ericae* UAMH 6735 (Fig. 6G–I). It is interesting to note that despite the colonisation intensity was not rigorously measured, the former fungal strain produced apparently higher colonisation levels (in terms of the number of the colonised rhizodermal cells) in all screened *Vaccinium* seedlings. The *Pinaceae*-derived *H. melinii* CBS 143705 produced no visible intraradical hyphal colonisation (Fig. 7A), despite that its inoculum was apparently viable during the course of the experiment as evidenced by the presence of fungal hyphae in the host rhizosphere (Fig. 7B). The *Pinaceae*-derived *H. bicolor* CBS 144009 very infrequently (much less than 1 % of the screened rhizodermal cells) formed intracellular hyphal coils (Fig. 7C, D) which are here interpreted, in terms of morphology, as ericoid mycorrhiza (cf. Vohník *et al.* 2007b). However, these sometimes morphologically differed from *H. hepaticicola* CBS 126291 and *R. ericae* UAMH 6735 in that the hyphal cells were shorter and as a result, the coils were less rounded (Fig. 7C) (cf. Vohník *et al.* 2013). None of the tested fungal strains colonised host vascular tissues (the central cylinder) as typical for *Ericaceae* endophytic fungi, *e.g.* dark septate endophytes (cf. Lukešová *et al.* 2015). Control plants not inoculated with fungal mycelium remained free of any visible hyphal colonisation (not shown). All inoculated plants remained healthy with no signs of fungal parasitism and, in terms of growth, performed better than the non-inoculated plants (not shown).

TAXONOMY

Hyaloscypha Boud., Bull. Soc. mycol. Fr. 1: 118. 1885.
Synonyms: *Eupezizella* Höhn., Mitt. bot. Inst. tech. Hochsch. Wien 3: 61. 1926 *apud* Huhtinen, Karstenia 29: 90. 1990.
Truncicola Velen., Monogr. Discom. Bohem.: 289. 1934 *apud* Huhtinen, Karstenia 29: 90. 1990.
Pseudaegerita J.L. Crane & Schokn., Mycologia 73: 78. 1981.
Fuscoscypha Svrček, Sydowia 39: 222. 1987 *apud* Baral *et al.*, Karstenia 49: 13. 2009.
Rhizoscyphus W.Y. Zhuang & Korf, Nova Hedw. 78: 481. 2004.
Meliniomyces Hambl. & Sigler, Stud. Mycol. 53: 12. 2005.

Lectotype species: *Hyaloscypha vitreola* (P. Karst.) Boud., Bull. Soc. mycol. Fr. 1: 118. 1885.

Notes: The synonymy of *Eupezizella* and *Truncicola* with *Hyaloscypha* was proposed by Huhtinen (1990). Baral *et al.* (2009) accepted the monotypic genus *Fuscoscypha* (Svrček 1986), typified by *F. acicularum* which is known only from the holotype, as a synonym of *Hyaloscypha* based on similar morphology of their type species. This synonymy is adopted in our study, however, it needs to be verified with molecular data. *Pseudaegerita corticalis*, the type species of *Pseudaegerita* (Crane & Schoknecht 1981), has long been known to be the asexual morph of *Hyaloscypha spiralis* (Abdullah & Webster 1983). Four ITS sequences (Cooper *et al.*, unpubl.) and two nrLSU sequences (Yamaguchi *et al.* 2012) of six different conidial isolates of *P. corticalis* form a strongly supported monophyletic clade with ascospore isolates of *H. spiralis* (Figs 1, 2) and thus prove their intimate relationship and that they belong to the life cycle of one organism at the molecular level. However, *Pseudaegerita* appears to be polyphyletic as two ITS sequences of *P. viridis* fall into the outgroup (Fig. 1).

The genus *Hyaloscypha* (*Hyaloscyphaceae*, *Leotiomycetes*) is delimited to fungi with sessile or shortly stipitate, white to whitish to grey-brown occasionally yellowish-brown apothecial ascomata when fresh possessing tapering, usually narrowly conical or conical-lageniform apothecial hairs with or without resinous exudates and blunt to tapering at the apex, cylindrical, stipitate, inoperculate asci with predominantly an amyloid apical annulus in Lugol's iodine and Melzer's reagent (euamyloid), although hemiamyloid reaction or abberations in euamyloidity occur rarely, filiform-cylindrical paraphyses without a yellow refractive vacuolar pigment and ellipsoidal, ellipsoidal-clavate to fusoid, hyaline, aseptate ascospores rarely with a middle septum developing upon aging (Huhtinen 1990). The conidiogenesis is either phialidic or holoblastic, occasionally thallic conidia are formed by disarticulation of existing hyphae. Some species form only sterile mycelia.

Based on phylogenetic evidence from four markers and in accordance with the principle of priority, *Meliniomyces* with *M. variabilis* as its type species, *Pseudaegerita*, typified by *P. corticalis*, and *Rhizoscyphus*, typified by *R. ericae*, are reduced to synonymy under *Hyaloscypha*. Our conclusion is supported by similar morphology of sexual *Hyaloscypha* and *Rhizoscyphus* and by a re-synthesis experiment with *H. hepaticicola* (see above) and its ability to form ericoid mycorrhiza.

Hyaloscypha melinii Vohník, Fehrer & Réblová, **sp. nov.** MycoBank MB825015. Figs 8A, 9.

Etymology: In honour of Elias Melin, a pioneer leader in mycorrhizal research.

Cultural characters: On MEA, colonies 27–31 mm diam after 28 d (16–18 mm after 14 d, 23–25 mm after 21 d), raised, circular, appearing waxy-mucoid. Aerial mycelium sparse, floccose with funiculate projections restricted to the centre and margins, the remaining mycelium moist, developing numerous radial folds, colony surface beige with a grey marginal ring; margin distinct, regular or weakly undulate; reverse dark beige. On MLA, colonies 28–30 mm diam after 28 d (18–20 mm after 14 d, 23–25 mm after 21 d), raised, circular. Aerial mycelium sparse, floccose with funiculate projections at the inoculation block and at the margins, appearing moist around the centre, with a narrow zone of diffused dark brown pigment and an ivory-grey broad zone of submerged growth, colony surface dark grey with white patches; margin distinct and regular; reverse dark grey to black. On PCA, colonies 20–23 mm diam after 28 d (15–17 mm after 14 d, 18–22 mm after 21 d), raised, circular. Aerial mycelium dense, cottony, sparse to almost cobwebby toward the margin with a broad zone of submerged growth, colony surface white with a dark grey marginal ring; margin

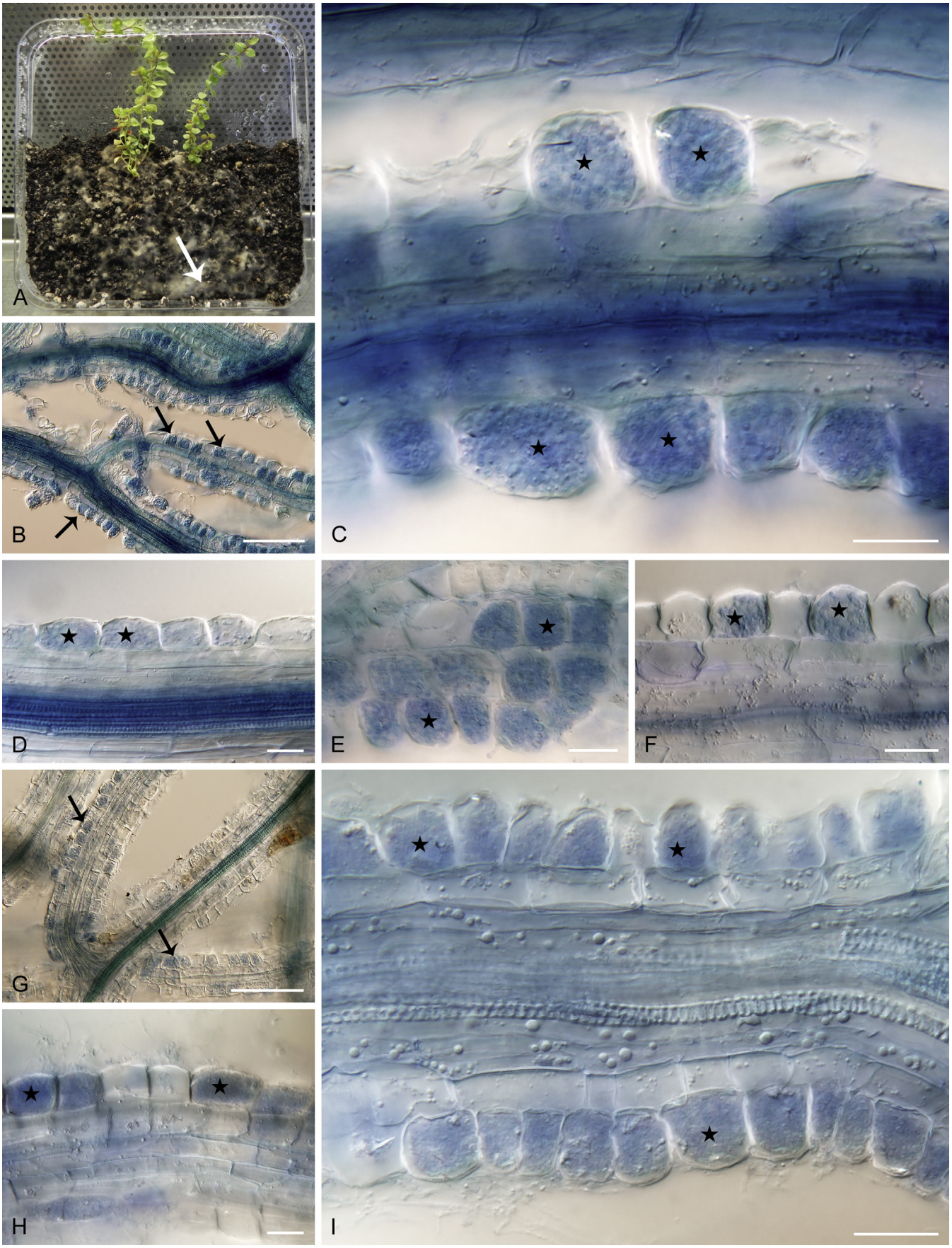


Fig. 6. Colonisation potential of *Hyaloscypha hepaticicola*/*Rhizoscyphus ericae* in *Vaccinium* roots. **A.** Experimental setup after opening the dish and removing moistened filter paper. Note abundant mycelium covering the surface of the cultivation substrate (arrow). **B, G.** The extent of colonisation within the whole root systems; plant cells with intracellular fungal hyphae stained blue with trypan blue in lactoglycerol are indicated by arrows. **C–F, H, I.** Dense intracellular hyphal coils typical for ericoid mycorrhiza, stained blue as above (asterisks). A–F. Bryophilous strain CBS 126291. G–I. Root-associated strain UAMH 6735 (as "*Rhizoscyphus ericae*"). Scale bars: B, G = 100 µm, C–F, H, I = 20 µm.

