Asperosporus subterraneus, a new genus and species of sequestrate Agaricaceae found in Florida nursery production

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Abstract: We describe a novel sequestrate genus and species, Asperosporus subterraneus gen. et sp. nov., found associated with nursery production of ferns in south Florida. This truffle species has a unique combination of morphological characters among described Agaricaceae in that it lacks a stipe or columella, has large, ornamented spores, the fresh sporocarps rapidly stain pink-red when cut or bruised, and they have a rancid smell. Although this fungus does not appear to be a direct plant pathogen, the hyphae of A. subterraneus produce a thick hydrophobic mycelial mat that binds the organic matter and therefore prevents water and fertilizer from being absorbed by plants, consequently causing wilting and chlorosis. Using morphological characteristics and phylogenetic reconstruction based on the internal transcribed spacer (ITS), partial large subunit nuclear ribosomal DNA (LSU), second largest subunit of RNA polymerase II (rpb2) and translation elongation factor 1-alpha (tef1) regions, we describe this taxon as a new genus and species in Agaricaceae.

INTRODUCTION

Agaricaceae is a large family of fungi containing species with diverse morphological characteristics, from spore color (white, green, dark brown, black, blue, pale-pink and pale-yellow) to sporocarp type (agaricoid, secotioid, gasteroid) (Lebel et al. 2004, Vellinga 2004b). While sporocarps from many genera in Agaricaceae bruise or stain red when fresh, this feature can be critical for species level identification in some taxa (Ge et al. 2018, Vellinga et al. 2010b). Here we refer to Agaricaceae as defined in Vellinga et al. (2011) but recently a more constrained delimitation of genera in Agaricaceae s.l. has been proposed which narrows the number of accepted taxa (Kalichman et al. 2020). Recently, several studies have revealed that sequestration is more prolific throughout the family than previously thought and that many of these sequestrate fungi are found in genera with mostly agaricoid fruiting forms (Lebel et al. 2004, Kropp et al. 2012, Lebel & Syme 2012, Ge et al. 2018). Since fungi with gasteroid sporocarp forms are generally hypoegenous, finding sufficient collections to describe new taxa is challenging and has resulted in multiple monotypic genera and descriptions based on few collections (Ge & Smith 2013). For instance, genera such as Amogaster (gasteroid, now considered in the genus Lepiota), and Barcheria (gasteroid) are only known from a small number of collections (Lebel et al. 2004, Ge & Smith 2013).

In 2017 and 2018, potted Boston fern (Nephrolepis exaltata) samples were submitted for disease diagnosis to the University of Florida Plant Diagnostic Clinic (Homestead, Florida) (Fig. 1A). The specimens from 2018 were not dried properly and therefore specimens were not kept nor were DNA sequences obtained. However, photos and notes taken at the time of submission confirm...
that the specimens correspond to the same fungus as the ones submitted in 2017. For both the 2017 and 2018 samples, the plants were excavated for root examination and several gasteroid basidiomata were observed (Fig. 1B). Tissues were examined under a light microscope using H₂O, 5 % KOH, and Melzer’s reagent. Spore dimensions are given in the following order length, width, and spore Q (length to width ratio) and spore measurements excluded spore ornaments, which were measured separately. All other morphological examinations (such as color and ornamentation) were based on 20–40 individual structures. Dried specimens were deposited at the University of Florida Fungal Herbarium under the accession number FLAS-F-68001.

