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A new species of *Cintractiella* (Ustilaginales) from the volcanic island of Kosrae, Caroline Islands, Micronesia

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Abstract

Cintractiella is an unusual genus of smut fungi containing two described species that produce sori as adventitious gall-like spikelets on members of tribe *Hypolytreae* (subfam. Mapanioideae, Cyperaceae). In September 200, during a botanical expedition on the volcanic island of Kosrae located in the eastern Caroline Islands and within the Federated States of Micronesia, a specimen of *Mapania pacifica* was collected displaying *Cintractiella*-like sori in adventitious spikelets on the host leaves. Sori were hypophyllous, occurring in groups of spikelets composed of olivaceous-brown scale-like leaves, 1–1.5 mm wide and up to 6 mm long. Microscopic comparison with the protologue and drawings of the type material of *C. lamii* show several differences in teliospore and sori characters between it and the newly collected material on *Mapania*. To our knowledge, this represents only the second known collection of any member of *Cintractiella* on vegetative organs of *Hypolytreae* and a third species for this genus and the only known smut species infecting *Mapania*, herein described as *Cintractiella kosraensis* **sp. nov.**

Keywords

Biodiversity, phytopathogens, sedges, South Pacific, Ustilaginomycotina, 1 new taxon

Introduction

There are strong correlations between the classification of smut fungi and the systematics of their host plants. For example, species of the smut genera *Anthracoidea*, *Aurantiosporium*, *Cintractia*, *Dermatosorus*, *Farysia*, *Kuntzeomyces*, *Leucocintractia*, *Moreaua*, *Orphanomyces*, *Schizonella*, *Testicularia*, *Trichocintractia* and *Ustanciosporium* exclusively infect members of Cyperaceae (Piepenbring 2001).

Cintractiella Boedijn, with only two known species, is an example of a smut genus that appears to be restricted to Cyperaceae, in this case wholly within the tribe *Hypolytreae*. *Cintractiella lamii* Boedijn, the type species of the genus, is only known from the locus classicus from Indonesia. The species produces sori in adventitious spikelets on leaves of a *Hypolytrum* sp. (Cyperaceae, subfam. Mapanioideae, tribe Hypolytreae). The type specimen was collected in Indonesia in 1920 and preserved in alcohol at Herb. Bogoriense (BO). Boedijn (1937) investigated the material thoroughly and described it as a new smut fungus in a new genus. Since that time, the fungus has not been recollected. Unfortunately, neither type material nor other collections of this species are available for study. The type specimen in Bogor was lost; only the empty glass vessel and label is present (Piepenbring 2001, Vánky 2003). Thus, our knowledge of this species is based on the original publication for *C. lamii* (Boedijn 1937, for a reproduction see Vánky 2013). The second species, *C. diplasiae* (Henn.) M. Piepenbr., was originally described as *Ustilago diplasiae* Henn., on *Diplasia karataefolia* L.C.Rich. (Hypolytreae). The type specimen was collected from Brazil and the species is also known from Venezuela on the same host species (Vánky 2003). In addition to differences in host plant and distribution, *C. diplasiae* differs from *C. lamii* in producing sori in the host inflorescences, rather than on the leaves.

In September 2009, an unusual smut fungus producing spikelets on the leaves of *Mapania pacifica* (Hosok.) T.Koyama (*Hypolytreae*) was discovered on the island of Kosrae within the Federated States of Micronesia, herein described as a third species of *Cintractiella, C. kosraensis* sp. nov. To our knowledge, *C. kosraensis* is the only smut species known to infect a species of *Mapania.*

Methods

Field surveys for botanical specimens were conducted on the island of Kosrae (5°20'N and 163°0'E) in September 2009. Due to the extreme steepness, inaccessibility and thickness of vegetation within this study region, survey transects were chosen intuitively and conformed to regional contours that were safely approachable. Herbarium voucher collections have been made in order to document common and rare plant taxa and for species identifications. Data for plant specimen vouchers are entered into the National Tropical Botanical Garden (PTBG) herbarium database. Specimens are being curated primarily at the Bishop Museum (BISH) and PTBG herbaria. Photographs of plants and habitats are curated by the NTBG and stored within a digital asset management

system (i.e. ResourceSpace). The NTBG maintains a checklist of vascular plant taxa observed within the study region (Microsoft Excel database). Latitude and longitude coordinates were recorded by a Garmin GPSmap 60CSx (Garmin corp., Olathe, Kansas, U.S) unit in Lat/Long decimal for herbarium specimen data. The new smut species was found along the summit ridge of Mt. Oma in Malem Municipality (Fig. 1A) on the indigenous sedge *M. pacifica* (Fig. 1B). Materials studied here were deposited in the Kriebel Herbarium (PUL) and National Tropical Botanical Garden (PTBG).

