New Phytologist

Letter

Endophytism and endolichenism in Pezizomycetes: the exception or the rule?

Introduction

Fungal endophytes live asymptomatically within plants and are widespread inhabitants of leaves and other organs (Wilson, 1995). Likewise, endolichenic fungi live asymptomatically within lichens, occurring in healthy lichen thalli worldwide (Arnold *et al.*, 2009). Endophytes and endolichenic fungi are ecologically similar, living in symbiosis with either a plant or the photobionts of lichens (Arnold *et al.*, 2009), and both functional groups represent the same major lineages of fungi (U'Ren *et al.*, 2012). As a whole, these fungi include diverse species whose life cycles often include pathogenic or saprobic phases (Porras-Alfaro & Bayman, 2011; Selosse, 2018; Terhonen *et al.*, 2019). Endophytes and endolichenic fungi occur from polar regions to the tropics (Arnold *et al.*, 2009), with most species transmitted horizontally (see Rodriguez *et al.*, 2009). Although most endophyte–host interactions have not been examined, some have positive impacts on the physiology, growth or stress tolerance of their hosts (e.g. Arnold *et al.*, 2003; Rodriguez *et al.*, 2009; Porras-Alfaro & Bayman, 2011). Endophytic and endolichenic fungi are especially common and diverse among the largest nonlichenized lineages of the subphylum Pezizomycotina (Ascomycota), with variation among host lineages and biomes in the relative abundance of the five most common classes in which endophytism is known (Sordariomycetes, Dothideomycetes, Leotiomycetes, Eurotiomycetes and Pezizomycetes; Arnold *et al.*, 2009).

Among these, fungi in the class Pezizomycetes are of special interest because the biology of many taxa is incompletely known. This class consists of one order (Pezizales), 23 families and an estimated 2000 species (Pfister & Healy, 2021). Species of Pezizomycetes include well-documented plant pathogens (Marek *et al.*, 2009), ectomycorrhizal (ECM) fungi (Tedersoo *et al.*, 2006) and saprobes (Hobbie *et al.*, 2001; Hansen & Pfister, 2006). Some species colonize specific substrates, acting as parasites of bryophytes (Döbbeler, 1997) or as specialized saprobes of dung (Pfister, 2015; Richardson, 2019) or postfire materials (Egger, 1986). Over the past decade, studies have shown that some species of Pezizomycetes are common as endophytes within bryophytes and occur frequently in lichen thalli (U'Ren *et al.*, 2010, 2019). The life cycles and trophic ecology of some Pezizomycete species are unclear or controversial, and the endophytic habit has not been considered an important ecological strategy across the class (Pfister, 2015). Yet evidence suggests a more complex story. For example, stable isotope analyses indicate that members of the genus *Morchella* (morels) are able to access dead organic matter (Hobbie *et al.*, 2001, 2016), but they also can live endophytically in roots (Baynes *et al.*, 2012) and in conifer needles (Baroni *et al.*, 2018). Likewise, the esteemed black truffles *Tuber melanosporum* and *T. aestivum* are ECM fungi, but in an interesting twist to truffle ecology, they have been shown to live as endophytes in roots of non-ECM plants (Schneider-Maunoury *et al.*, 2020).

While studying Pezizomycetes from an evolutionary and functional perspective, we noted that rDNA sequences from many Pezizomycete endophytes were available in GenBank, but that their phylogenetic affinities were not defined. Many sequences were generated from living cultures isolated from diverse plants and lichens sampled across the globe by Arnold and collaborators at the University of Arizona (UA) and maintained there as part of the Robert L. Gilbertson Mycological Herbarium (Myco-ARIZ; e.g. Hoffman & Arnold, 2010; U'Ren *et al.*, 2010, 2012, 2014, 2019; Lau *et al.*, 2013; Sandberg *et al.*, 2014; Massimo *et al.*, 2015; Huang *et al.*, 2016, 2018a; U'Ren & Arnold, 2016; Bowman & Arnold, 2018; Oita *et al.*, 2021a,b).

The many DNA barcode matches among endophytes in GenBank and our unpublished sequences from fruit bodies suggested that endophytism and endolichenism might be more common, phylogenetically dispersed and ecologically important among Pezizomycetes than documented previously. Therefore, we assembled the available ecological and phylogenetic data on endophytic and endolichenic Pezizomycete species to ask: (1) Of the estimated 2000 Pezizomycete species known to date, how many occur as endophytes or endolichenic fungi?; (2) In which lineages does endophytism or endolichenism occur across the Pezizomycetes?; and (3) What are the main nutritional modes of endophytic or endolichenic Pezizomycete species when they are outside their hosts?