Small pieces of basidiomata were ground in liquid nitrogen in 1.5 mL Eppendorf tubes and DNA was extracted using the CTAB extraction method (Doyle & Doyle 1987). PCR amplification was completed with the following primer pairs and thermocycler conditions according to each reference: the internal transcribed spacer (ITS) ITS1f/4 (White et al. 1990, Gardes & Bruns 1993), large subunit nuclear ribosomal DNA (LSU) LROR/LR6 (Gilvagys & Hester 1990), RNA polymerase II subunit 2 (rpb2) BRPB2-6F and bRPB2-7.1R (Matheny 2005), and the translation elongation factor 1-α locus (tef1) EF-983F/EF1-1567R (Rehner & Buckley 2005). PCR products were run on a 1.5 % agarose gel using SYBR Green and sequenced at MCLab (San Francisco, CA). Bi-directional reads were trimmed, assembled, and checked for quality using Geneious v. 2020.1.2. Sequences were aligned with MAFFT v. 7.450 (Katoh et al. 2010) with reduced stringency settings by allowing gaps within aligned regions. Topologies were compared and inspected for conflict. Conflict was assumed to be significant if a group of taxa was supported at 70 % as monophyletic with one locus but supported as nonmonophyletic by another locus [reciprocal 70 % ML bootstrap support criterion (Reeb et al. 2004)].

Maximum Likelihood (ML) phylogenies based on the ITS and LSU regions, and on the concatenated LSU + rpb2 + tef1 dataset were constructed using RaxML v. 8.2.10 (Stamatakis 2014) under the GTR+ gamma evolutionary model. Branch support values were estimated using 1 000 bootstrap (BS) replicates. The same alignments were used to infer phylogenies using Bayesian analysis with MrBayes v. 3.2.6.x64 (Huelsenberg 2004). jModeltest v. 2.1.10 (Darriba et al. 2012) was used to estimate the model of evolution for each region and the GTR+I+G model of evolution was assigned to the LSU and rpb2 regions and the GTR+G model of evolution to the tef1. The concatenated matrix was partitioned by locus prior to phylogeny inference. Posterior probabilities (PP) were determined using 10⁶ generations with sampling every 1 000 generations. The first 25 % of trees were discarded as the burn in. Sequence data was deposited in NCBI (Table 1), and alignments and trees were deposited on the Open Science Framework (OSF) at https://osf.io/7gn8S/?view_only=08df5d71e46b4c086b12388fc775a9c7e.

**RESULTS**

The ITS and LSU sequences generated from specimens were compared to the NCBI and UNITE (Abarenkov et al. 2010) nucleotide databases, using different BLAST strategies (including/excluding environmental sequences and restricting the search to sequences generated from type specimens). Results based on the ITS sequence (701 bp) yielded top BLAST hits to members of the genus *Coniolepiota* (e.g., *HM488757, KGC2S531, and HM488755*) but only with approximately 87 % similarity. Results based on the LSU sequence (868 bp) yielded top BLAST hits to *Chlorophyllum molybdites* (KU049687, KU049686, and KU049684) with approximately 98 % similarity. Since BLAST matches provided limited resolution on the identity and taxonomic placement of this novel truffle, we performed a preliminary phylogenetic analysis based on the ITS and LSU regions.

Phylogenetic reconstruction using the ITS region (909 characters / 36 taxa) placed the novel fungus within the *Agaricus*+satellite clade (*sensu* Vellinga et al. 2011), which includes *Agaricus* and several other closely related genera such as *Clarkeinda*, *Coniolepiota*, *Eriocybe*, *Heinemannomyces*, and *Hymenagaricus* (BS: 75 %; PP: 0.98). Overall, the resulting topologies from the ML and BI analyses were similar and differences in relationships among taxa were unsupported by BS or PP statistics (Supplementary Fig. S1). The ML analysis placed the novel taxon as sister to *Eriocybe chionea* and the BI placed it sister to *Lepiota* cf. *fuscovinacea*, in both cases with no support.

The phylogenetic tree based on the LSU region (861 characters / 44 taxa) resolved the novel fungus as an unsupported lineage sister to *Agaricus* (Supplementary Fig. S2). The analysis based on LSU provided weak support for most relationships across the family. No conflict was found between the ML and BI analysis. The novel lineage was not recovered as closely related to any of the known sequestrate *Agaricaeae* taxa in either the ITS or LSU analyses.

