The root-symbiotic *Rhizoscyphus ericae* aggregate and *Hyaloscypha* (Leotiomyctes) 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, nLSU, mtSSU, and rpd2 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 monophyly 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*. *Pseudagenta*, 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 melini* 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 *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 saprobic, bryophilous, 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*, *Pseudagenta*. Sexual-asexual connection.

**Taxonomic novelties:** New species: *Hyaloscypha melini* Vohník, Fehrer & Rébllová

New combinations: *Hyaloscypha bicolor* (Hambl. & Sigler) Vohník, Fehrer & Rébllová, *Hyaloscypha finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Rébllová, *Hyaloscypha variabilis* (Hambl. & Sigler) Vohník, Fehrer & Rébllová, *Hyaloscypha vralstadiæ* (Hambl. & Sigler) Vohník, Fehrer & Rébllová.

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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 (EM) fungus which inhabits *Ericaceae* hair roots worldwide (Bruzzone 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...
(plant-associated biotrophic lifestyle). Apart from molecular sequencing and ecology, *Rhizoscyphus*, typified by *R. ericae*, is delimited from *Hymenoscyphus* by discord 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 & Kriegsteiner (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 (Bruzzone 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, Huhntinen 1990) encompassing predominately lignonicolous 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 *Cheirimycella*, *Clathropsphaeria*, *Monodictys*, *Pseudaegerita* and *Philalophora*-like fungi (Descals & Webster 1976, Abdulla & Webster 1983, Huhntinen 1990, Hosoya & Huhntinen 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 *Cephalozia 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 described 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.

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 CellID (Olympus) and QuickPhoto Micro 2.3 software (Promicra Ltd., Czech Republic). All images were
Cultures were maintained on Modified Leonian's agar (MLA) (Mallow 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 re-synthesis experiment was performed with V. 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 2-3 months. Vaccinium seeds of local origin were extracted from dried fruits, surface sterilized with 10 % SAVO (common household bleach, Unilever CR 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, rpb2, 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 rpb2, only Hyaloscypha spp., but not representatives of the REA were available. Species availability and sequence variation among Hyaloscypha spp. for nrLSU, mtSSU and rpb2 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 authors teams used a largely overlapping set of molecular markers, but different regions of the rpb2 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 rpb2 regions (5–7 and 7–11) as well as for nrLSU and mtSSU. Novel nrLSU, mtSSU and rpb2 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 rpb2