We generated comprehensive phylogenies of Pezizomycetes based on 3315 sequences from the internal transcribed spacer region of nuclear ribosomal DNA (ITS1-5.8s-ITS2 nrDNA; hereafter ITS) and 1102 sequences of the large subunit nrDNA (28S) from fruit bodies, endophytes, and environmental sequences. We used maximum-likelihood (ML) phylogenetic analysis to determine operational taxonomic units (OTUs) (Table 1), but also compared our phylogeny-based OTUs with those recovered from a clustering approach based on 97% sequence similarity (Supporting Information Table S1). We included representatives of 3784 ITS sequences from the UA endophyte collection as well as new and reference sequences from Pezizomycete fruit bodies (Tables 1, S2). We preferentially incorporated available sequences from type specimens and used representative sequences in combination with BLAST to obtain additional sequences of endophytic and endolichenic species (see flow chart in Fig. S1 for graphic of methods).

Table 1 Overview of endophytic Pezizomycetes based on previous reports and new data.

Phylogenetic lineage	Number of endophyte OTUs in ITS trees	Host(s)	ITS trees ($n = 35$)	Asexual spores?	Documented genera with endophytes	Representative reports of endophytes
Ascobolaceae	5	Angiosperm, Gymnosperm, Bryophyte, Lichen	Fig. S2 (Ascobolaceae)	Yes	*Ascobolus*	Johnston *et al*. (2017), Schneider-Maunoury *et al*. (2020), Su *et al*. (2010)
Ascodesmidaceae	8	Angiosperm, Gymnosperm	Fig. S3 (Ascodesmidaceae)	Yes	*Ascodesmis*, *Eleutherascus*, *Lasiobolus*, *Trichobolus*, Undetermined 1–3	Sieber (1985)
Coprotaceae	4	Angiosperm, Lichen	Fig. S4 (Coprotaceae)	Unknown	*Coprotus*	*New report, this study*
Chorioactidaceae	4	Angiosperm, Gymnosperm, Bryophyte, Lichen	Fig. S5 (Chorioactidaceae)	Yes	*Desmazierella*	Martinović *et al*. (2016), Bußkamp *et al*. (2020)
Discinaceae	8	Gymnosperm, Bryophyte, Lichen	Fig. S6 (Discinaceae)	Yes	*Gyromitra*	Soca-Chafre *et al*. (2011)
Morchellaceae	7	Angiosperm, Gymnosperm, Lichen	Figs S7–S8 (*Morchella esculenta* group, *Morchella elata* group)	Yes	*Morchella*	Baynes *et al*. (2012)
Otideaceae	2	Lycopod, Bryophyte, Lichen	Fig. S9 (*Otidea*)	Unknown	*Otidea*	*New report, this study*
Pezizaceae	27	Angiosperm, Gymnosperm, Bryophyte, Lichen	Figs S10–S17 (*Chromelosporium*, *Geoscypha*, *Iodophanus*, *Lepidotia*, *Malvipezia*, *Mattirolomyes*, *Peziza* s.s., *Plicaria*)	Yes	*Chromelosporium*, *Daleomyces*, *Geoscypha*, *Iodophanus*, *Lepidotia*, *Malvipezia*, *Mattirolomyces*, *Peziza*, *Plicaria*, Undetermined 1	Shipunov *et al*. (2008), Botella & Diez (2011), Baynes *et al*. (2012), Gomes *et al*. (2019), Bußkamp *et al*. (2020), Raudabaugh *et al*. (2020)
Pseudombrophilaceae	4	Angiosperm, Gymnosperm, Lichen	Fig. S18 (Pseudombrophilaceae)	Yes	*Pseudombrophila*	Gomes *et al*. (2018), Deng *et al*. (2019), Yang *et al*. (2020)
Pulvinulaceae	2	Angiosperm, Gymnosperm, Bryophyte, Lichen	Fig. S19 (Pulvinulaceae)	Unknown	*Pulvinula*	Tedersoo *et al*. (2013), Bizabani (2015), Wijeratne *et al*. (2015)
Pyronemataceae	51	Angiosperm, Gymnosperm, Fern, Lycopod, Bryophyte, Lichen	Figs S20–S28 (*Lasiobolidium*, *Perilachnea*, *Pyronema*, *Scutellinia*, *Smardaea*, *Sphaerosporella*, *Tricharina*, *Trichophaeopsis*, *Wilcoxina*)	Yes	*Aleuria*, *Anthracobia*, *Ascorhizoctonia*, *Byssonectria*, *Lasiobolidium*, *Paratricharina*, *Perilachnea*, *Pseudotricharina*, *Pyronema*, *Pyropyxis*, *Scutellinia*, *Smardaea*, *Sphaerosporella*, *Spooneromyces*, *Tricharina*, *Trichophaea*, *Trichophaeopsis*, Undetermined 1–8	Sieber (1985), Sun & Guo (2010), Baynes *et al*. (2012), Tedersoo *et al*. (2013), Qadri *et al*. (2014), Gomes *et al*. (2019), Bußkamp *et al*. (2020), Hughes *et al*. (2020), Raudabaugh *et al*. (2020), Sadatri (2021)
Rhizinaceae	1	Lycopod, Bryophyte, Lichen	Fig. S29 (Rhizinaceae)	Yes	*Rhizina*	Raudabaugh *et al*. (2020)
Sarcoscyphaceae	7	Angiosperm, Gymnosperm, Bryophyte, Lichen	Fig. S30 (Sarcoscyphaceae)	Yes	*Pithya*, *Pseudopithyella*	Ganley & Newcombe (2006), Mapperson (2014), Schlegel *et al*. (2018)
Sarcosomataceae	27	Angiosperm, Gymnosperm, Fern, Lycopod, Bryophyte, Lichen	Figs S31–S34 (*Donadinia*, *Plectania*, *Pseudoplectania*, *Urnula*)	Yes	*Donadinia*, *Galiella*, *Plectania*, *Pseudoplectania*, *Sarcosoma*, *Urnula*, Undetermined 1	Ganley & Newcombe (2006), Mapperson (2014), Qadri *et al*. (2014), Sanz-Ros *et al*. (2015), Gomes *et al*. (2018), Lagarde *et al*. (2018), Yoshino *et al*. (2020), Zhang & Zhang (2020)