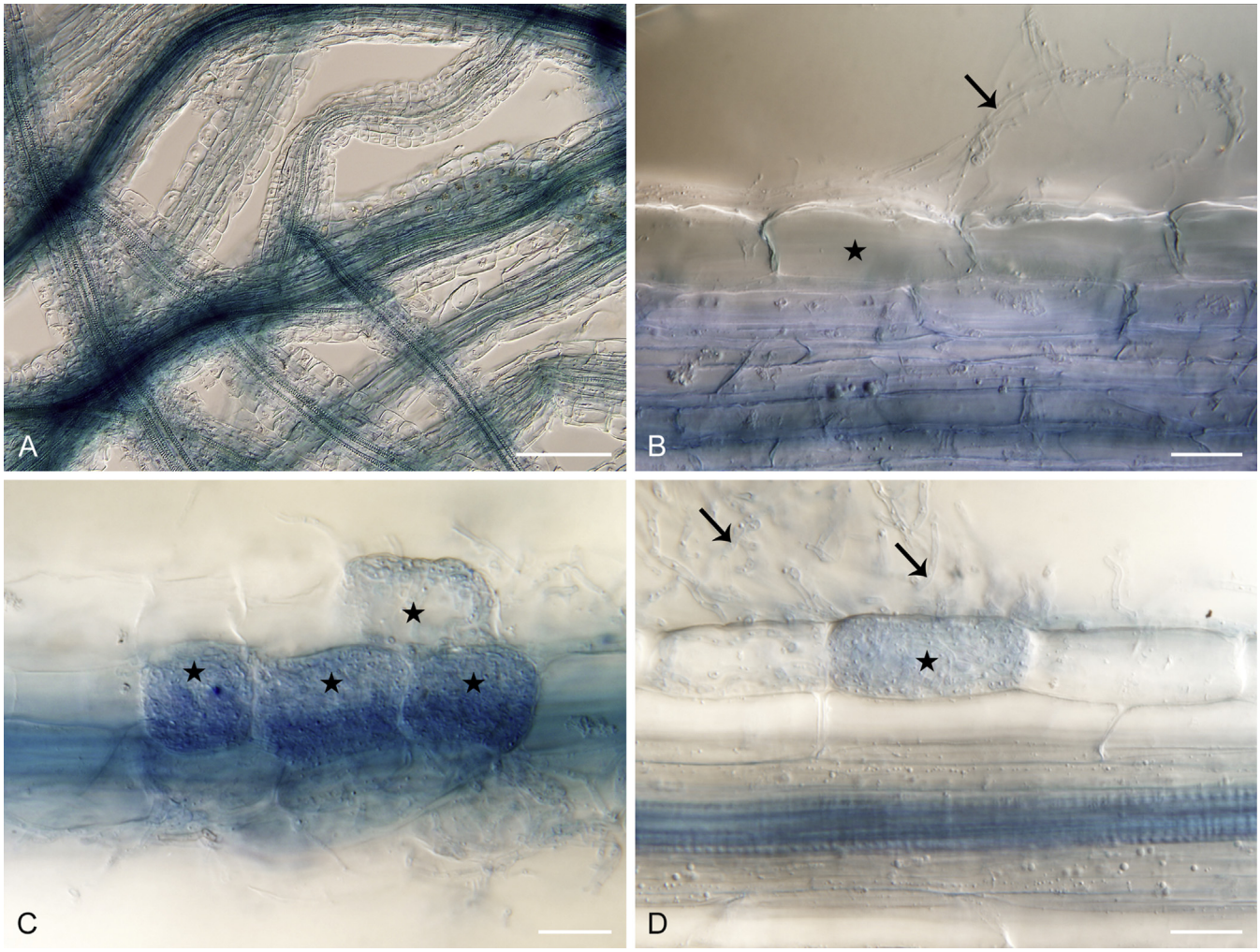


Fig. 7. Colonisation potential of *Hyaloscypha melinii* and *H. bicolor* in *Vaccinium* roots. **A.** The whole root system is free of visible fungal colonisation. **B.** Empty rhizodermal cell without fungal colonisation (asterisk); arrow indicates extraradical mycelium attached to the root surface. **C, D.** Dense intracellular hyphal coils resembling ericoid mycorrhiza (asterisks) stained blue with trypan blue in lactoglycerol; arrows indicate extraradical mycelium attached to the root surface. A, B. *Hyaloscypha melinii* CBS 143705. C, D. *Hyaloscypha bicolor* CBS 144009. Scale bars: A = 100 µm, B–D = 20 µm.

distinct and regular to weakly undulate; reverse black. On PDA, colonies 20–23 mm diam after 28 d (13–16 mm after 14 d, 16–18 mm after 21 d), raised, circular, appearing waxy-mucoid. Aerial mycelium sparse, floccose with funiculate projections, colony surface beige to pale pink becoming pale beige toward the margin, developing numerous radial folds; margin undulate; reverse dark beige. Sporulation absent on all media.

Specimens examined: **Czech Republic**, Southern Bohemia, Bohemian Forest National Park (Šumava Mts.), a spot between Březník and Modrava, 1075 m a.s.l., N°49.000, E°13.483, isolated from a basidiomycetous ectomycorrhizal root tip of a *Picea abies* seedling (*i.e.* likely endophytic), 4 Aug. 2005, L. Mrnka & M. Vohník SM7-2 (**holotype**, dried culture PRA-13668, culture ex-type CBS 143705); ibid., L. Mrnka & M. Vohník SM7-1 (living culture is no longer viable).

Notes: For isolation details of the type strain and the *in vitro* colonisation potential of *H. melinii* CBS 143705 in *P. abies* and *V. myrtillus* see Vohník *et al.* (2013). This taxon may be rare even in its original region; no isolates of this species were obtained from *Ericaceae* and *Pinaceae* hosts during a more extensive sampling at a site in the same area (Vohník *et al.* unpubl.).

Hyaloscypha bicolor (Hambl. & Sigler) Vohník, Fehrer & Réblová, **comb. nov.** MycoBank MB825016. Figs 8B, 10.
Basionym: *Meliniomyces bicolor* Hambl. & Sigler, Stud. Mycol. 53: 16. 2005.

Conidiophores on MMN 53–73 µm long, 2.5–3.5 µm wide, mostly semi-macronematous rarely macronematous, mononematous, branched, dark brown, septate; branches consisting of subcylindrical cells 3.5–6(–7) × 3–4(–5) µm, bearing metulae with conidiogenous cells. *Conidiogenous cells* (16–)18–25(–29) × 2.5–3(–3.5) µm (mean ± SD = 20.2 ± 3.7 × 3.0 ± 0.3 µm), terminal, integrated, phialidic, born on metulae, single or most often in groups of two or in small penicillate clusters, cylindrical or cylindrical-lageniform, tapering to *ca.* 1.5(–2) µm just below the collarette, pale brown, subhyaline toward the collarette; *metulae* pale brown, thin-walled, (5.5–)6–10(–11) × 2.5–3.5 µm (mean ± SD = 7.9 ± 1.8 × 2.8 ± 0.2 µm); *collarette* darker, flaring, wedge-shaped (3–)3.5–4.5 µm deep and 3–4 µm wide; the pigment in collarette disappearing with age. *Conidia* (5.5–)6–7(–7.5) × 3–4 µm (mean ± SD = 6.7 ± 0.4 × 3.7 ± 0.3 µm), L:W ratio (1.5–)2:1, ellipsoidal to clavate or dacryoid, with a broadly rounded apical end and truncate, narrowly conical basal end, hyaline, aseptate, smooth-walled. *Chlamydospores* absent.