The concatenated alignment based on LSU, rpb2 and tef1 consisted of 2 215 characters and comprised 29 taxa. This dataset included 902 bp for LSU, 766 bp for rpb2 and 547 bp for tef1. ML and BI analyses generated phylogenies with non-conflicting
### Table 1. GenBank accession numbers for taxa analyzed in this study. Bold taxa are sequences generated in this study.

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topologies. Bootstrap support values and posterior probabilities were congruent for major nodes and recovered currently accepted phylogenetic relationships among genera within the Agaricaceae [for taxonomic revisions of the family see Vellinga (2004b), Vellinga et al. (2011), and Kalichman et al. (2020)]. The Bayesian analysis recovered (PP: 0.97) the novel taxon as an independent lineage within the Agaricus+satellite clade (Fig. 2). Phylogenies based on matrices with and without ambiguously aligned regions resulted in similar topologies. The multilocus phylogeny based on the concatenated alignment with ambiguously aligned regions is shown in Supplementary Fig. S3.

**Taxonomy**

**Asperosporus** Karl.-Ayala, Gazis & M.E. Sm., gen. nov. MycoBank MB 838905.

*Etymology:* From the Latin “asper” (rough) and “sporus” (spores), for the highly ornamented spores.

Distinguished from other genera in Agaricaceae by a combination of the following characters: Basidiomata hypogeous and sequestrate, globose to subglobose, stipitate, and friable when dry. Gleba loculate, brownish, turning pink-red when freshly cut or bruised, hydrophobic, drying powdery and friable. Basidiospores subglobose to ellipsoid, pale yellow-orange when young but turning brown with age, thick-walled, ornamented, sterigmal remnants attached, and strongly dextrinoid in Melzer’s reagent. Basidiomata producing a rancid odor when fresh. Clamp connections absent.

*Type species:* *Asperosporus subterraneus* Karl.-Ayala, Gazis & M.E. Sm.

*Asperosporus subterraneus,* Karl.-Ayala, Gazis & M.E. Sm., sp. nov. MycoBank MB 838906. Figs 1, 3A–F.

*Etymology:* From the Latin “sub” (below) and “terra” (earth), for the habit of fruiting below the soil.

*Diagnosis:* Basidiomata 5–30 mm diam, globose to irregular, Table 1. (Continued).

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</table>
astipitate, light tan but drying dark brown and staining pink-red when handled, producing a rancid odor when fresh. *Peridium* thin and friable when dry. *Gleba* loculate, staining pink-red when exposed to air, friable and hydrophobic when dried. *Basidia* 1–3 spored in fresh specimens, collapsing upon drying. *Spores* 16–22 × 12–18 μm, subglobose or ellipsoid, ornamented with warts, thick walled, strongly dextrinoid, often with sterigma remnants remaining attached. The genus is presently monotypic.

**Macroscopic features:** *Basidiomata* 5–30 mm diam, globose to irregular, lacking a stipe, surface smooth, at first white or light tan but becoming brownish with age, staining pink-red where handled or bruised (Fig. 1C), drying darker brown, particularly where bruised. *Peridium* irregular, friable when dry (Fig. 1D), sometimes sloughing off completely where handled, staining dark brown to black in Melzer’s reagent. No response in KOH when dried. *Gleba* compressed, irregular, loculate (Fig. 3A), lacking a columella, light tan at first with occasional veins of white trama tissue and pockets of darker brown spores, staining pinkish or red when cut and exposed to air, reddish staining more notable in younger specimens, turning dark brown, powdery, delicate and hydrophobic upon drying. *Odor* rancid when fresh, indistinctly fungal when dry. *Taste* not determined.