Table 1 (Continued)

Phylogenetic lineage	Number of endophyte OTUs in ITS trees	Host(s)	ITS trees (*n* = 35)	Asexual spores?	Documented genera with endophytes	Representative reports of endophytes
Tarzettaceae	8	Angiosperm, Gymnosperm, Lycopod, Bryophyte, Lichen	Fig. S35 (Tarzettaceae)	Yes	*Geopyxis*, *Hydnocystis*	Ganley & Newcombe (2006), Soca-Chafre *et al.* (2011), Tedersoo *et al.* (2013), Qadri *et al.* (2014), Wang *et al.* (2016)
Tuberaceae	2	Angiosperm	Fig. S36 (Tuberaceae)	Yes	*Tuber*	Schneider-Maunoury *et al.* (2020)
Undetermined lineage	1	Angiosperm, Lichen	Fig. S37 (Geomorium)	Unknown	Undetermined 1	*New report, this study*
16 families, 1 undetermined family lineage	168				50 genera and 14 undetermined generic lineages	

Only taxa from living, healthy plants and lichens are reported. Seven additional families of Pezizomycetes had no documented endophytes. Described families are listed except for the undetermined lineage, which is related to Tuberaceae and Geomoriaceae but not resolved in either family (see Fig. 2). Previous documentation of asexual spores, which may be important for colonization of plant tissues, is noted at the family level.

In order to examine placement of endophytes in Pezizomycetes, we first assembled a single 28S rDNA alignment with representatives of Pezizomycete lineages and ecological modes, including 266 endophytic and endolichenic sequences, and performed ML analyses via RAxML with 1000 bootstraps. The 50% majority rule tree (Figs 1, S2) was used in conjunction with Hansen & Pfister (2006) and Pfister (2015) as a guide for placement of endophytic and endolichenic taxa (Table 1). The ITS locus often is useful for species-level identification (Schoch *et al.*, 2012; Kõljalg *et al.*, 2013) and many endophyte studies generate only ITS sequences. Accordingly, we compiled 35 separate ITS alignments for individual families or lineages within families of Pezizomycetes, including 1046 endophytic or endolichenic sequences (Figs S3–S37). Details of methods, sequence alignments and accession numbers are provided in Notes S1; Fig. S1; Tables S1, S2.

We detected endophytic and endolichenic species in 50 Pezizomycete genera and in 14 lineages that could not be assigned confidently to a genus. Together these represented ≥ 160 OTUs distributed across ≥ 16 families (Table 1; Figs 1, S2–S37). Some families had endophytism or endolichenism represented in only one or two genera (e.g. *Desmazierella* in Chorioactidaceae, *Pseudombrophila* in Pseudombrophilaceae), whereas others had species with these lifestyles in many genera, including Pezizaceae (27 OTUs in nine genera) and Pyronemataceae (45 OTUs in 17 genera; see also Tedersoo *et al.*, 2013). Our conservative OTU delimitations based on ITS phylogenies yielded 160 OTUs whereas a clustering-based approach yielded 216 OTUs. However, the two methods yielded similar inferences regarding the ecology and phylogenetic distribution of Pezizomycete endophytes and endolichenic fungi (Table S1). The results presented here are based on the phylogenetic approach.