Cultural characters: On MEA, colonies 19–23 mm diam after 28 d (10–12 mm after 14 d, 14–15 after 21 d), raised, circular. Aerial mycelium abundant, dense, woolly, colony surface grey, paler around the centre, developing several radial folds; margin distinct and regular; reverse dark grey to black. On MLA, colonies 19–20 mm diam after 28 d (11–12 mm after 14 d, 14–16

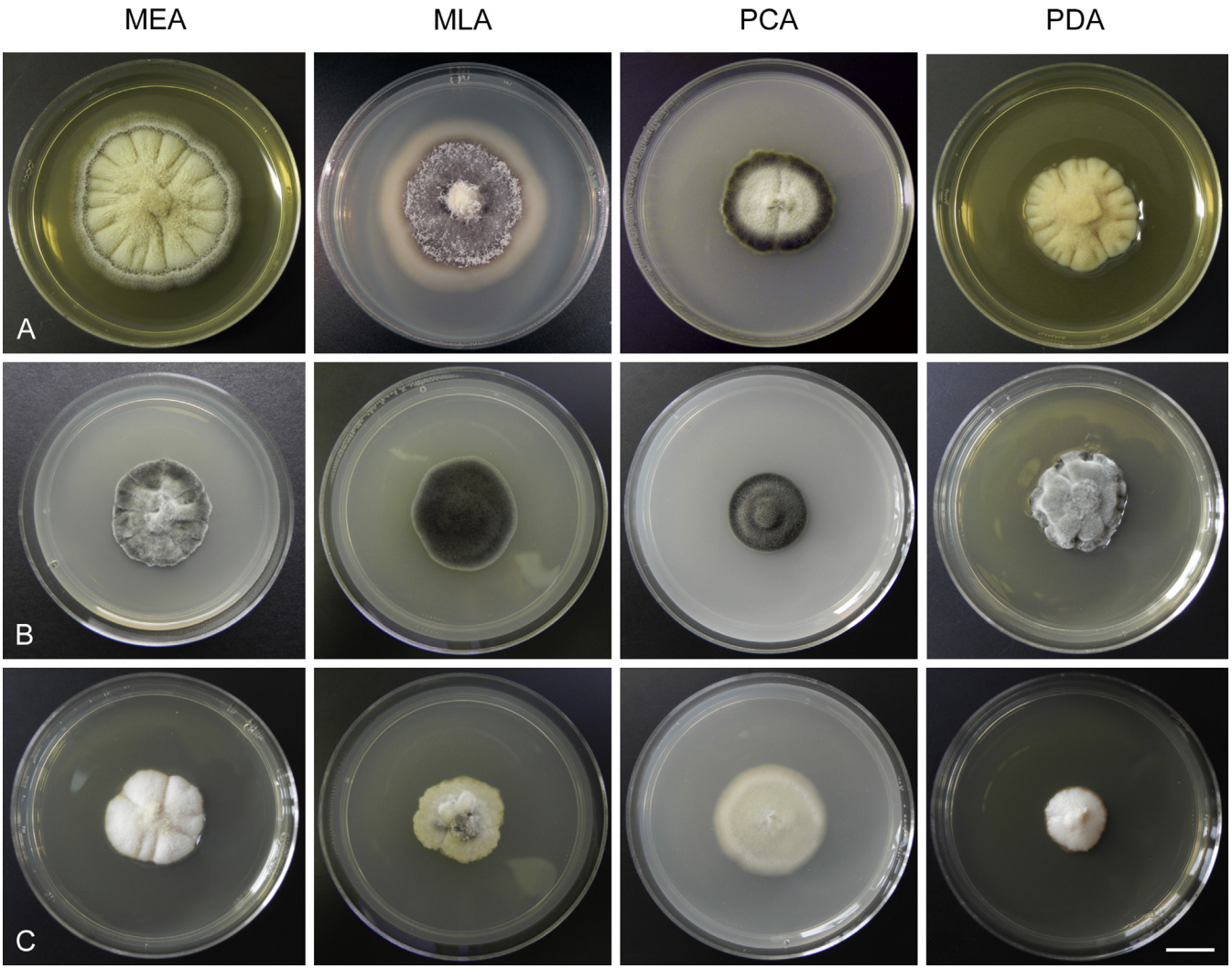


Fig. 8. Colonies of *Hyaloscypha* spp. on MEA, MLA, PCA and PDA after 28 d. **A.** *Hyaloscypha melinii* CBS 143705 ex-type. **B.** *Hyaloscypha bicolor* CBS 144009. **C.** *Hyaloscypha aureliella* CBS 126298. Scale bar: A–C = 1 cm.

after 21 d), concave, circular. Aerial mycelium abundant, dense, woolly, colony surface dark grey with a pale grey marginal zone; margin distinct and regular; reverse black. On PCA, colonies 15–16 mm diam after 28 d (9–10 mm after 14 d, 12–13 after 21 d), concave, raised at the centre, circular. Aerial mycelium abundant, dense, woolly, dark grey with a darker marginal zone consisting of decumbent hyphae and a zone of submerged growth; margin distinct and regular; reverse black. On PDA, colonies 18–20 mm diam after 28 d (11–12 mm after 14 d, 15–16 after 21 d), concave, circular. Aerial mycelium abundant, dense, cottony, developing numerous radial folds, colony surface grey; margin distinct, weakly undulate; reverse grey. Sporulation after 18 mo on MMN at 6 °C.

Specimens examined: **Czech Republic**, Northern Bohemia, Lužické Mts., a spot near Ptačinec Mt., N°50.8560703, E°14.6174975, isolated from a *Cenococcum geophilum*-like ectomycorrhiza of a *Picea abies* seedling (*i.e.* probably endophytic in this ectomycorrhiza), 16 Sep. 2015, M. Vohník REA-3 (dried culture PRA-13608, living culture CBS 144009). **Norway**, Telemark, Kragero, isolated from roots of *Quercus robur* seedlings, 1998, T. Vrålstad ARON 2893.S (living culture CBS 116123 = UAMH 10108). **United Kingdom**, England, North Yorkshire, isolated from roots of a *Nothofagus* sp. seedling, 1998, A. Taylor (**ex-type** strain CBS 116122 = UAMH 10107).

Notes: The ex-type and other strain CBS 116123 of *H. bicolor* were initially isolated as sterile mycelia from roots of deciduous trees (*Fagaceae*), while other strains including our specimen CBS 144009 were isolated from coniferous roots (*Pinaceae*). Although from variable sources (including non-*Picea* hosts), together with *H. vraolstadiae* they were informally labelled as derived from/forming the *Piceirhiza bicolorata* ectomycorrhizal morphotype (cf. Brand *et al.* 1992, Vrålstad *et al.* 2000). With the aid of ITS and nrSSU sequence data they were distinguished as two separate species and placed in *Meliniomyces* (Hambleton & Sigler 2005), which is also corroborated by nrLSU, mtSSU and *rpb2* phylogenies (Figs 2–4). The conidiogenesis of *H. bicolor* was observed for the first time, induced during a prolonged incubation at 6 °C.

Hyaloscypha bicolor is remarkably similar to *H. finlandica* based on morphology of conidiophores, phialides and conidia, but the latter species differs from it by wider, dark brown and thick-walled doliiform cells of branches, narrower collarette and smaller conidia (Wang & Wilcox 1985). A comparison of morphological diagnostic characters of both species is provided in Table 2. All molecular markers investigated here group these species together, yet they are distinguishable with ITS, nrLSU and *rpb2*, and branch support is significant with the most variable markers, ITS and *rpb2*.

Hyaloscypha finlandica (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová, **comb. nov.** MycoBank MB825017.

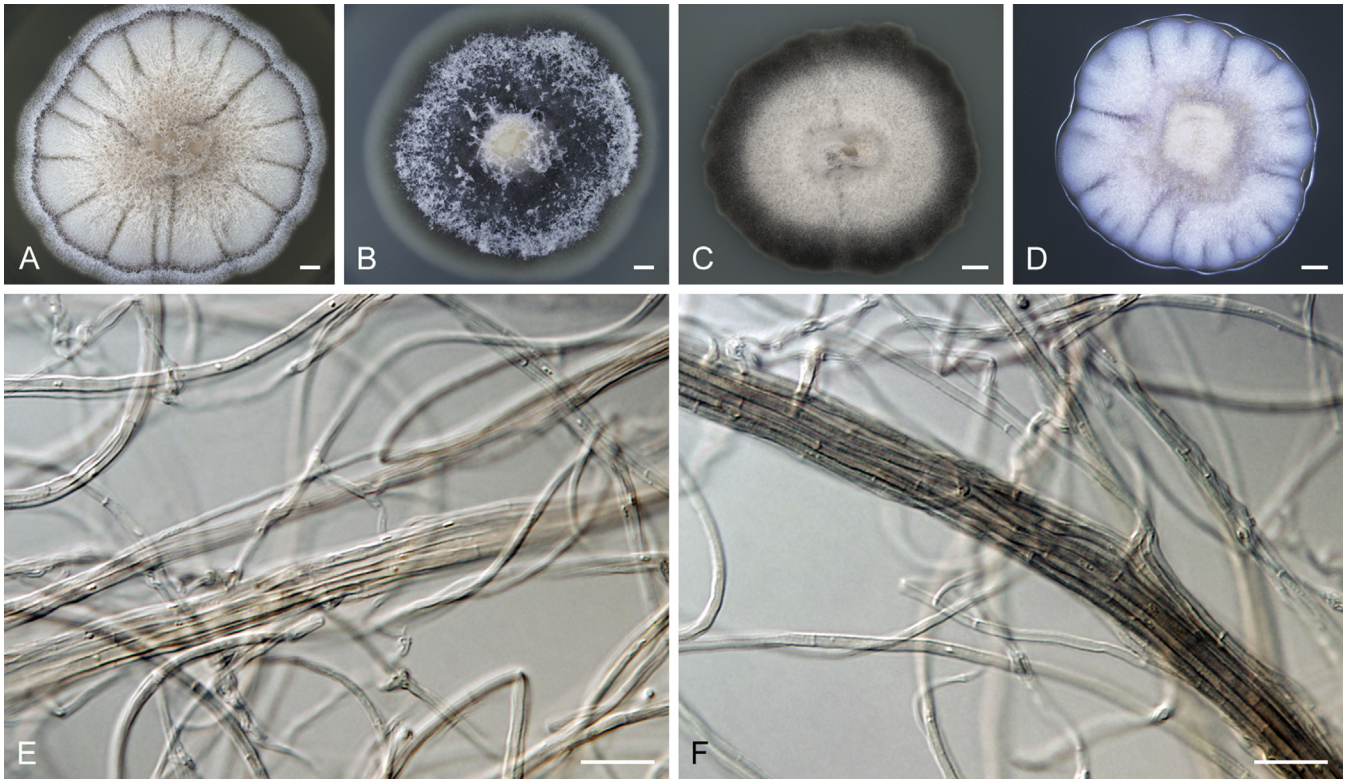


Fig. 9. *Hyaloscypha melinii* CBS 143705 ex-type. **A–D.** Colony details on MEA, MLA, PCA and PDA after 28 d. **E, F.** Vegetative hyphae, single or aggregated and forming funiculi, on MLA, 28 d. Scale bars: A–D = 2 mm, E, F = 10 µm.