**Microscopic features:** *Basidiospores* 16–22 × 12–18 μm (av. 18 × 15 μm) at maturity, globose, subglobose or ellipsoid (Q = 1–1.47 μm, mean Q = 1.21 μm); walls 1–3.5 μm thick (av. = 1.8 μm); restricted at the sterigmal attachment point and apiculate, notably ornamented with larger pyramidal to irregular warts, up to 1 μm tall and 1 μm wide. Immature spores are notably smaller, mostly 12–15 × 10–12 μm, and with smaller spines that are 0.5 × 0.5 μm and clearly separated from one another.
Sterigma remnants often remaining attached and clearly visible in many spores (Fig. 3E) but more common in young spores, most 4–5 μm × 1–2 μm but sometimes up to 9 μm long. Pale yellow-orange when young but becoming darker orange-brown at maturity when observed using KOH or water, strongly dextrinoid in Melzer's reagent with mature spores turning notably darker than younger spores, highly variable in size and shape.

**Basidia** 1–3 spored, difficult to find and see, collapsing in mature dried specimens (Fig. 3F).

**Cystidia** not observed.

**Peridium** (100–)150–250(–350) μm thick, composed of loosely interwoven (Fig. 3B) and irregularly branched and septate, single layered, hyphae 3–5 μm diam, occasionally swelling up to 10 μm; arrangement of hyphae mostly tangled and irregular but occasional bands of hyphae parallel to the exterior near the peridial surface, light yellow-brown, some hyphae strongly dextrinoid. **Trama** tissue 75–200 μm thick, composed of irregularly shaped, elongated and inflated hyaline hyphae, 10–26 μm diam. **Subhymenium** approximately 10–40 μm thick, comprised of densely packed interwoven hyphae with cells 12–14 μm diam that are brown to orange-brown. **Clamp connections** absent on all hyphae. No response to KOH.

**Habitat and distribution:** Found in south Florida growing in soil of potted nursery plants with poor drainage. Specimens thus far found only in association with Boston Fern (*N. exaltata*) which was planted in a Canadian peat moss and Florida pine bark mixed potting soil during the winter months.

**Typus:** USA, Florida, Tropical Research and Education Center Plant Diagnostic Clinic, Homestead, Miami-Dade Co., 21 Dec. 2017, R. Gazis, MES-3094 (holotype FLAS-F-68001).

**Additional collection examined:** USA, Florida, Tropical Research and Education Center Diagnostic Clinic, Homestead, Miami-Dade Co., 4 Dec. 2018, R. Gazis, 180871 (Photos deposited in MycoBank, but specimens not dried properly and therefore discarded).
Fig. 3. Microscopic features of *Asperosporus subterraneus* specimen FLAS-F-68001 (holotype) **A.** Loculate gleba **B.** Peridium hyphae. **C.** Tangled peridium hyphae. **D.** Ornamented spores with thick spore walls. **E.** Subhymenium with basidiospores, some of which have retained sterigma remnants (indicated with arrows). **F.** Basidium with basidiospores. Scale bars: **A–C** = 60 μm, **D–F** = 20 μm.
DISCUSSION

Morphology

Several puffballs in Agaricaceae s.l. (i.e., Bovista, Calvatia, Lycoperdon) have features that are similar to those of *A. subterraneus*, such as the highly ornamented, powdery brown spores and a hydrophobic surface. However, these puffballs do not stain red when handled and typically produce aboveground basidiomata (Vellinga, 2004b, Larsson & Jeppson 2008, Jeppson et al. 2012). Furthermore, most of these puffball species have spores that are relatively small (e.g. less than 10 μm diam) whereas the spores of *A. subterraneus* are much larger (av. 18 × 15 μm) (Miller & Miller 1988). A pink-red staining reaction of the basidiomata is prominent in many genera of the Agaricaceae, including in species of *Agaricus, Barcheria, Chlorophyllum, Cystolepiota, Leptia, Leucoagaricus, Leucocoprinus, Macrospallioiota, and Melanophyllum* (Pagel 1985, Vellinga 2010, Vellinga et al. 2010, 2011, Zhao et al., 2010, 2016, Ge et al. 2018). However, the combination of red staining basidiomata and ornamented spores is unique to *A. subterraneus* within Agaricaceae. *Leucoagaricus* (Agaraceae) also contains red staining taxa without clamp connections (Vellinga et al. 2010a, Dutta et al. 2021) but these taxa are all agaricoid. Morphologically, *A. subterraneus* could be mistaken for *Barcheria willisiana* (Agaraceae) which is a fragile, astipitate, sequestrate fungus that contains an enclosed, loculate gleba, stains red when bruised, lacks clamp connections, and contains thick-walled globose spores that are strongly dextrinoid in Melzer’s reagent (Lebel et al. 2004). However, *Barcheria* is currently known only from Australia and this taxon is easily differentiated from *A. subterraneus* by its smooth spores, scaly peridium, and basidia that remain intact upon drying.