Notably, we detected endophytism or endolichenism in two lineages for which this mode was previously undocumented: the ECM genus *Otidea* (Otideaceae) (Fig. S10) and dung saprobe genus *Coprotus* (Coprotaceae) (Fig. S6). In both genera we detected endophytic and endolichenic isolates from multiple hosts, suggesting that endophytism is a regular feature of their biology. Although a few Pezizomycete lineages with endophytic or endolichenic members were detected in only a limited range of hosts (e.g. Ascodesmidaceae was only isolated from vascular plants), most Pezizomycetes have a broad endophytic host range (as reported in detail by U'Ren *et al.*, 2019). Endophytic and endolichenic Pezizomycetes are from lineages with a variety of trophic strategies, including saprobes, plant pathogens and mycorrhizal fungi (Tables S2, S4), but also include taxa for which the trophic strategies remain unresolved or unknown (Table S5).

One particularly striking finding was an OTU that could not be assigned to a known family. This OTU is represented by five isolates from angiosperm leaves and lichens in an Alaskan boreal forest (USA) (U'Ren *et al.*, 2019). It represents a unique lineage nested between Tuberaceae and Geomoriaceae, both of which consist exclusively of ECM species (Fig. 2; Notes S1; Tables S1–S3).

The vast majority of Pezizomycete isolates in the culture-based UA dataset came from lichens (78%). Only one family (Ascodesmidaceae) was never detected in lichens, suggesting that lichens are important hosts for Pezizomycete species (Table S4). However,

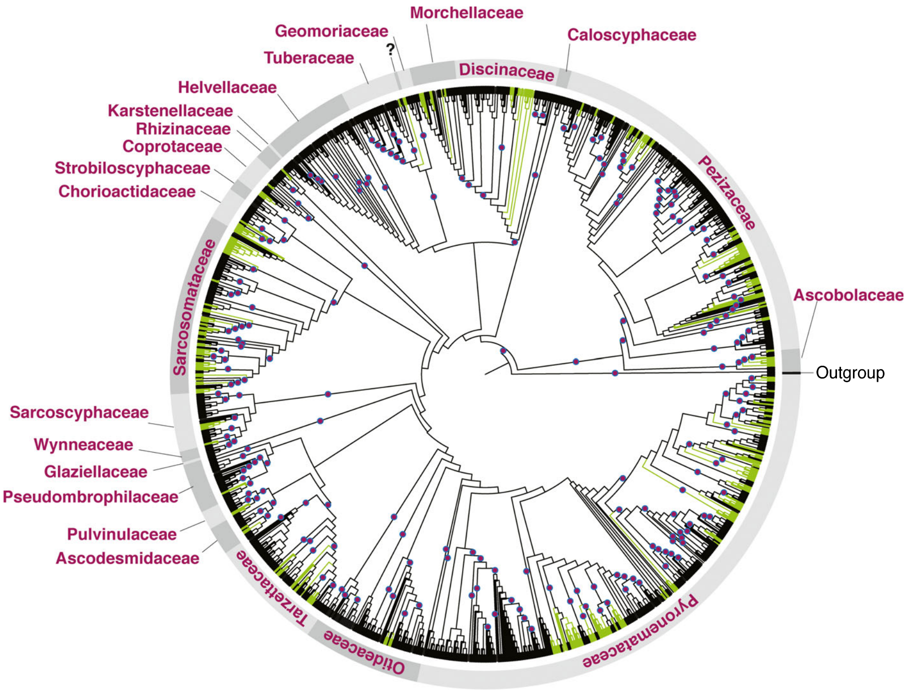

Fig. 1 Maximum likelihood phylogeny based on 28S sequences of Pezizomycetes highlighting the placement of endophytic and endolichenic fungi. Text for taxa detected as endophytic or endolichenic are green, and taxa not known as endophytic or endolichenic are black. All nodes with ≥ 70% bootstrap support are shown with a purple circle. The phylogram is a 50% majority rule tree with support based on 1000 bootstrap replicates and taxa in the Orbiliomycetes used as the outgroup. Families within Pezizomycetes are demarcated by gray shaded regions in the outer circle. The exception is an undetermined lineage related to Tuberaceae and Geomoriaceae (designated by a question mark; see Fig. 2).

despite the importance of lichens as hosts, no Pezizomycete families were restricted to lichens alone and OTUs from most Pezizomycete families were detected in many photosynthetic hosts. For example, the most frequently isolated species were from Sarcosomataceae (Table S4), a family of largely saprobic taxa. At the genus level *Pseudoplectania* (Sarcoscomataceae) was especially well represented, comprising nearly 40% of Pezizomycete isolates in the UA dataset (Table S5).