Basionym: *Phialophora finlandica* C.J.K. Wang & H.E. Wilcox [as *'finlandia'*], Mycologia 77: 953. 1985.
Synonyms: *Cadophora finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew [as *'finlandia'*], Mycotaxon 87: 147. 2003.
Chloridium paucisporum C.J.K. Wang & H.E. Wilcox, Mycologia 77: 956. 1985.

Specimens examined: **Czech Republic**, Southern Bohemia, Bohemian Forest National Park, Modrava, isolated from a *Picea abies* ectomycorrhiza, 2005, L. Mrnka CFI-3 (living culture CCF 3579). **Finland**, Suonenjoki, isolated from roots of a *Pinus sylvestris* seedling, 15 Jul. 1975, C. J. K. Wang (**holotype** of *P. finlandica*, dried culture FAG-15, culture ex-type CBS 444.86).

Notes: For description and illustration see Wang & Wilcox (1985). Similar to *H. bicolor*, phialidic conidiogenesis of *H. finlandica in vitro* was induced by cold treatment during incubation of MMN agar plates at 5 °C for a period of 6–12 mo (Wang & Wilcox 1985); for additional growth details at low temperature see Wilcox *et al.* (1974). For ectomycorrhiza formation between *H. finlandica* CFI-3 (CCF 3579) and *Picea abies* see Mrnka *et al.* (2009), for ectendomycorrhiza formation between the same partners see Vohník *et al.* (2013).

Chloridium paucisporum was described for an ectendomycorrhizal root-isolate of *Pinus resinosa* (Wang & Wilcox 1985) and was based on former observation and experiments of Wilcox *et al.* (1974). The ITS sequence (Alberton *et al.* 2010) of the type strain CBS 445.86 is almost identical to the type strain of *H. finlandica* (Fig. 1, Table 1) indicating that these taxa are conspecific. Morphologically, both species are highly similar and the sizes of their phialides and conidia overlap. Based on the evidence of DNA sequence data and morphology, *C. paucisporum* is accepted as a synonym of *H. finlandica*. Other *Chloridium* spp. belong to the *Chaetosphaeriales* (Gams & Holubová-Jechová 1976, Réblová & Winka 2000).

Hyaloscypha variabilis (Hambl. & Sigler) Vohník, Fehrer & Réblová, **comb. nov.** MycoBank MB825018.
Basionym: *Meliniomyces variabilis* Hambl. & Sigler, Stud. Mycol. 53: 12. 2005.

Specimens examined: **Canada**, Alberta, Jasper National Park, Outpost Lake, isolated from *Rhododendron albiflorum* roots, 29 Aug. 1994, S. Hambleton S-70Ac, (ex-type culture UAMH 8861). **Czech Republic**, Southern Bohemia, Bohemian Forest National Park, Modrava, isolated from *Picea abies* ectomycorrhiza, 2003, M. Vohník MVA-1 (living culture CCF 3583).

Notes: For description, illustration and growth details see Hambleton & Sigler (2005). For details on re-syntheses and mycorrhizal experiments see Vrålstad *et al.* (2002) and Vohník *et al.* (2007a,b).

Hyaloscypha vraolstadiae (Hambl. & Sigler) Vohník, Fehrer & Réblová, **comb. nov.** MycoBank MB825019.
Basionym: *Meliniomyces vraolstadiae* Hambl. & Sigler, Stud. Mycol. 53: 18. 2005.

Specimens examined: **Canada**, Quebec, Duparquet Lake, isolated from *Cenococcum geophilum* mycorrhizae of *Abies balsamea*, 17 May 2007, G. Kernaghan & G. Patriquin ARSL 170507.36 (living culture UAMH 11203). Nova Scotia, Cape Breton Highlands National Park of Canada, Mount MacKenzie, isolated from *Cenococcum geophilum* mycorrhizae of *Picea glauca*, 7 Sep. 2007, G. Kernaghan & G. Patriquin ARSL 070907.12 (living culture UAMH 11204). **Norway**, Akershus, Eidsvoll, isolated from *Betula pubescens* seedling roots, 1998, T. Vrålstad (ex-type culture CBS 116126 = UAMH 10111, CBS 116127 = UAMH 10112).

Notes: For description, illustration and growth details see Hambleton & Sigler (2005). For details on re-synthesis and mycorrhizal experiments see Vrålstad *et al.* (2002). Isolates attributed to this species form two well distinguished subclades in ITS analyses (Fig. 1) as in Vrålstad *et al.* (2002), which may be indicative of cryptic species. Whether or not morphological differences between the two groups of *M. vraolstadiae* can be

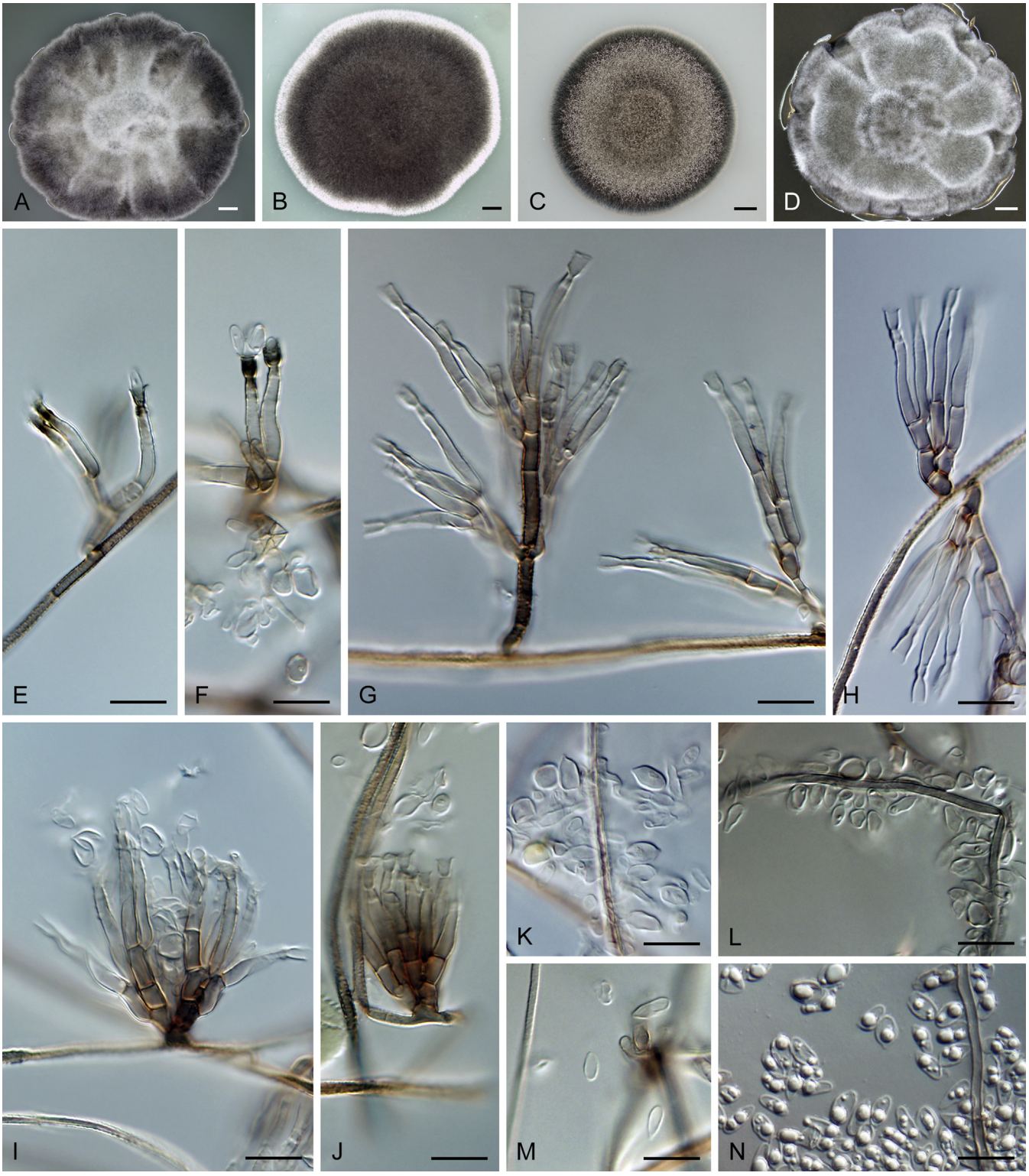


Fig. 10. *Hyaloscypha bicolor* CBS 144009. **A–D.** Colony details on MEA, MLA, PCA and PDA after 28 d. **E–J.** Conidiophores with phialides, on MMN2, 18 mo. **K–N.** Conidia, on MMN2, 18 mo. Scale bars: A–D = 2 mm, E–N = 10 µm.

Table 2. Diagnostic morphological characters of *H. finlandica* and *H. bicolor*.

Taxon	Strain	Phialides	Collarettes (depth x width)	Conidia	Conidia L:W ratio	Cells of branches	Reference
H. finlandica	CBS 444.86	(15–)18–20(–29) × (2–)2.5–3 µm	(3–)4–5 × 2–2.5(–3) µm	4.5–6 × 1.5–2 µm	3–4:1	Doliiform, 6–8 × 4–7 µm	Wang & Wilcox (1985)
H. bicolor	CBS 144009	(16–)18–25(–29) × 2.5–3(–3.5) µm	(3–)3.5–4.5 × 3–4 µm	(5.5–)6–7(–7.5) × 3–4 µm	(1.5–)2:1	Subcylindrical, 3.5–6(–7) × 3–4(–5) µm	This study

found that would justify the description of subclade 4 as a new species exceeds the scope of this paper.