Initial molecular and morphological similarities suggested that *A. subterraneus* was closely related to *Chlorophyllum* (Agaricaceae). *Chlorophyllum* is a diverse genus that contains several taxa that are sequestrate and stain upon handling as well as taxa that are associated with disturbed, nutrient-rich landscapes (Vellinga 2004a, Ge et al. 2018). Recently, more species of sequestrate *Chlorophyllum* have been discovered, such as *C. lusitanicum* (Carlavilla et al. 2018) and *C. levantinum* (Loizides et al. 2020). However, sequestrate fungi in this genus typically have smooth spores and a well-defined columnella or stipe. Kropp et al. (2012) described three new species of sequestrate taxa from western North America and erected a new genus, *Cryptolepiota*, that were later transferred to *Leptia* (Agaricaceae) (Lebel & Vellinga 2013). All of these taxa are subglobose, astipitate, sequestrate fungi that are fragile when dry. *Asperosporus* can be differentiated from species of “Cryptolepiota” by the pink-red staining reactions, rancid odor when fresh, ornamented spores and basidia that collapse upon drying. While spore ornamentation can be used to differentiate *Asperosporus* from most *Leptia* species, there are some sequestrate *Leptia* species with ornamented spores. However, all of these taxa these (e.g. *L. iberica, L. olbia, L. smurfiorum*, and *L. tulostomoides*) are secotioid and retain their stipe at maturity (Vidal et al. 2015). The sequestrate fungus *Neosecomium macrosporum* (Agaricaceae) also has ornamented spores and a powdery gleba (Lizárraga et al. 2012). While there are currently no publicly available sequence data for *Neosecomium macrosporum*, this sequestrate fungus differs from *A. subterraneus* in that *N. macrosporum* has a columnella, a thick peridium, smaller and more globose spores, and flatter ornamentation that give the spores a cracked appearance. *Agaricus* (Agaricaceae) is a large genus of saprotrophic fungi containing more than 400 species in six subgenera and 21 sections (Zhao et al. 2016, Chen et al. 2017). This genus has species with diverse basidiomata types, including many different agaricoid, secotoid, and gasteroid taxa. Newly described sequestrate taxa (Lebel & Syme 2012, Lebel 2013) as well as species that were more recently transferred from other obsolete genera (e.g. *Longula texensis = Agaricus deserticola*) have expanded the diversity of this genus. Many taxa in *Agaricus* also stain or bruise red when fresh and all lack clamp connections (Zhao et al. 2016). However, species of *Agaricus* can be differentiated from *Asperosporus* by their smooth spores that lack the ornamentation seen in *A. subterraneus*.