More generally, one conspicuous and recurrent phylogenetic pattern is that both endophytes and endolichenic fungi are common in clades containing well-characterized saprobes from wood, dung or postfire substrates, but infrequent in closely related ECM or plant pathogenic clades, as noted for Pyronemataceae by Tedersoo *et al.* (2013). For example, endophytism and endolichenism were commonly detected for *Geopyxis*, a genus of Tarzettaceae with putatively biotrophic, weakly parasitic and/or pyrophilous species (Egger, 1986; Vrålstad *et al.*, 1998), but not detected for the ECM sister genus *Tarzetta* (Fig. S35). Likewise, endophytism and endolichenism was commonly detected for species of saprobic Pezizaceae (e.g. *Peziza* s.s., *Plicaria* and *Geoscypha*) (Figs S13, S17, S18) but rarely or never detected for ECM species such as those in *Legaliana* or *Ruhlandiella* (Fig. S11). In the Discinaceae, endophytism and endolichenism was common for species of *Gyromitra*, a genus of putative saprobes, but absent in species of the ECM sister genus *Hydnotrya* (Hobbie *et al.*, 2001) (Fig. S7).

Although endophytes were documented only rarely among ECM and pathogenic clades of Pezizomycetes, we detected endophytism and endolichenism in four lineages known previously only for ECM lifestyles (*Otidea* and several ECM Pezizaceae), and endophytism also was reported in species of the ECM genera *Sphaerosporella* (Hughes *et al.*, 2020) and *Tuber* (Schneider-Maunoury *et al.*, 2018). Although many ECM fungi are difficult or impossible to culture (Tedersoo *et al.*, 2010), these endophytic or endolichenic isolates grow well in pure culture and thus enable future research in genomics, experimental manipulation and secondary metabolites. There is evidence from *Sphaerosporella* that endophytes of needles and colonization of ECM roots may rarely occur in the same individual host, but that the mode of infection for these two organs is different (Hughes *et al.*, 2020). Additional studies are needed, perhaps employing culturing, resynthesis, inoculation and isotopic methods, to substantiate whether Pezizomycete endophytes with identical ITS sequences to those on their ECM host roots are actually the same genotype and are playing similar ecological roles. New approaches that allow visualization of the extent and morphology of fungal colonization, such as fluorescence in situ hybridization (Schneider-Maunoury *et al.*, 2020), will be especially helpful in future work.

By contrast, other ECM Pezizomycetes (e.g. *Otidea,* some ECM Pezizaceae) were not detected as endophytes in their ECM hosts but were instead found in bryophytes and lichens, suggesting possible compartmentalization of different trophic modes on different hosts. A similar pattern also was found among the two lineages of plant pathogens where endophytism was detected. The endophytic state of *Pithya cupressina* is putatively a dormant pathogen because this fungus is considered the cause of twig die-back on *Juniperus*, but it also was found as an endophyte in healthy tissue of the same *Juniperus* species. By contrast *Rhizina undulata*, a root pathogen of Pinaceae, was endophytic only in lycopods and ferns. Interestingly, no bryophyte parasites (such as *Octospora* or *Lamprospora*) were detected as endophytic or endolichenic in any sampled host, including mosses. These patterns suggest that host preferences, compartmentalization and the ability to colonize different hosts or host organs may be species- or lineage-specific.