Hyaloscypha aureliella (Nyl.) Huhtinen, Karstenia 29: 107. 1990. Figs 8C, 11.
Basionym: *Peziza aureliella* Nyl., Not. Sällsk. Fauna et Fl. Fenn. Förh., Ny Ser. 10: 49. 1868.
Synonyms: *Dicoccum microscopicum* P. Karst., Meddn Soc. Fauna Flora fenn. 14: 91. 1887.
Cheiromycella microscopica (P. Karst.) S. Hughes, Can. J. Bot. 36: 747. 1958.

Conidiophores on MMN2 9–18 µm long, 2.5–4(–5) µm wide, semi-macronematous, monilioid, consisting of oblong to subglobose cells, simple or branched, hyaline, thin-walled, smooth, arising from vegetative hyphae, often reduced to single conidiogenous cells. *Conidiogenous cells* 4.5–6.5(–7.5) µm long, 3–4.5 µm wide (mean ± SD = 5.5 ± 1.1 × 3.8 ± 0.7 µm), terminal and lateral integrated in the conidiophore or discrete arising from vegetative hyphae, mono- or polyblastic, subglobose, ellipsoidal to ellipsoidal-conical, hyaline, thin-walled. *Conidia* phragmosporous 12–14.5(–16.5) × 5–5.5 µm (mean ± SD = 13.8 ± 1.9 × 5.2 ± 0.2 µm), 1–3-septate, most conidia cheiroid with a total length of 12–16.5(–18) µm, composed of a subglobose to rhomboid 1-celled base 5.5–6.5 × 4.5–5.5(–6) µm (mean ± SD = 5.7 ± 0.3 × 5.5 ± 0.5 µm) and two arms, 9–12(–13) × 4.5–5.5 µm (mean ± SD = 10.2 ± 1.6 × 5.1 ± 0.3 µm), arms subequal in length to distinctly unequal, divergent or non-divergent, composed of 2–3 cells, constricted at the septa, medium brown, base tends to be paller than the arms, smooth, rounded apically, base rounded to truncate.

Cultural characters: On MEA, colonies 17–20 mm diam after 28 d (12–14 mm after 14 d, 15–17 after 21 d), raised, circular, appearing waxy mucoid. Aerial mycelium sparse, with funiculate projections at the centre and margins, remaining mycelium of a moist appearance, developing several deep folds, colony surface whitish to ivory with a pale salmon orange pigment at the centre, beige at the margin; margin distinct and regular to weakly undulate; reverse beige. On MLA, colonies 17–18 mm diam after 28 d (11–12 mm after 14 d, 14–16 after 21 d), concave, circular. Aerial mycelium sparse, with funiculate projections at the centre, cottony to cobwebby at the margin, colony surface creamy to ivory with irregular white patches, grey at the centre; margin distinct and slightly undulate; reverse white. On PDA, colonies 13–14 mm diam after 28 d (9–10 mm after 14 d, 11–12 after 21 d), concave, circular, appearing waxy-mucoid. Aerial mycelium at the centre of the colony, sparse, colony surface beige with a thin pale brown ring at the margin; margin distinct and regular, slightly filiform; reverse beige. On PCA, colonies 22–24 mm diam after 28 d (13–15 mm after 14 d, 18–20 after 21 d), slightly

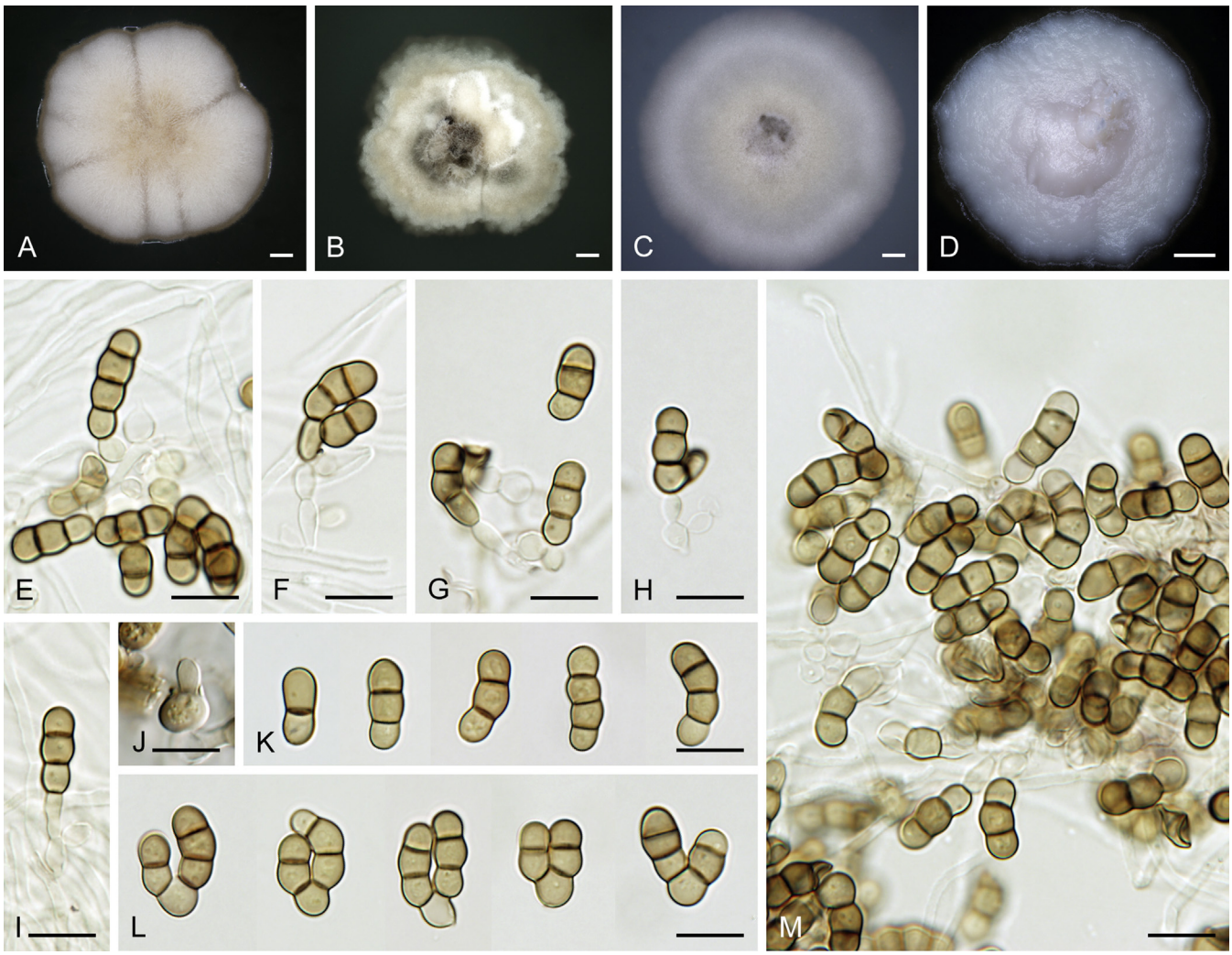


Fig. 11. *Hyaloscypha aureliella* CBS 126298. **A–D.** Colony details on MEA, MLA, PCA and PDA after 28 d. **E–J.** Conidiogenous cells with conidia, on PCA, 28 d. **K–M.** Conidia, on PCA, 28 d. Scale bars: A–D = 2 mm, E–M = 10 µm.

raised, circular. Aerial mycelium dense, cottony, colony surface beige to ivory with a paler marginal ring; margin distinct and regular; reverse beige. Sporulation observed only on PCA and MLA after 28 d and previously also on MMN2 after 45 d at 25 °C in darkness, sparse at the centre of the colony.

Specimen examined: **United Kingdom**, Scotland, Cairngorms National Park, Anagach wood, on decaying wood, S. Huhtinen (TUR 172136, culture CBS 126298).

Notes: For full synonymy, description and illustration of the sexual morph see Huhtinen (1990) and Quijada *et al.* (2017), for full synonymy and nomenclatural comments of the asexual morph see Braun *et al.* (2009). Among *Hyaloscypha* species, *H. aureliella* is similar to *H. fuckelii* in morphology and size of ascospores, but it is distinguished from the latter by yellowish-brown to brown resinous granules on apothecial hairs with a wider apex and the frequent presence of amyloid nodules in the excipulum. Both species also differ in conidiogenesis, which is holoblastic in *H. aureliella* and phialidic in *H. fuckelii* (Huhtinen 1990). Also, while *H. fuckelii* belongs to the core group of *Hyaloscypha*, *H. aureliella* is genetically fairly divergent (Fig. 5).

The *Cheiromycella microscopica* asexual morph was repeatedly obtained from ascospore isolates of *H. aureliella* in axenic culture (Huhtinen 1990, this study). *Cheiromycella* is delimited to dematiaceous hyphomycetes producing sporodochia on the host and cheiroid conidia formed on mono- or polyblastic conidiogenous cells. The genus consists of three species described from wood and leaves of deciduous trees, but except *C. microscopica* no other sexual-asexual relationship has been reported (Braun *et al.* 2009).

Hyaloscypha hepaticicola (Grélet & Croz.) Baral *et al.* [as '*hepaticola*'], Karstenia 49: 7. 2009.
Basionym: *Trichopeziza hepaticicola* Grélet & Croz. [as '*hepaticola*'], in Grélet, Bull. trimest. Soc. mycol. Fr. 41: 85. 1925.
Synonyms: *Pezizella ericae* D.J. Read, Trans. Br. Mycol. Soc. 63: 381. 1974.
Hymenoscyphus ericae (D.J. Read) Korf & Kernan, Mycologia 75: 919. 1983.
Rhizoscyphus ericae (D.J. Read) W.Y. Zhuang & Korf, Nova Hedw. 78: 481. 2004.
Pezoloma ericae (D.J. Read) Baral, Acta Mycol. 41: 16. 2006.
Scytalidium vaccinii Dalpé *et al.*, Mycotaxon 35: 372. 1989.