Asperosporus subterraneus is characterized by a unique combination of morphological features that also differentiates it from other known sequestrate fungi outside Agaricaceae. Sequestrate fungi with fully enclosed basidiomata typically lose their ability to forcibly discharge their spores. Evidence of this can be seen in fungi that have poorly reviving basidia when dried and have spores with sterigma remnants (Trappe et al. 2009). Sterigma attachments was seen in *Asperosporus subterraneus* and in other sequestrate fungi such as *Guyanagaster necrorhiza* (*Physalacriaceae*) (Henkel et al. 2010). *Guyanagaster necrorhiza* is similar to *A. subterraneus* in that both taxa have strongly dextrinoid, thick-walled, ornamented spores and a well-defined gleba. However, the spores of *G. necrorhiza* are spiny, globose and light tan with pink tones and the peridium is highly warted (Henkel et al. 2010). Another genus with similarities to *Asperosporus* is *Octaviania* (Boletaceae). *Octaviania* basidiomata have sporocarps that often bruise when handled (blue, green, or black but occasionally red) and have globose to subglobose spores with pyramidal ornamentation and dextrinoid reactions (Orihara et al. 2012). However, species of *Octaviania* generally have smaller spores with more conspicuous ornaments and a gleba that never becomes powdery at maturity. The truffle *Xerocomellus macmurchyi* (Boletaceae) is also similar to *A. subterraneus* in its powdery gleba and in the dextrinoid spores that are similar in size and ornamentation (Smith et al. 2018). However, both *Octaviania* and *Xerocomellus* belong to the Boletales and are ectomycorrhizal whereas *A. subterraneus* belongs to the Agaricales and is likely saprotrophic. Many previously described truffle-like fungi with ornamented brown spores were originally placed within the genus *Hymenogaster*. However, morphological and molecular analyses have shown that *Hymenagaster sensu lato* contains many different unrelated taxa (Bougher & Castellano 1993, Smith et al. 2018). We have reviewed the descriptions of taxa in *Hymenagaster sensu lato* (Dodge & Zeller 1934) and were unable to locate any species that match the description of *A. subterraneus*.

Phylogenetic placement

Several challenges arise when attempting to place *A. subterraneus* into a phylogenetic context. No close matches (above 90%) were found in nucleotide databases (NCBI and UNITE) between ITS sequences of *A. subterraneus* and previously collected specimens or environmental sequences generated through metabarcoding studies. Maximum Likelihood and Bayesian analyses based on the LSU and multilocus datasets placed *Asperosporus subterraneus* as sister to *Agaricus* within the Agaricaceae sensu lato (sensu Vellinga et al. 2011) without support (Fig. 2, Supplementary Fig. 2018).
Ecology of Asperosporus subterraneus

Asperosporus subterraneus has thus far only been found in association with potted Boston Fern plants submitted to the University of Florida Plant Diagnostic Clinic in Homestead, Florida in 2017 and 2018. The hyphae of A. subterraneus extensively colonize the substrate, thereby generating a hydrophobic mat which binds the organic matter and prevents the plant’s roots from accessing water and nutrients. There is no evidence to suggest that A. subterraneus is a plant pathogen because there was no obvious root contact with the fungus. However, A. subterraneus can cause plants to develop wilting and chlorosis that make them unmarketable. The dense hyphal mats and ample basidiomata found within the substrate suggest that this fungus can become prolific in nursery settings and that the source of inoculum is likely the potting soil. Thus far basidiomata have not been found in soil or pots without plants. The south Florida ornamental industry generally grows plants in outdoor shade houses that allow ambient rainfall, temperature, and humidity to impact the growth of plants. The planting containers likely create suitable conditions for this fungus to fruit during plant production. Asperosporus subterraneus has been a recurrent problem in ornamental nurseries in south Florida and is thought to be associated with potting soil containing a mix of peat moss and pine bark (R. Gazis, pers. obs.). A robust understanding of the lifestyle, growth requirements, and dispersal of this fungus will help develop effective management recommendations for nurseries to prevent financial losses due to unmarketable plants. There are several fungi in the Agaricaceae that are common in nursery settings or in indoor potted plants. For example, Leucocoprinus birnbaumii, commonly called the “yellow house plant mushroom” is frequently found in indoor potted houseplants (Vellinga 2004a). Some disturbance-adapted Agaricaceae species have been found far outside their known natural distributions. For example, Smithiomycetes mexicanus, known from tropical regions in Florida and Brazil, was found growing in Belgium around swimming pools with a sub-tropical landscape (Vellinga 1999). The nutritional mode for Asperosporus subterraneus remains unknown but we can infer it as being saprotrophic because it falls within the Agaricaceae, close to the Agaricus clade, which only contains saprotrophic species (Vellinga 2004b). Although this newly described fungus was not found in a natural environment and we do not know the geographic origin of this fungus, we still introduce this taxon as novel based on morphological characteristics and molecular evidence. Furthermore, the morphological characteristics and molecular placement of this truffle highlight the high diversity and unique combinations of morphological characteristics of sequestrate fungi within the Agaricaceae.