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 (Storchová 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 μL of each primer. All cycling conditions consisted of 25 cycles with 95 °C for 5 min for pre-denaturation and 72 °C for 10 min for final extension and were done on a Mastercycler gradient (Eppendorf Czech & Slovakia, Ricany 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 rp2 gene were amplified: Region 5–7 used by Han et al. (2014) was amplified with primers rRPB2-5F (Liu et al. 1999) and rRPB2-P7R (Hansen et al. 2005), and region 7–11 used by Baral et al. (2009) was amplified with primers rRPB2-7cF and rRPB2-11ar (Liu et al. 1999). Cycling conditions for both rp2 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 mtLSU. 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 rp2 sequences (MH018926–MH018960) generated during this study and homologous sequences of representatives of Hylloscypha and other members of the Leotiomyctes 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 ≥ 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 Rhi- zoscyphus (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 Hylloscypha spp. were retrieved from GenBank and added manually to the alignment of the R. ericae aggregate. Additionally, we obtained cultures of all named Hylloscypha 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 Hylloscypha 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 Hylloscypha 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) |
|                  | orim148                      | 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     |              |              |            |            | Manson (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)                            |
| *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     | EU940354   |            | Baral *et al*. (2009), Stenroos *et al*. (2010) |
| *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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| 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          | NR_121279    | MH018941 | MH018934 | MH018948 | MH018954   | Grünig et al. (2002), this study             |
| Isotype*            | PRF15        | DQ485204 |        |        |            |            |                                                 |
|                     | B54J12       | EF093155 |        |        |            |            |                                                 |
|                     | FAG 15       | AF011327 |        |        |            |            |                                                 |
|                     | ARON 2948.S  | AJ292202 |        |        |            |            |                                                 |
|                     | IFM 50530    |        |        |        |            |            |                                                 |
| **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     |        |        |        |            |            |                                                 |
| CBS 116123          | AJ292203     |        |        |        |            |            |                                                 |
| ARON 2810.S         | AJ308340     |        |        |        |            |            |                                                 |
| C51.7               | X611538      |        |        |        |            |            |                                                 |
| ARON2965.S          | AJ430122     |        |        |        |            |            |                                                 |
|                     | MBl-1        | EF093180 |        |        |            |            |                                                 |
|                     | NY077        | KM216335 |        |        |            |            |                                                 |
|                     | LVR4069      | AY579413 |        |        |            |            |                                                 |
| **M. variabilis**   | UAMH 8861 Type* | AY762619 | MH018944 | MH018937 | MH018950 | MH018957 | Hambleton & Sigler (2005), this study |
|                     | MV-S-4       | EF093166 |        |        |            |            |                                                 |
|                     | ARON 2879.S  | AJ292201 |        |        |            |            |                                                 |
|                     | LF10A16D9    | JQ272355 |        |        |            |            |                                                 |
| **M. vraolstadiae** | CBS 116126, UAMH 10111 Type* | AJ292199 | MH018945 | MH018938 | MH018951 | MH018958 | Vrålstad (2002), this study |
|                     | UAMH 11203   | MH018933 |        |        |            |            |                                                 |
|                     | CBS 116127, ARON2917.S | AJ292200 |        |        |            |            |                                                 |
|                     | ARSL 070907.12 | HQ157928 |        |        |            |            |                                                 |
|                     | ARSL 230507.46 | HQ157836 |        |        |            |            |                                                 |
|                     | FG34P1       | FN678887 |        |        |            |            |                                                 |
| **H. melini sp. nov.** | SM7-2, CBS 143705 Type* | EF093175 | MH018946 | MH018939 | MH018952 | MH018959 | Vörhnik et al. (2013), this study |
|                     | SM7-1        | EF093174 |        |        |            |            |                                                 |
|                     | ECSR075      | KM678388 |        |        |            |            |                                                 |
|                     | GMU_LL_03_G4 | KC180693 |        |        |            |            |                                                 |
| **Meliniomyces sp.** | UAMH 6735 Type* | NR111110 | MH018947 | MH018940 | MH018953 | MH018960 | Vrålstad (2002), this study |
|                     | ARON 3024.S  | AJ430126 |        |        |            |            |                                                 |
|                     | ARON 2888.S  | AJ308337 |        |        |            |            |                                                 |
| Isolate 21          | AF069439     |        |        |        |            |            |                                                 |
| UAMH 8680           | AY762622     |        |        |        |            |            |                                                 |
| C43.4               | X611525      |        |        |        |            |            |                                                 |

* Reference for type isolate.
| Taxon                      | Source/type | ITS     | nrLSU     | mtSSU     | RPB2 (5–7) | RPB2 (7–11) | Reference                             |
|---------------------------|-------------|---------|-----------|-----------|------------|------------|---------------------------------------|
| D. J. Read 100            | AF151089    |         |           |           |            |            | McLean et al. (1999)                  |
| pkc29                     |             | AY394907|           | AM887699  |            |            | Lim et al. (2003), unpublished        |
| strain 111                |             |         |           |           |            |            | Turnau et al. (2007)                  |
| Scytalidium vaccinii      | UBCM5       | AF081435|           |           |            |            | Monreal et al. (1999)                 |
|                           |             |         |           |           |            |            | Monreal et al. (1999)                 |
| Eriocid endophyte         | UAMH 5828 Type  | AF081439|           |           |            |            | Sharples et al. (2000), unpublished   |
| Fungal sp.                | 3.44.4J     | KJ649999|           |           |            |            | Sarjala et al. (2014), unpublished    |
| Calluna vulgaris          | Fungus agrKH180 | FM172867|           |           |            |            | Pietrowski et al. (2008), unpublished |
|                           |             |         |           |           |            |            |                                      |
| Epacris microphylla       | Fungus 13   | AY268197|           |           |            |            | Williams et al. (2004)                |
| root associated           |             |         |           |           |            |            |                                      |
| Epaclid root endophyte    | RK1-11      | AY279179|           |           |            |            | Williams et al. (2004)                |
| Epaclid root endophyte    | RK2.4       | AY279181|           |           |            |            | Williams et al. (2004)                |
| cf. H. ericae agg.        | ARON 3014.S | AJ430121|           |           |            |            | Vrálstad (2001), Vrálstad et al. (2002) |
| Salix root associated     | UBCra264    | AF149070|           |           |            |            | Millar et al. (1999), unpublished     |
| fungus                    |             |         |           |           |            |            |                                      |
| Amicodisca castaneae      | KUS-F51377  | JN033389| JN086692  | JN086766  | JN086843   | Han et al. (2014)                     |
| Arachnopediza 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  | JN086991  |            | Han et al. (2014)                     |
| Ascocoryne cylichnium     | KUS-F52351  | JN086709| JN086782  |            |            | Han et al. (2014)                     |
| A. sarcoideae             | AFTOL-ID 1834| FJ176886| FJ238369  |           |            | Schoch et al. (2009)                  |
| Chloridiun 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   | Spatafora et al. (2006), Schoch et al. (2009) |
| Cyathicula microsora      | M267        | EU940165| EU940240  | EU940304  |            | Baral et al. (2009), Stenroos et al. (2010) |
| Dematiascuspya delicata   | TNS-F17834  | JN086739| JN086808  | JN086883  |            | Han et al. (2014)                     |
| Hymenoscyphus leuconia    | KUS-F52474  | JN086719|           |           |            | Han et al. (2014)                     |
| H. necticioida            | CBS 597.77  | JN086884| JN086761  | JN086836  |            | Han et al. (2014)                     |
| Hymenoscyphus 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) |