Specimens examined: **Finland**, Etelä-Häme, Tammela, Liesjärvi National Park, grid 6730:3329, on *Lophozia* and *Ptilidium*, 4. Jul. 2005, Nieminen 10 (TUR 180982, living culture CBS 126283); ibid., Varsinais-Suomi, Kemiö, Gästerby, Solbacka, grid 66862:32639, on *Ptilidium*, 4. Aug. 2006, Kukkonen 24 (TUR 180981, living culture CBS 126291). **United Kingdom**, England, Yorkshire, Bolsterstone, isolated from *Calluna vulgaris* roots, Jul. 1970, D.J. Read (**holotype** of *Pezizella ericae*, IMI 182065, dried culture UAMH 6652, living culture UAMH 6735).

Notes: For description and illustration of the sexual morph see Read (1974), Hambleton *et al.* (1999) and Baral *et al.* (2009), for asexual morph and growth details in axenic culture see Dalpé *et al.* (1989) and Hambleton & Sigler (2005). Because no ex-type culture of the holotype of *H. hepaticicola* exists (France, Provence-Alpes-Côte d'Azur, Var dept., Notre Dame des Anges near Pignans, on stems of living *Cephaloziella byssacea*, Jun.1924, de Crozals; not examined), two non-type strains of *H. hepaticicola* derived from recent collections on *Lophozia* and *Ptilidium* (Baral *et al.* 2009) were examined. Phylogenetic analyses of four markers of these two isolates and of the ex-type strains of *R. ericae* and *S. vaccinii* strongly support their conspecificity; based on the priority, both latter species are reduced to synonymy with *H. hepaticicola*. However, without appropriate material for epitypification [should preferably be on *Cephaloziella divaricata* (syn.: *C. byssacea*) from southern France], we refrain from selecting an epitype and ex-epitype strain from the two specimens of *H. hepaticicola* analysed in this study or other recent herbarium material collected in Finland (Baral *et al.* 2009). These isolates were selected by Baral *et al.* (2009) to represent the species in their *Hyaloscypha* phylogeny. In the same publication, *H. hepaticicola* was described and illustrated based on three collections labelled H.B. 6377, H.B. 7111 and H.B. 7120. We examined herbarium specimens of the two isolates of *H. hepaticicola* and confirm they match the description and illustration provided by Baral *et al.* (2009, figs 2–4).

Under specific growth conditions, 0–1-septate thallic conidia are formed in pure culture ranging from hyaline to subhyaline or subhyaline to yellow-brown or olive-brown depending on the agar medium used (Dalpé *et al.* 1989, Hambleton & Sigler 2005).

Although *H. hepaticicola* is mainly known to fruit on bryophytes, our re-synthesis experiment with the originally bryophilous strain CBS 126291 confirmed its ability to form ericoid mycorrhiza (Fig. 6). Vice versa, during previous re-synthesis experiments of the ex-type strain of *R. ericae* (UAMH 6735), it was verified that apothecial ascomata can also form on superficial roots and in nearby soil (Read 1974). Furthermore, *R. ericae* was isolated from rhizoids of *Cephaloziella* spp. (Chambers *et al.* 1999, Upson *et al.* 2007).

DISCUSSION

REA = *Hyaloscypha*: phylogenetic evidence and species relationships

All phylogenetic analyses and molecular markers unequivocally show REA and *Hyaloscypha* as a strongly supported monophylum. The finding that core REA members *Rhizoscyphus*, based on *R. ericae*, and *Meliniomyces*, typified by *M. variabilis*, are congeneric with *Hyaloscypha* brings new perspectives to mycorrhizal research and sheds new light upon the taxonomy of the long-standing problematics of the so called *Rhizoscyphus ericae* aggregate (originally *Hymenoscyphus ericae* aggregate). In our phylogeny, *Hyaloscypha* constitutes a core group to which the majority of the analysed species belong. In the absence of the ex-type strain of *H. vitreola*, the lectotype species of *Hyaloscypha* (Huhtinen 1990), it is represented in our multigene phylogeny by two non-type strains collected in Finland (Baral *et al.* 2009). The distribution of known asexual morphs of *Hyaloscypha* spp. does not seem to form any pattern. On the most basal branches of the *Hyaloscypha s. str.* clade (Figs 1, 4, 5) reside species with predominantly holoblastic (*H. monodictys*, *H. spiralis*) but also phialidic (*H. fuckelii*) conidiogenesis in contrast to *H. bicolor* and *H. finlandica* also producing phialidic conidia but which reside on upper branches in the ITS phylogram (Fig. 1). The thallic conidiogenesis is limited to a single clade of *H. hepaticicola* whose position varies in the ingroup.

Although species relationships of the ingroup are largely unresolved except for one subgroup (Fig. 5, bottom), several new species pairs were found that assign a traditional REA member to a particular species of *Hyaloscypha*. The most

prominent example is *H. hepaticicola* and its *Rhizoscyphus ericae* synonym linked with the asexual state originally described as *Scytalidium vaccinii* (Fig. 1). Another case is the assignment of sterile *H. variabilis* (syn. *Meliniomyces variabilis*) with a sexual *Hyaloscypha* sp. (represented by strains M19, M25) (Schoch *et al.* 2012). Their sequence similarities, in addition to strongly supported branches indicate conspecificity. However, either intraspecific differences are higher in this group or they form closely related sister taxa, which cannot be decided based on the available data. Further examples are outlined above for individual datasets, but in all those cases, asexual or sexual *Hyaloscypha* spp. or both are undescribed.

Rhizoscyphus ericae = *Hyaloscypha hepaticicola*: evidence from ericoid mycorrhizal re-synthesis

Ericoid mycorrhiza has long been viewed as a domain of ascomycetous mycobionts, and especially of the typical ascomycetous ErM fungus *R. ericae* (Smith & Read 2008). Recently, however, basidiomycetous sheathed ericoid mycorrhiza formed by *Kurtia argillacea* (*Hymenochaetales*) has been described and re-synthetized *in vitro* (Vohník *et al.* 2012, Kolařík & Vohník 2018), and a serendipitoid (*Serendipitaceae*, *Sebacinales*) strain derived from *Vaccinium* hair roots has been experimentally proven as ericoid mycorrhizal (Vohník *et al.* 2016). This together with the fact that *R. ericae* is absent in the roots of *Ericaceae* at many locations worldwide (*e.g.* Bruzone *et al.* 2015, Lorberau *et al.* 2017) may eventually lessen its central position in the ErM research. Nevertheless, to date it is by far the most investigated and best understood ErM mycobiont with global distribution, including South America (Bruzone *et al.* 2017), South Africa (Kohout & Tedersoo 2017) and Australia (Midgley *et al.* 2017). It was thus unexpected that the well-researched ericoid mycorrhizal *R. ericae* and the bryophilous *H. hepaticicola* should be the same entity, the latter being found in a symbiosis with a liverwort and formally described nearly fifty years earlier. On the other hand, *R. ericae* is well known from rhizoids of various liverworts (Chambers *et al.* 1999, Upson *et al.* 2007, Kowal *et al.* 2016), and re-syntheses confirmed that liverwort-derived *R. ericae* can form ericoid mycorrhizae in the *Ericaceae* (Upson *et al.* 2007, Kowal *et al.* 2016), similarly to our results with the bryophyte-derived *H. hepaticicola* CBS 126291 and *Vaccinium* (Fig. 6). Thus, the experimental evidence supports the molecular/morphological evidence showing that *R. ericae* and *H. hepaticicola* are conspecific.

In *V. myrtillus* roots, the *Pinaceae*-derived *H. bicolor* CBS 144009 formed intracellular hyphal coils interpreted here as ericoid mycorrhiza, which is in agreement with our previous results with the *Pinaceae*-derived *H. bicolor* CCF 3582 (GenBank EF093180) and the same host (Vohník *et al.* 2013). Since *H. bicolor* can form the so-called *Piceirhiza bicolorata* ectomycorrhizal morphotype with suitable hosts (Vrålstad *et al.* 2000), it might connect co-occurring ErM and ectomycorrhizal plants via shared mycelium. While this phenomenon has been demonstrated to some extent *in vitro* (Villareal-Ruiz *et al.* 2004), the observations from a more realistic outdoor experiment (Kohout *et al.* 2011) and natural habitats (Bruzone *et al.* 2015, 2017) hint against an ecological significance of such possible shared mycelial networks. Additionally, it is important to note that the colonisation pattern of *H. bicolor* CBS 144009 differed from the typical ErM pattern produced by the tested *H. hepaticicola*/*R. ericae* isolates, both in terms of morphology and frequency. Further research is apparently needed to elucidate this intriguing relationship between ericoid mycorrhizal and ectomycorrhizal plants and their root mycobionts.

REA species: phylogenetic placement and taxonomic consequences

Evidence from molecular and morphological data and the re-synthesis experiment confirm that *H. hepaticicola* and *R. ericae* are conspecific. *Hyaloscypha hepaticicola* inhabits many ecological niches and includes ericoid mycorrhizal symbionts, saprobes on arboreous litter or soil and also bryosymbiotic strains fruiting mostly on living hepatics and liverworts. These life styles are represented by several isolates in our phylogenetic analyses.

The newly described species *H. melinii* (Figs 7–9) producing only sterile mycelium in culture is genetically clearly distinct from all other previously known members of REA and available *Hyaloscypha* species. In the combined dataset (Fig. 5), it falls into the subclade including *H. vitreola*, *H. variabilis*, *H.* cf. *bulbopilosa*, and *Hyaloscypha* spp. (strains M19, M20, M288) but it does not show close relationships with any particular species in any of the datasets.