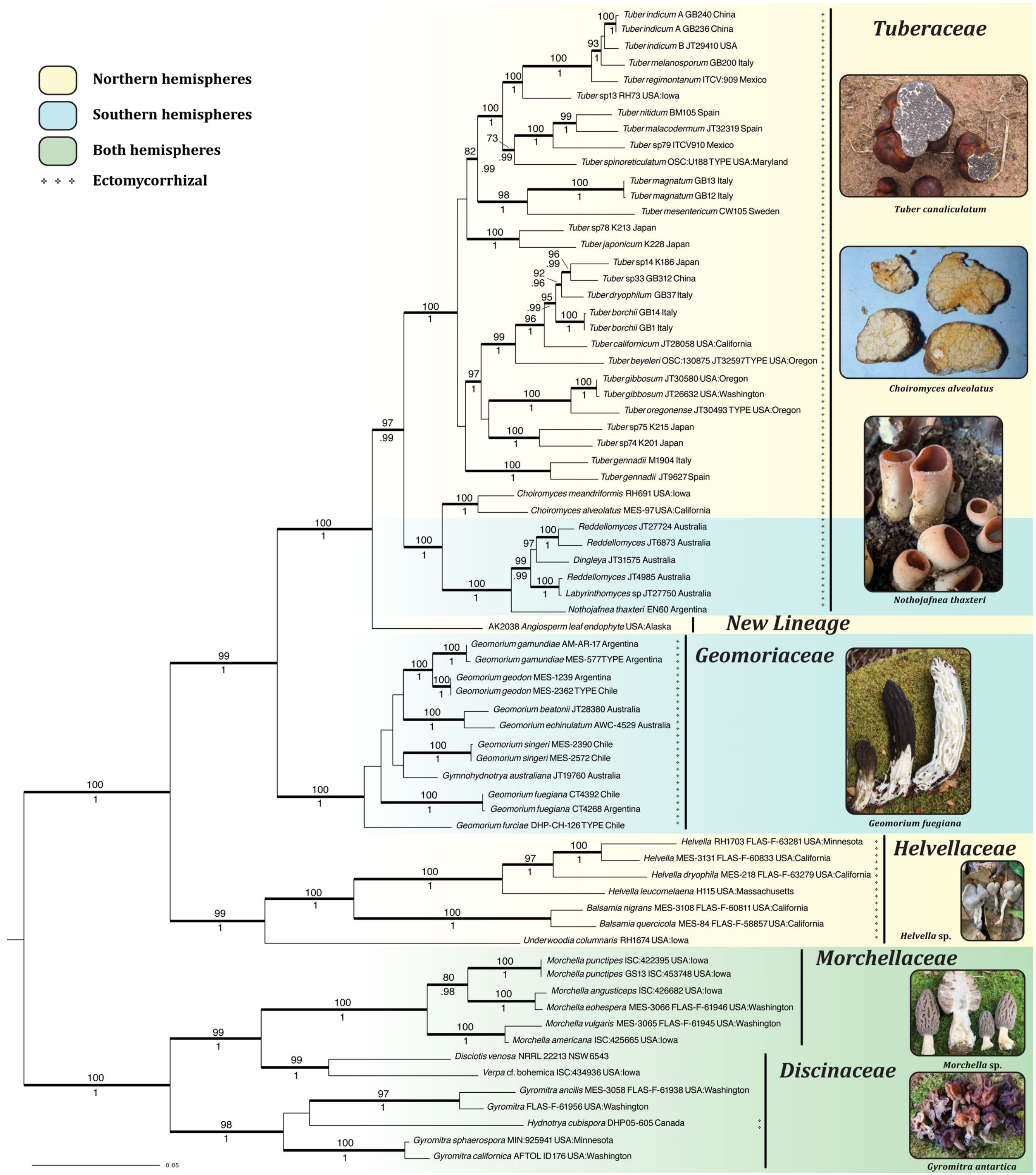

Fig. 2 Multigene phylogeny based on maximum likelihood and Bayesian analyses of 28S rDNA, *rpb2*, and *tef*1 of Discinaceae, Geomoriaceae, Helvellaceae, Morchellaceae, Tuberaceae, and the undetermined lineage comprised of endophyte and endolichenic isolates. The phylogram is a 50% majority rule tree with support based on 1000 bootstrap replicates. Posterior probabilities from Bayesian analysis was based on 20 million generations with sampling every 1000 generations, and the first 25% discarded as burn-in. Taxa in the Morchellaceae and Discinaceae were used as the outgroup. Values displayed on branches represent bootstrap support ≥ 70% (above) and posterior probabilities ≥ 0.95 (below). Northern Hemisphere taxa are highlighted in yellow, Southern Hemisphere taxa are highlighted in blue, and lineages found in both hemispheres are highlighted in green. Fruit body photographs of taxa representative of each major clade include *Choiromyces alveolatus* (Tuberaceae), *Geomorium fuegiana* (Geomoriaceae), *Gyromytra antarctica* (Discinaceae), *Helvella* sp. (Hevellaceae), *Morchella* sp. (Morchellaceae), *Nothojafnea thaxteri*, and *Tuber canaliculatum* (Tuberaceae).

Notably, endophytic and endolichenic species were particularly common among clades of fire-adapted and pyrophilous Pezizomycetes, including 24 OTUs from 19 genera (Table S6). Raudabaugh *et al.* (2020) found that pyrophilous taxa such as *Anthracobia melaloma*, *Ascorhizoctonia praecox*, *Pyronema omphalodes* and *R. undulata* are common as endophytes but appear to be rare to absent in soil. After wildfires, however, these fungi fruit prolifically on soil and burnt plant debris (e.g. Petersen, 1970; Reazin *et al.*, 2016; Bruns *et al.*, 2020). Likewise, in a study of endophytes of *Bromus tectorum* (cheatgrass), Baynes *et al.* (2012) identified several pyrophilous Pezizomycetes (e.g. *Peziza ostracoderma*, *Pyronema domesticum*, *Morchella eximia* and *M. snyderi*). Subsequent experiments showed that endophytic *Morchella* species increased *B. tectorum* growth and enhanced seed survival following fire, highlighting a previously unstudied benefit of this symbiosis. U'Ren *et al.* (2012) and Huang *et al.* (2016) reported the dominance of Pezizomycete endophytic and endolichenic species in Arizona forests where fire is common, indicating that this may be a widespread phenomenon. Our analysis revealed additional pyrophilous fungi that can be endophytic or endolichenic, including *Geoscypha tenacella* and *Pyropyxis rubra*. Available evidence suggests hidden roles of some pyrophilous Pezizomycetes as plant symbionts, setting the stage for studies of ecological effects of endophytism on host plants and endolichenism on host lichens.