(continued on next page)
In addition, we included four available sequences of *Pseudaegeira corticalis* (Cooper et al., unpublished), an asexual morph of *Hyaloscypha spiralis* (Abdullah & Webster 1983) and two sequences of *Pseudaegeira viridis* (Cooper et al., unpublished, Bruzone et al. 2017) along with highly similar sequences of *Coleophoma cylindrospora* (Crous et al. 2014). Four sequences of *Hymenoscyphus mon torrents* from different studies were added because of the morphological similarity to *R. ericae*. A sequence of the type strain of *Chloridium paucisperatum* (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 *Hyaloscypha* ceae 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 RAxML 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 nrSSU 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. (orlm148) (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. paludososa from Baral et al. (2009) for which no ITS data were available, were included in this and most further datasets. Two sequences of Pseudaegetera 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_10M110, KJ425314, Welc et al., unpubl.), which probably also represents C. finlandica according to sequence similarity with the type, and one sample labelled R. ericae (EF653875, 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 Pro-liferodiscus, Arachnepoziza, Ascocoryne and Hyalopeziza. One sample of Arachnepoziza varieipiosa (EU940086, M337) from Baral et al. (2009) is probably misidentified, because its nrLSU sequence is identical to that of Pezoloma clifera (see also Stenroos et al. 2010) and divergent from four other species of Arachnepoziza. 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 uninformative indels in the alignment and may contain mistakes; and one sequence of Ascocoryne saroceans (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 sarcoceans (FJ176886), Hyphodiscus hymenophilus (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 albocyphalina 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 (JN068800) 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. albocyphalina strain TNS-F-1733 was again identical with TNS-F-17335 (like above for mtSSU), but not with H. albocyphalina 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
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 rpb2 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 rpb2, 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 Hymenoscypha 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 Hymenoscypha 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 rpb2 sequence was available (EU940360). Similar sequences for comparison in this dataset, and the sequence was excluded as a potential artefact based on the ITS results.

The rpb2 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 rpb2 datasets were available were concatenated for combined phylogenetic analysis. As outgroup, 18 taxa representing 10 genera were used. The mtISSU 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 Hymenoscypha (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 Hymenoscypha s. str. Two species of Hymenoscypha (H. albohyalina and H. aureliella) fall outside this group; we treat them here as Hymenoscypha s. lat. One sample named Hymenoscypha sp. 2-13c (Long et al. 2013) appears among the outgroup and is most likely misidentified.

Usually, multiple accesses of the same species of Hymenoscypha 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 accesses (JN033423, JN033451) of Hymenoscypha 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. Hymenoscypha spiralis and Pseudoaegerita 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 Melinomyces 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 Melinomyces 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. hepaticola and Scytalidium vaccinii...
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.
combined dataset of nrLSU, mtSSU, rpb2 (5–7 & 7–11)

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.

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)

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Meliniomycetes variabilis falls into a strongly supported clade along with Hyaloscypha sp. (M19, M25); (ii) three accessions of Hyaloscypha daedaeae 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 are “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 Arachnopeziella and most likely belong to that genus. Like before, well-supported branches are formed by (i) R. ericae and H. hepaticcola, (ii) Meliniomycetes 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 Pseudoaeginta corticis 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 species may be erroneous. Results of nrLSU and ITS based phylogenies are expectedly similar because these markers are linked. Some species may be erroneous.