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Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES


**Asperosporus subterraneus gen. et sp. nov.**

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We refer you to the above-mentioned references for a comprehensive understanding of the phylogenetic relationships and molecular evidence supporting the description of **Asperosporus subterraneus**. This fungus is part of an updated phylogenetic tree based on the ITS region, which recovers many of the satellite taxa in the clade together with the description of more unknown taxa. The phylogenetic tree is rooted with the supported sister lineage sister to Agaricus within the Agaricaceae (Supplementary Fig. S1). Many of the satellite taxa in the Agaricus clade, such as Coniolepiota, Eriocybe, and Heinemannomyces and Psuedolepiota are monotypic genera, with limited collections and sequence data, and some of them have been described from a single locality in Asia. Heinemannomyces is a monotypic genus but recently has been shown to fall within Hymenagaricus (Hussain et al. 2018). Moreover, phylogenetic relationships among members of this clade remain unresolved (Watling 1998, Vellinga et al. 2011, Ge & Yang 2017) and unsupported nodes may be a reflection of incomplete taxon sampling. Therefore, future efforts to increase taxon sampling may be an important component to improve our accurate phylogenetic placement and understanding of relationships within this group (Som 2015). This includes increasing sequence availability of additional loci from known taxa in this clade together with the description of more unknown taxa.


**Supplementary Material:** [http://fuse-journal.org/](http://fuse-journal.org/)

**Fig. S1.** Maximum likelihood tree based on ITS dataset. Branch support values >75 % and Bayesian posterior probabilities > 0.90 are indicated. Symbols with closed circles represent gasteroid taxa, symbols with half circles represent secotoid taxa, and all other taxa are agaricoid. *Limacella glioderma* was used as an outgroup.

**Fig. S2.** Maximum likelihood tree based on LSU dataset. Branch support values >75 % and Bayesian posterior probabilities > 0.90 are indicated. Symbols with closed circles represent gasteroid taxa, symbols with half circles represent secotoid taxa, and all other taxa are agaricoid. *Limacella glioderma* was used as an outgroup.

**Fig. S3.** Maximum likelihood tree based on concatenated LSU, *rpB2*, and *tef1* dataset with ambiguously aligned regions excluded. Branch bootstrap support values >75 % and Bayesian posterior probabilities > 0.90 are indicated. *Limacella delicata*, *Limacella glioderma* and *Limacella guttata* were used as an outgroup.
Supplementary Fig. S1. Maximum likelihood tree based on ITS dataset. Branch support values >75 % and Bayesian posterior probabilities > 0.90 are indicated. Symbols with closed circles represent gasteroid taxa, symbols with half circles represent secotoid taxa, and all other taxa are agaricoid. Limacella glioderma was used as an outgroup.
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Figure S3

**Supplementary Fig. S3.** Maximum likelihood tree based on concatenated LSU, rpb2, and tef1 dataset with ambiguously aligned regions excluded. Branch bootstrap support values >75 % and Bayesian posterior probabilities > 0.90 are indicated. *Limacella delicata*, *Limacella glioderma* and *Limacella guttata* were used as an outgroup.