These and other hypotheses will be testable in the future due to living fungal libraries such as the UA culture collection. Metagenomics and other culture-free tools have been and will continue to be critical for elucidating plant–fungi interactions, especially because these methods typically detect a far greater diversity of fungi than culture-based methods alone (U'Ren *et al.*, 2019). However, studies such as ours highlight the importance of maintaining living endophytic and endolichenic fungus cultures and generating ITS and 28S DNA to identify them (see U'Ren *et al.*, 2019 for benefits and drawbacks of molecular vs culture-based detection). Fungal cultures can be used for diverse purposes: to test nutritional requirements, characterize novel metabolites, sequence genomes and transcriptomes, and inoculate plants to study the effects on plant and fungal fitness (e.g. Wijeratne *et al.*, 2012; Sarmiento *et al.*, 2017; Torres-Cruz *et al.*, 2017; Huang *et al.*, 2018b; Harrington *et al.*, 2019).

In this study, we identified endophytic and endolichenic species from an impressive 16 of 23 recognized Pezizomycete families, as well as a lineage that likely represents a new family (undetermined lineage in Fig. 2). Our results suggest that endophytism and endolichenism may indeed be the rule rather than the exception across families of Pezizomycetes. Our conservative phylogenetic approach to OTU delimitation detected a minimum of 160 OTUs of endophytic and endolichenic Pezizomycetes. This is equivalent to *c.* 8% of the estimated 2000 species in this class, but these are spread across *c.* 70% of the families. Given that only a small fraction of potential hosts and geographical areas have been sampled for endophytes or endolichenic fungi, the number of OTUs is probably a marked underestimate. Notably, relatively few studies have broadly sampled lichens wherein Pezizomycete species are dominant (but see U'Ren *et al.*, 2012, 2019). It seems probable that more sampling will detect additional Pezizomycete species as cryptic residents in plants and lichens from biomes ranging from tropical forests to polar deserts, as illustrated by culture-based and culture-free studies (e.g. Higgins *et al.*, 2007; U'Ren *et al.*, 2019; Oita *et al.*, 2021a).

The ecologies of many rare or understudied species of Pezizomycetes in genera such as *Carbomyces*, *Eremiomyces*, *Glaziella*, *Hydnocystis*, *Kalaharituber*, *Pseudotricharina* and *Sowerbyella* remain mysterious and currently unclear (Læssøe & Hansen, 2007; Tedersoo *et al.*, 2010; Tedersoo & Smith, 2013). Although the trophic nature for most endophytic Pezizomycetes outside their hosts is putatively saprobic, endophytism and endolichenism also appear to be a normal part of the life history in many ECM and pathogenic species. Functional roles during their endophytic or endolichenic phase are unknown for any of these trophic groups and require further investigation. Our results suggest that future studies to elucidate the lifestyles of these poorly known Pezizomycetes should look first to the nearest plants and lichens to see what fungi might be living inside.

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Author contributions

MES and RAH conceived the study; MES, AEA, DHP, RAH and GB obtained funding and contributed resources to the work; MES, RAH, BL, DHP and GB collected specimens and generated DNA sequences from museum specimens; AEA and Y-LH isolated endophytes, maintained fungal cultures and generated DNA sequences from endophyte cultures, in collaboration with authors who submitted cultures to the UA collection (see References and Acknowledgements); MES, RAH and BL compiled data, completed analyses, generated figures and deposited specimens and sequences; DHP provided advice and reviewed all taxonomy and nomenclature; and MES and RAH wrote the manuscript with input from all authors (MES, RH, DHP, BL, GB, AEA and Y-LH).

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Data availability

Data in this manuscript are publicly available on GenBank and via OSF (see link in manuscript).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Flow chart outlining the basic steps for assembling data on Pezizomycetes endophytes and endolichenics.

Fig. S2 Phylogeny of endophytic fungi in Pezizomycetes based on 28S sequences analyzed with ML. This figure is an expanded, linear version of Fig. 1 that includes more detailed annotation.

Fig. S3 Phylogeny based on ITS sequences of Ascobolaceae and related endophytic or endolichenic fungi analyzed with ML.

Fig. S4 Phylogeny based on ITS sequences of Ascodesmidaceae and related endophytic or endolichenic fungi analyzed with ML.

Fig. S5 Phylogeny for Coprotaceae and related endophytic or endolichenic fungi analyzed with ML.