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 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 Arachnopeziella as in nrLSU; R. ericae groups with H. hepaticcola, 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.

rp2b phylogenies

Both trees based on different regions of the rp2b 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 rp2b regions 7–11 (Fig. 4B), namely unclear basal relationships among ingroup taxa and relatively long branches of Hyaloscypha s. lat. species (in rp2b only H. aureliella).

For both regions of rp2b, largely non-overlapping sets of ingroup and outgroup taxa were available with the exception of the newly generated REA sequences. Hyaloscypha aff. paludosa and Arachnopeziella 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 Arachnopeziella. Both trees show the close relationship of M. bicolor and C. finlandica, and Fig. 4B shows the associations of R. ericae/H. hepaticcola and M. variabilis/Hyaloscypha sp. (M19) also revealed by other markers (data for the other rp2b region are not available for these Hyaloscypha species).

Concerning ingroup relationships, rp2b 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. daedaeae, in ITS also H. epiporia and H. alniseda, however, the clade is supported only in BA. Thus, only rp2b 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 rp2b (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 Hyalopeziella and the Amicodiscal/Dematoscypha clade. “Hyaloscypha aff. paludosa” based on four specimens evidently belongs to Arachnopeziella according to its
position in the tree, although 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 rp2b. 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 EnM 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 144009 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 mycorriza (cf. Vohník et al. 2007b). However, these sometimes morphologically differed from *H. hepaticicola* CBS 126291 and *R. ericae* UAMH 6735 in that the hyphal coils 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. 1894 apud Huhtinen, Karstenia 29: 90. 1990. Pseud eag eritia J.L. Crane & Schokn., Mycologia 73: 78. 1981. Fuscoscypha Svrček, Sydowia 39: 222. 1887 apud Baral et al., Karstenia 49: 13. 2009. Rhizoscyphus W.Y. Zhuang & Korv, 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. *Pseudae gentia corticalis*, the type species of *Pseudae gentia* (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 (Yamagu chi 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, *Pseudae gentia* appears to be polyphyletic as two ITS sequences of *P. viridis* fall into the outgroup (Fig. 1).

The genus *Hyaloscypha* (Hy aloscyphaceae, Leptomycetes) 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 aberrations in euamyloidity occur rarely, filiform-cylindrical paraphyses without a yellow refractive vacular 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, *Pseudae gentia*, 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 fuscous 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 fuscous 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.
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 (Sumava Mts.), a spot between Brezník 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 & Reblová, 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)6–10(–11) × 2.5–3(–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. 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.
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 Plácinec 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, Kragerø, 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 doliform 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. 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.
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, Rěblová & Winka 2000).

Hyaloscypha variabilis (Hambl. & Sigler) Vohník, Fehrer & Rěblová, 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-70Ae, (ex-type culture UAMH 8961). 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 & Rěblová, 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. Patruquin ARSL 170507.38 (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. Patruquin 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

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Fig. 9. Hyaloscypha melini 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.
Table 2. Diagnostic morphological characters of *H. finlandica* and *H. bicolor*.

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

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.
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. Sälsk. 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, moniloid, consisting of oblong to sub-globose 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 paler 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 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 126286).

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 cheiridoid 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-axial species relationship has been reported (Braun et al. 2009).

Hyaloscypha hepatitisica (Grélet & Croz.) Baral et al. [as ‘hepaticola’]. Karstenia 49: 7. 2009.

Basionym: Trichopeziza hepatitisica 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 67: 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 vaccini Dalpé et al., Mycotaxon 35: 372. 1989.

Specimens examined. Finland, Etelä-Härme, Tammela, Liesjärvi National Park, grid 6730:3329, on Lophozia and Pilidiom, 4. Jul. 2005, Niminen 10 (TUR 180982, living culture CBS 126283); ibid., Varsinais-Suomi, Kemiö, Gasterby, Solbacka, grid 6686:32639, on Pilidiom, 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. hepatitisica exists (France, Provence-Alpes-Côte d’Azur, Var dept., Notre Dame des Anges near Pignans, on stems of Cephaloziella byssacea, Jun. 1924, de Crozals; not examined), two non-type strains of H. hepatitisica derived from recent collections on Lophozia and Pilidiom (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. vaccini strongly support their conspecificity; based on the priority, both latter species are reduced to synonymy with H. hepatitisica. 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. hepatitisica 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. hepatitisica 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. hepatitisica 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. hepatitisica 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 monophyly. 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 problems 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 Hya-