Spores were mounted in lactic acid in glycerol. Light microscopic analyses were performed using a Nikon Eclipse 80i microscope (Nikon corp., Tokyo, Japan). Photomicrographs were obtained with a DS-Fi1 Nikon camera. Measurements are of a minimum of sixty randomly selected spores.

Taxonomy

***Cintractiella kosraensis* Aime, M.Abbasi & K.R.Wood sp. nov.**
MycoBank No: MB826716
Fig. 2

Diagnosis. Differs from the similar *Cintractiella lamii* in having thin walled mostly depressed-globose spores with no visible germ pore and in lacking the hard, cylindrical curved mass of spores and hypertrophic parenchymatic tissue on the leaves, characteristic of *C. lamii*.

Type. CAROLINE ISLANDS: The State of Kosrae: Malem Municipality, Mount Oma, 410 m alt., on *Mapania pacifica* (Hosok.) T. Koyama, 4 Sep 2009, K.R.Wood 13895 (holotype: PTBG-070102; isotype: PUL F2910).

Description. Sori amphigenous, mostly hypophyllous, clustered in groups of spikelets, each composed of olivaceous-brown, scale-like leaves, 1–1.5 mm wide, up to 6 mm long (Fig. 2A–B). Spore mass black, initially agglutinated and surrounded by a thin hyaline membrane, with no hard cylindrical body; at maturity, exposed at the opened tip of the spikelet. Spores single, mostly depressed-globose, globose or semi-globose, (28–) 35–44 µm in diameter, with no visible germ pore, wall dark reddish-brown, (1.2–) 1.5–2.5 (–3) µm thick, minutely reticulate (Fig. 2C–D). Spore germination not known.

Distribution and ecology. *Cintractiella kosraensis* sp. nov. is only known from the type location along the summit ridge of Mt. Oma in Malem Municipality and type host–the indigenous sedge *M. pacifica*–on the volcanic island of Kosrae, located in the eastern Caroline Islands and within the Federated States of Micronesia in the general vicinity of 5°20'N, 163°0'E (Lorence and Wood 2012, Figure 1A).

Etymology. *kosraensis* = for the island of Kosrae, where this species was discovered.

Specimens examined. Caroline Islands. The State of Kosrae: Malem Municipality, Mount Oma, 410 m alt., on *M. pacifica*, 4 Sep 2009, K.R.Wood 13895 (holotype: PTBG-070102; isotype: PUL F2910).

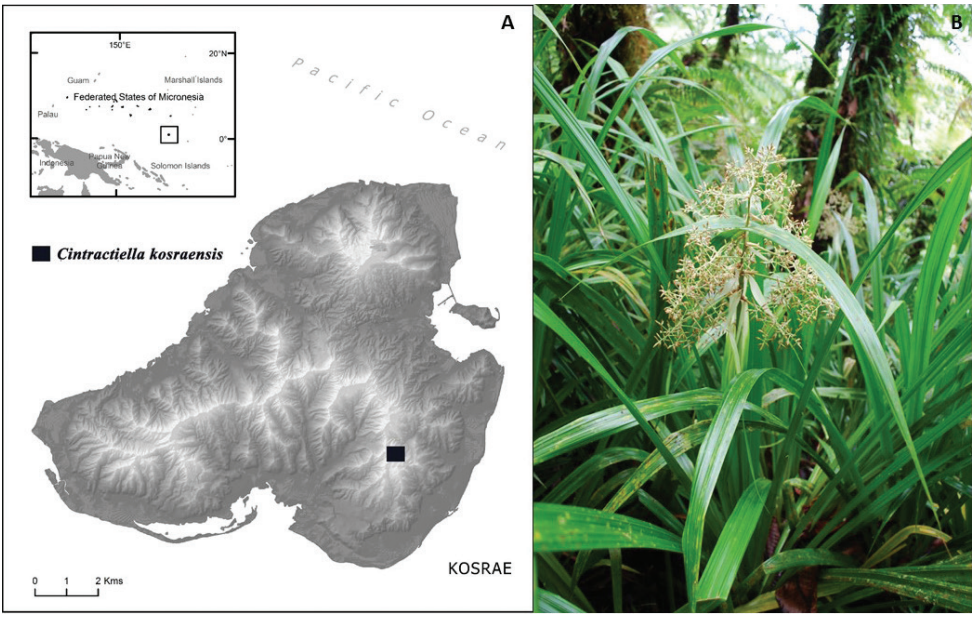

Figure 1.A Type location of *Cintractiella kosraensis* on the island of Kosrae, Federated States of Micronesia **B** The indigenous host, *Mapania pacifica*, occurring along the summit ridge to Mt Oma.