Fig. S6 Phylogeny based on ITS sequences of Chorioactidaceae and related endophytic or endolichenic fungi analyzed with ML.

Fig. S7 Phylogeny based on ITS sequences of Discinaceae endophytic or endolichenic fungi analyzed with ML.

Figs S8 and S9 Phylogeny based on ITS sequences of Morchellaceae endophytic or endolichenic fungi analyzed with MLood.

Fig. S10 Phylogeny based on ITS sequences of Otideaceae and related endophytic or endolichenic fungi analyzed with ML.

Fig. S11 Phylogeny based on ITS sequences of Pezizaceae (pro parte) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S12 Phylogeny based on ITS sequences of Pezizaceae (*Daleomyces*, *Malvipezia*) and related endophytic or endolichenic fungi analyzed with ML.

Fig. S13 Phylogeny based on ITS sequences of Pezizaceae (*Geoscypha*) and related endophytic or endolichenic fungi analyzed with ML.

Fig. S14 Phylogeny based on ITS sequences of Pezizaceae (*Iodophanus*) and related endophytic or endolichenic fungi analyzed with ML.

Fig. S15 Phylogeny based on ITS sequences of Pezizaceae (*Lepidotia*) and related endophyte analyzed with ML.

Fig. S16 Phylogeny based on ITS sequences of Pezizaceae (*Mattirolomyes, Elderia*) and related endophytes analyzed with ML.

Fig. S17 Phylogeny based on ITS sequences of Pezizaceae (*Peziza sensu stricto*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S18 Phylogeny based on ITS sequences of Pezizaceae (*Plicaria*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S19 Phylogeny based on ITS sequences of Pseudombrophilaceae and related endophytic and endolichenic fungi analyzed with ML.

Fig. S20 Phylogeny based on ITS sequences of Pulvinulaceae and related endophytic and endolichenic fungi analyzed with ML.

Fig. S21 Phylogeny based on ITS of Pyronemataceae (pro parte) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S22 Phylogeny based on ITS of Pyronemataceae (pro parte) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S23 Phylogeny based on ITS of Pyronemataceae (pro parte) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S24 Phylogeny based on ITS of Pyronemataceae (*Lasiobolidium*) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S25 Phylogeny based on ITS of Pyronemataceae (*Perilachnea*) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S26 Phylogeny based on ITS of Pyronemataceae (*Jafnea, Pyropyxis, Smardaea*) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S27 Phylogeny based on ITS of Pyronemataceae (*pro parte*) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S28 Maximum likelihood analysis of ITS of Pyronemataceae (*Trichophaea, Wilcoxina*) for placement of endophytes and endolichenic fungi analyzed with ML.

Fig. S29 Phylogeny based on ITS sequences of Rhizinaceae and related endophytic and endolichenic fungi analyzed with ML.

Fig. S30 Phylogeny based on ITS sequences of Sarcoscyphaceae and related endophytic and endolichenic fungi analyzed with ML.

Fig. S31 Phylogeny based on ITS sequences of Sarcosomataceae (*Donadinia*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S32 Phylogeny based on ITS sequences of Sarcosomataceae (*Galiella*, *Plectania*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S33 Phylogeny based on ITS sequences of Sarcosomataceae (*Pseudoplectania, Sarcosoma*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S34 Phylogeny based on ITS sequences of Sarcosomataceae (*Urnula*) and related endophytic and endolichenic fungi analyzed with ML.

Fig. S35 Phylogeny based on ITS sequences of Tarzettaceae and related endophytic and endolichenic fungi analyzed with ML.

Fig. S36 Phylogeny based on ITS sequences of Tuberaceae analyzed with ML.

Fig. S37 Phylogeny based on ITS sequences of Geomoriaceae and an undetermined lineage of related endophytic and endolichenic fungi analyzed with ML.

Notes S1 Additional method details for culture work, molecular work, and phylogenetic analyses.

Table S1 Number of sequences, characters and endophyte OTUs included in the rDNA and multilocus analyses.

Table S2 GenBank numbers for newly accessioned sequences and their sequence sources, herbaria of deposit, and geographical localities.

Table S3 GenBank numbers for newly accessioned sequences and their sequence sources, herbaria of deposit, and geographical localities.

Table S4 Synopsis of the 3784 records of endophytic and endolichenic Pezizomycete isolates in the UA database showing both the phylogenetic placement to the family level and the recorded host associations.

Table S5 Synopsis of the most frequently isolated endophytic or endolichenic Pezizomycetes in the UA database, enumerated by genus in a particular type of host.

Table S6 Endophytic or endolichenic species of Pezizomycetes that are obligately pyrophilous or commonly fruit after burns or volcanic eruptions.

Key words: endophytes, Pezizales, phylogenetics, pyrophilous fungi, symbiosis, systematics.

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