loscypha (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 Hya-

loscypha 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. hepatitisica 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. hepaticola* and its *Rhizoscyphus ericae* synonym linked with the asexual state originally described as *Scytophium 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 hepaticola: 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 & Reed 2008). Recently, however, basidiomycetous sheathed ericoid mycorrhiza formed by *Kurtia argillacea* (*Hymenochaetales*) has been described and re-synthesized *in vitro* (Vohnik et al. 2012, Kolarik & Vohník 2018), and a serendipitous (*Serendipitaceae, Sebacinales*) strain derived from *Vaccinium* hair roots has been experimentally proven as ericoid mycorrhizal (Vohnik 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 well-researched *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. hepaticola* and *R. ericae* are conspecific. *Hyaloscypha hepaticola* inhabits many ecological niches and includes ericoid mycorrhizal symbionts, saprobes on arboreal 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. bulbipila, 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 *Philalophora*-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 *Ploetnenulaceae* (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, Rěbolová et al. 2015).

**Hyaloscypha** taxonomy, phylogenetic evidence and open questions

The genus *Hyaloscypha* was monographed by Huhtinen (1990) and segregated from morphologically similar *Harnatocanthoscypha* 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; *Eupeziella* 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 amylod reaction in the hairs and/or excipula. Although Index Fun-gorum 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 documented with molecular tools for various groups that are not convincingly alignable to each other. The only *Monodictys* species, *M. monodictys* var. *fuscifuckelii* (Huhtinen 1990), *H. aureliella* and *Phialophora*-like (*H. hepaticola* 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*), *H. aureliella* and *Cheiromycella zalesski* (Descals & Webster 1976). Most of these species were analysed in this study. Four of them belong to the core group of *Hyaloscypha* (*H. fuckelli*, *H. hepaticola*, *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 fuckelli* 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 *Derma-teaceae*; 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 *Clauussenomyces atrovirens* (Tympaniaceae) (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. hepaticola*, the type species of *Scytalidium* is not congeneric with *Hyaloscypha*; based on mSSU 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. montropae*. It is morphologically similar to *H. hepaticola* with which it also shares plant-associated and saprophic lifestyles. ITS sequences of five strains of *R. montropae* are available in GenBank (as *Hymenoscyphus montropae*); four have been isolated as members of root fungal communities of *Tsuga canadensis*, *Vaccinium uliginosum*, and *Saix 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. montropae* and fungi assigned to the REA. According to BLAST searches (97 % similarity) and its position in the ITS tree (Fig. 1), *R. montropae* is conspecific or at least congeneric with *Cya-thicula microsperma* M267 (Baral et al. 2009), which is included as an outgroup in our study. Given these facts, *R. montropae* 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 *Eupeziella* and studied with DNA sequence data. It forms cheiroid and phragmosporous conidia in vitro (Fig. 11); its asexual morph is *C. microsporica*, 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 *Pseudaegerita* (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 phylogene-tic 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 *Eupeziella*.
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 Pseudoeurotium, however, its highest sequence similarity is shared with mostly unpublished environmental samples. In the ITS tree (Fig. 1), it groups near Pseudoaegerita viridis and Coleophoma clyndrospora (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 Arachnipeziza according to all datasets for which sequence data were available. However, we refrain from making a formal combination of H. paludosa in Arachnipeziza 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 Cla-throsphearia 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 (Solits 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 (JN086880 and JN086875, Han et al. 2014) of strain TNS-F-17333 (= NBRC 106631). According to ITS and nrLSU it belongs to H. albocyphalina whereas it is identical to H. sp. TNS-F-17335 based on mtSSU and rpb2, 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 rpb2 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. multicityp genes, heterozygosity, hybrids) or contamination of samples. Two rpb2 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 (Chvtek 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. Réblóvá 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. hepaticola (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 Hylloscypha comprise H. albohyalina, H. spiralis and H. monodictys or H. fuckelli and H. aniseda 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, Pseudoegerita, Monodictys, Phialophora, Scytalidium), and wrongly assigned names or misidentified strains are revealed (e.g. ‘Hyaloscypha’ sp. 2-13c, ‘Archnoppeiza 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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