Sterile root-associated isolates previously accommodated in *Meliniomyces* (Hambleton & Sigler 2005) are nested among sexual *Hyaloscypha* species in our phylogenies, however they remain without closer relationship except one case discussed above. These fungi were provisionally labelled according to the type of mycorrhiza they formed as "*Piceirhiza bicolorata*", "Hemlock mycorrhiza" or "Salal mycorrhiza" (Vrålstad *et al.* 2000, Hambleton & Sigler 2005) or according to the cultural morphotype as "Variable White Taxon" (Hambleton *et al.* 1999) or "Sterile white 1" (Summerbell 1989). The simple cultural morphology and a general lack of distinguishing characters have made it challenging to differentiate individual genotypes or suggest their conspecificity until these issues have been facilitated by molecular data and sequence comparison (*e.g.* McLean *et al.* 1999, Chambers *et al.* 2000, Vrålstad *et al.* 2000, 2002, Hambleton & Sigler 2005). Based on phylogenetic evidence from four markers (Figs 1–5), *M. bicolor*, *M. variabilis* and *M. vraolstadiae* are accepted in *Hyaloscypha* and new combinations are proposed. Our strain of *H. bicolor* (CBS 144009), whose sequences always clustered with those of the ex-type strains of *H. bicolor* and *H. finlandica*, was observed sporulating *in vitro* for the first time (Fig. 10, Table 2). The sporulation in both species is typically protracted and induced by cold treatment (Wilcox *et al.* 1974, Wang & Wilcox 1985, this study).

A new combination of *Cadophora finlandica* in *Hyaloscypha*, including its *Chloridium paucisporum* synonym, is proposed. In each dataset (Figs 1–5), the type strains of *H. bicolor* and *H. finlandica* show very similar, but distinguishable sequences, which always form well-supported groups (in ITS, support is only significant in BA, which is most likely due to the large intraspecific variation of strains diverging from the type strains). Although *Cadophora* with *C. fastigiata* as the type species was established for *Phialophora*-like fungi, ITS sequence data suggest the genus is polyphyletic (Harrington & McNew 2003). While the core of *Cadophora* was recovered as an *incertae sedis* lineage in the *Leotiomycetes* with affinities to the *Dermateaceae*, recently

referred to the *Ploettnerulaceae* (Kirschstein 1923), its segregates were disposed to four genera in three fungal classes (Hughes 1958, Schol-Schwarz 1970, Vijaykrishna *et al.* 2004, Crous *et al.* 2007, Grünig *et al.* 2009, Day *et al.* 2012, Réblová *et al.* 2015).

Hyaloscypha taxonomy, phylogenetic evidence and open questions

The genus *Hyaloscypha* was monographed by Huhtinen (1990) and segregated from morphologically similar *Hamatocanthoscypha* and *Phialina* using a combination of diagnostic morphological, chemical and ecological characters. Their separation was later corroborated with molecular data by Han *et al.* (2014). Huhtinen (1990) segregated *Hyaloscypha* into two subgenera; *Eupezizella* with four species and two varieties was distinguished from *Hyaloscypha* by the presence of resinous exudates on predominantly blunt apothecial hairs lacking a dextrinoid reaction, preference to softwood and occasional amyloid reaction in the hairs and/or excipula. Although Index Fungorum lists 224 species and variety names of *Hyaloscypha*, only a small fraction has been studied with DNA sequence data or isolated in axenic culture.

Besides numerous sterile isolates, *Hyaloscypha s. str.* encompass up to three types of asexual spore structures including phialidic, holoblastic and thallic conidia. The sexual-asexual connections of *Hyaloscypha* have been so far experimentally confirmed only for *H. aureliella* and *Cheiromycella microscopica* (Huhtinen 1990), *H. fuckelii* var. *fuckelii* and *Phialophora*-like (Huhtinen 1990), *H. hepaticicola* and *Scytalidium vaccinii* (as *R. ericae*, Egger & Sigler 1993, Hambleton & Sigler 2005), *H. monodictys* and *Monodictys* sp. (as *H. albohyalina* var. *monodictys*, Hosoya & Huhtinen 2002), *H. spiralis* and *Pseudaegerita corticalis* (as *H. lignicola*, Abdullah & Webster 1983), and *H. zalewski* and *Clathrosphaerina zalewski* (Descals & Webster 1976). Most of these species were analysed in this study. Four of them belong to the core group of *Hyaloscypha* (*H. fuckelii*, *H. hepaticicola*, *H. monodictys*, *H. spiralis*), one to *Hyaloscypha s. lat.* (*H. aureliella*). Several *Monodictys* sp. ITS sequences available in GenBank form three groups that are not convincingly alignable to each other. The only reliably identified sample is *M. arctica* from type material along with other almost identical sequences of that species. However, these sequences are not alignable to our dataset and certainly not similar to *Hyaloscypha monodictys*. *Monodictys* is obviously a polyphyletic genus and its treatment is beyond scope of this paper. *Hyaloscypha fuckelii* forms *in vitro* hyaline phialides with a hardly perceptible collarette unlike the dematiaceous phialides with a conspicuous, darker, wedge-shaped collarette of *H. bicolor* and *H. finlandica*. The polyphyletic nature of *Phialophora* and *Phialophora*-like fungi (*e.g.* Gams 2000) and *Cadophora* (Harrington & McNew 2003), whose segregates fall into *Hyaloscypha* has been documented with molecular tools for various taxonomic groups.

Pseudaegerita is formally reduced to synonymy with *Hyaloscypha* in this study; however, it shows a polyphyletic concept. Members of this genus inhabit fresh water environments or very damp shadowy places and are characterised by holoblastic conidia composed of a highly branched system of torulose cells. *Pseudaegerita* consists of eight species, but DNA sequence data are available only for *P. corticalis*, the type species, and *P. viridis* (Abdullah & Webster 1983). While *P. corticalis* (= *H. spiralis*) groups in *Hyaloscypha s. str.*, *P. viridis* is a member of *Dermateaceae*; based on ITS sequences of two strains (Cooper *et al.*, unpubl., Bruzone *et al.* 2015, Fig. 1) it shows some similarity with the ex-type and another non-type strain of *Coleophoma cylindrospora* (Crous *et al.* 2014). Another asexual-sexual connection was experimentally proved between *Pseudaegerita* sp. and *Claussenomyces atrovirens* (*Tympanidaceae*) (Fisher 1985).

A polyphyletic concept of *Scytalidium*, based on *S. lignicola*, has also been recognized. Although *S. vaccinii* is accepted as a synonym of *H. hepaticicola*, the type species of *Scytalidium* is not congeneric with *Hyaloscypha*; based on nrSSU sequences it was shown to be distantly related to the REA (Hambleton & Sigler 2005).

Although *Rhizoscyphus* is treated as a generic synonym of *Hyaloscypha*, it contains a second species, *R. monotropae*. It is morphologically similar to *H. hepaticicola* with which it also shares plant-associated and saprobic lifestyles. ITS sequences of five strains of *R. monotropae* are available in GenBank (as *Hymenoscyphus monotropae*); four have been isolated as members of root fungal communities of *Tsuga canadensis*, *Vaccinium uliginosum*, and *Salix arctica*, and only one strain was associated with roots of *Monotropa uniflora* (UAMH 6650, Egger & Sigler 1993). The authors have already pointed out the high sequence divergence (24 %) between *R. monotropae* and fungi assigned to the REA. According to BLAST searches (97 % similarity) and its position in the ITS tree (Fig. 1), *R. monotropae* is conspecific or at least congeneric with *Cyathicula microspora* M267 (Baral *et al.* 2009), which is included as an outgroup in our study. Given these facts, *R. monotropae* is not accepted in *Hyaloscypha*.

Of the *Hyaloscypha* species analysed in this study, four were shown outside the *Hyaloscypha s. str.* clade (Figs 1, 5). Two species, *H. aureliella* and *H. albohyalina*, are either sisters to the core group, or nest among the most closely related outgroup genera, or show long isolated branches in basal positions if they group with *Hyaloscypha s. str.*/REA. *Hyaloscypha aureliella* is the only species assigned by Huhtinen (1990) to the subgenus *Eupezizella* and studied with DNA sequence data. It forms cheiroid and phragmosporous conidia *in vitro* (Fig. 11); its asexual morph is *C. microscopica*, the type species of *Cheiromycella* (Hughes 1958). One fungal strain isolated from live xylem of *Betula pendula* (as "*Hymenoscyphus* sp. olrim148") apparently belongs to *H. aureliella* (Fig. 1). Although the asexual morph of *H. albohyalina* var. *albohyalina* is unknown, two of its varieties, var. *monodictys* and var. *spiralis*, recently elevated to the species level by Han *et al.* (2014) and grouping within the core of *Hyaloscypha* (Fig. 5), were linked with two different asexual morphs, *i.e. Monodictys* and *Pseudoaegerita* (see above). Based on limited sampling and partly inconsistent results from different markers, we treat *H. albohyalina* and *H. aureliella* as *Hyaloscypha s. lat*. Both show isolated lineages in phylogenetic trees and accordingly, they may actually represent two different genera distinct from *Hyaloscypha*. For that reason, we refrain from formally accepting *Cheiromycella* in *Hyaloscypha* as its synonym and we do not propose new combinations for two other *Cheiromycella* spp. which are not corroborated by molecular data. However, the generic name *Cheiromycella* is available and recommended for use to accommodate *H. aureliella* and related fungi if its separate position from *Hyaloscypha s. str.* is confirmed with more concentrated sampling including also other species of the subgenus *Eupezizella*.

Other "*Hyaloscypha*" species, whose sequences were retrieved from GenBank and studied with our datasets, do not belong to this genus, even in its widest sense. "*Hyaloscypha* sp. 2-13c" is a misidentified sample whose ITS sequence (KC790474, Long *et al.* 2013) shows 93–94 % similarity with species of *Pseudeurotium*, however, its highest sequence similarity is shared with mostly unpublished environmental samples. In the ITS tree (Fig. 1), it groups near *Pseudaegerita viridis* and *Coleophoma cylindrospora* (*Dermateaceae*). Three collections tentatively identified as *Hyaloscypha* aff. *paludosa* (Stenroos *et al.* 2010), although no description or illustration was provided (herbarium material deposited in TUR), evidently belong to *Arachnopeziza* according to all datasets for which sequence data were available. However, we refrain from making a formal combination of *H. paludosa* in *Arachnopeziza* based on these specimens until the holotype of *H. paludosa* is examined and representative specimens are studied with molecular DNA data. *Hyaloscypha zalewskii*, experimentally confirmed to form *Clathrosphaerina zalewskii* asexual state *in vitro* (Descals & Webster 1976), which is characterised by holoblastic, clathrate hollow conidia, was not included in our study. A single available ITS sequence (EF029222) of a strain derived from conidia from New Zealand material (ICMP 15322, Cooper *et al.*, unpubl.) suggests it is unrelated to *Hyaloscypha*.

Despite the large number of characters, relationships among outgroup genera are generally not well resolved (either not at all, or only in some analyses), not even in the combined dataset (Fig. 5). Similarly, ingroup relationships were not much better resolved than in analyses based on individual markers. Moreover, many ingroup taxa had to be omitted from combined analyses because they were only available for part of the datasets. Thus, combining datasets did not result in considerably improved resolution of species relationships. High levels of homoplasy or putatively rapid radiation of ingroup taxa and conflicting placements of outgroup taxa with different markers may be responsible for this outcome. Detailed targeted taxonomic work, denser taxon sampling and additional markers may help to improve the resolution of species relationships. However, resolving infrageneric relationships within *Hyaloscypha* is beyond the scope of this study. Nevertheless, we present the most comprehensive and detailed phylogenetic analysis focused on *Hyaloscypha*, which may serve as a sound basis for further taxonomic work on the genus.

Impact of sequence data quality on phylogeny

While focusing on a taxonomic revision of the REA, we were able to sort out many problematic issues in terms of sequence quality, obvious or likely confusion or misidentification of samples in public databases. In the course of dataset assembly, we had to rely to a large degree on previously published sequence data from various sources. Careful inspection of this data revealed a number of problems that will inevitably lead to misinterpretations or to artefacts of tree construction if they go undetected. Apart from erroneously assigned names and apparently poor sequence read quality, which are common problems that can be dealt with comparably easily, we also detected more serious cases that have been overlooked in previous publications; these comprise (i) a chimeric sequence, (ii) confused samples of allegedly the same strain, and (iii) numerous polymorphic bases at phylogenetically informative positions.

(i) Chimeras are artefacts produced by PCR-mediated recombination. In the particular case, one sequence of *R. ericae* [AJ430176, "cf. *Hymenoscyphus ericae* agg.", Vrålstad (2001)] was found to be a chimera between *R. ericae* and *Cadophora luteo-olivacea* that may have resulted from contamination of the source material or as a PCR contamination by previously amplified samples. As genus *Cadophora* is not a member of the REA, this sequence part (ca. 120 bp in ITS2) was very divergent compared to all other sequences included in the present study. In phylogenetic analyses, chimeras or recombinants end up either somewhere between clades, or basal to clades matching the longest or the most variable or diagnostic part of the sequence, depending on the recombination point (Soltis *et al.* 2008, Kaplan *et al.* 2018). By this, they may not only be confused with isolated or new lineages as in Vrålstad *et al.* (2002) and Hambleton & Sigler (2005) in the particular case, but they are also affecting topology and branch support of "good" clades.

(ii) Confusion of samples of different molecular markers for the same strain may go undetected depending on the analysis. Han *et al.* (2014) have apparently overlooked such an artefact concerning sequences ITS and nrLSU (AB546939, AB546938, Hosoya *et al.* 2011) and mtSSU and *rpb2* (JN086800 and JN086875, Han *et al.* 2014) of strain TNS-F-17333 (= NBRC 106631). According to ITS and nrLSU it belongs to *H. albohyalina* whereas it is identical to *H.* sp. TNS-F-17335 based on mtSSU and *rpb*2, which occupy very divergent positions in the tree according to all markers. The tree based on a combined dataset in Han *et al.* (2014) grouped *Hyaloscypha* strains TNS-F-17333 and TNS-F-17335 together, but with relatively large divergence and unequal branch lengths of tips. Their sister relationship reflects the high variation and large number of characters in both mtSSU and *rpb*2 that constitute the majority of the signal in the combined dataset whereas their alleged differences were contributed by ITS and nrLSU. When analysing a single dataset, an unusual placement might be recognized depending on the taxon sampling. However, if phylogenetic analyses based on individual markers are not performed and assessed for plausibility, erroneous placements will follow in combined datasets that may even receive 100 % support as in the described case.

(iii) Polymorphisms within sequence reads can be caused by technical difficulties (poor sequencing quality), actual intragenomic differences (*e.g.* multicopy genes, heterozygosity, hybrids) or contamination of samples. Two *rpb*2 sequences of *H. vitreola* (FJ477057) and *Hymenoscyphus fructigenus* (EU940365) (Baral *et al.* 2009) were found to exhibit large numbers of polymorphisms (28 and 37, respectively) which by far exceed reasonable noise levels. Their alternative character states (bases) happened to reflect differences between taxa at the particular positions, *i.e.* at phylogenetically informative positions (without suggesting particular mixes). Whether an inadvertent mixture or PCR contamination of samples or the uncritical use of a software consensus function has led to this outcome cannot be decided. To include such data into phylogenetic analyses can merge clades or decrease their support or resolution, and the sequences may end up in basal or unresolved positions depending on the degree of overall phylogenetic signal (Chrtek *et al.* 2009).

To sum up, working on data that are largely retrieved from public databases requires much care and critical assessment in order to identify potential errors. Here, we have identified a

number of erroneously assigned taxa in different datasets and many sequences that are problematic for some reason, which will be helpful for others who work on these fungi to avoid spurious conclusions or artefacts in tree construction.

Species concepts and genetic divergence

What constitutes a species is a long-standing question as reflected by numerous, often conflicting species concepts independent of the group of organisms studied. The intrinsic difficulty is to transform a snapshot of an evolutionary process into a static classification system. Groups that are notoriously poor in morphological characters or may not always show them as in the case of asexual morphs or vegetative states add another layer of complexity to a general problem. Molecular data have helped immensely to inform taxonomic decisions, especially in such groups. Yet the question remains how species shall be treated if morphology, the degree of sequence identity of molecular markers or the position in phylogenetic trees are equivocal or in conflict with each other. For example, micromorphology may clearly distinguish two entities, but sometimes the difference is not so conspicuous or does not exist, sometimes species are clearly distinguished by colony characters, sometimes by a primary sequence (*e.g.* Réblová *et al.* 2015, 2018). Thus, different species may not only have different features of how and when they have formed and split from sister taxa, but also the nature of their morphological or molecular diagnostic characters may differ from case to case. Examples from this study again illustrate that there is no universal way to look at things, not even within closely related groups.

Hyaloscypha bicolor and *H. finlandica* are very closely related according to molecular and morphological characters, yet distinguishable in both (Fig. 1, Table 2). Therefore, although *H. bicolor* is paraphyletic with respect to *H. finlandica* (including *Chloridium paucisporum*) in phylogenetic analysis, we consider it justified to treat them as different species of which the latter appears to constitute a relatively recently diverged lineage. A similar pattern was described in a group of sterile lichens (Bayerová *et al.* 2005). The most contrasting case is *H. spiralis* (including *Pseudaegerita corticalis*) where large intraspecific genetic variation is observed (Figs 1, 2), which may be interpreted as an indication for a relatively old and potentially widespread species. With respect to morphological characters and clade monophyly, species status is undisputed, however, this species shows a high intraspecific sequence variation (up to 4.2 % *p*-distance without indels) in the ITS region. Thus, the common practise of molecular species identification based solely on a percentage of sequence identity in BLAST searches is not always suitable to identify species boundaries reliably as was also shown for South American strains of *R. ericae* / *H. hepaticicola* (Bruzone *et al.* 2017).

On the other hand, the power of molecular sequence data in taxonomy is best seen when morphological convergence or the lack of appropriate characters fail to reveal independent species (*e.g.* Fehrer *et al.* 2008) or higher order taxa. Examples for *Hyaloscypha* comprise *H. albohyalina*, *H. spiralis* and *H. monodictys* or *H. fuckelii* and *H. alniseda* that constitute fairly divergent species instead of mere varieties of the same species (Han *et al.* 2014, this study). In the same way, many polyphyletic genera are here identified or confirmed as such according to distant phylogenetic position (*Pezoloma*, *Cadophora*, *Hymenoscyphus*, *Rhizoscyphus*, *Pseudaegerita*, *Monodictys*, *Phialophora*, *Scytalidium*), and wrongly assigned names or misidentified strains are revealed (*e.g.* '*Hyaloscypha*' sp. 2-13c, '*Arachnopeziza variepilosa*' M337, '*H. albohyalina* var. *spiralis*' M259, '*H. minuta*' G.M. 2015-04-06.2, see also the compilation of datasets in Materials and Methods). Also, higher than usual genetic divergence in combination with non-monophyly can give hints that strains attributed to the same species may actually belong to different ones (*H.* cf. *bulbopilosa*) or that taxa may represent different genera (*H. aureliella*, *H. albohyalina*) and thus generate working hypotheses for further research.

CONCLUSIONS

In the process of proving the identity of the root-symbiotic *Rhizoscyphus ericae* aggregate with the inoperculate discomycetes of the genus *Hyaloscypha*, we also encountered many problematic molecular data and taxonomic treatments. Some of them could be successfully solved in the frame of this paper whereas others will require particular targeted studies. With the present paper we provide a much improved basis for future work on these genera and strongly advocate a comprehensive approach for species identifications or taxonomic decisions that critically considers morphological, primary sequence as well as phylogenetic evidence and discourage taxonomic decisions based on insufficient or low quality data.

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