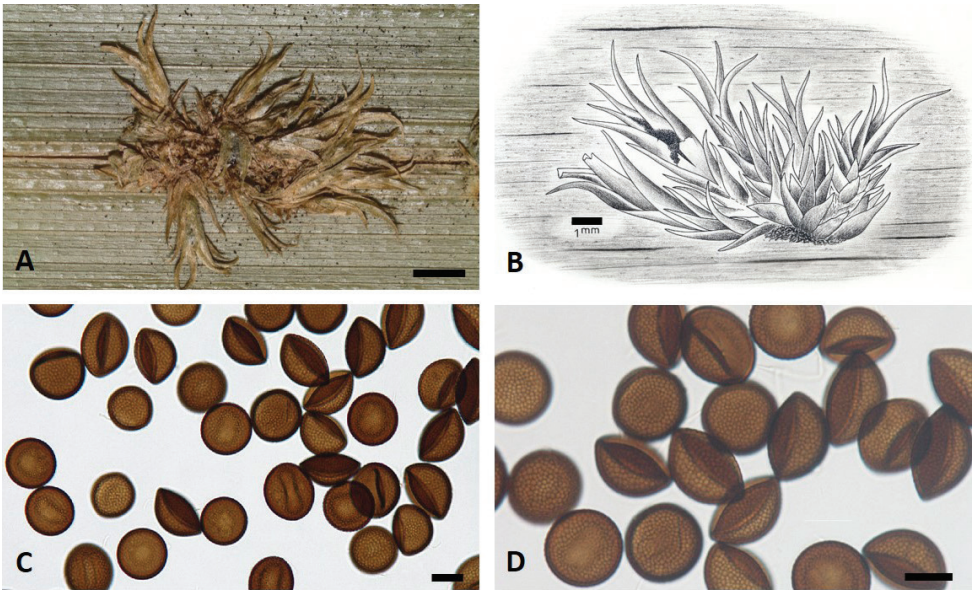

Figure 2. *Cintractiella kosraensis* (holotype, PTBG-070102) **A–B** sori on leaf as a photomicrograph (**A** Scale bar: 2 mm) and a line drawing (**B** Scale bar: 1 mm). **C–D** teliospores (Scale bars: 25 μm).

Discussion

Cintractiella is an unusual genus amongst smut fungi that produces sori in adventitious spikelets on vegetative or generative organs of members of tribe *Hypolytreae* (subfam. Mapanioideae, Cyperaceae). Only two other species have been described: *C. diplasiae* and *C. lamii*. *Cintractiella diplasiae* differs from *C. kosraensis* in producing sori in the host inflorescences and also producing teliospores with walls covered by blunt, rather densely situated, rarely confluent warts of variable sizes (Vánky 2003). *Cintractiella lamii* produces masses of teliospores in "peculiar galls", i.e. adventitious branches with scale-like leaves, growing out of hypertrophic parenchymatic tissue on the abaxial side of the lamina of leaves, similar to *C. kosraensis*. However, in *C. lamii,* these are agglutinated and protrude as a column from the tips of the branches, whereas no column is formed in *C. kosraensis*. Teliospores are also diagnostic: in *C. lamii* these are globose, more or less flattened at one side, 29–36 µm and dark brown with a germpore and spore walls that are 3–4 µm thick and finely reticulate (Piepenbring 2001).

All three known members of *Cintractiella* parasitise members of Mapanioideae in Cyperaceae. The only report of *C. lamii* is from *Hypolytrum* sp. from Indonesia; *C. diplasiae* is found on *Diplasia karataefolia* in Brazil, Trinidad and Venezuela (Vánky 2003). To our knowledge, *C. kosraensis* is the first smut fungus known to infect a species of *Mapania*.

Ideally, the description of new taxa is supported by abundant material from multiple collections. However, especially when considering microfungi from remote locales, these optima often cannot be met. Nonetheless, description of new species, even from limited material, adds to our understanding of fungal diversity (Kurtzman 2010) and highlights regions and lineages for which in-depth studies are needed. Most of the South Pacific islands remain under-explored for fungi, although these also appear rich in rare and endemic taxa (e.g. Kijpornyongpan and Aime 2016). Importantly, newly discovered taxa from rare lineages were shown to harbour the majority of novel genes in comparative genomic studies in smut fungi (Kijpornyongpan et al. 2018), highlighting the urgency in documenting this diversity before it disappears.

Acknowledgements

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RESEARCH ARTICLE

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Two new species of *Geejayessia* (Hypocreales) from Asia as evidenced by morphology and multi-gene analyses

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Abstract

Two new species of *Geejayessia* are introduced, based on materials collected from central China. *Geejayessia clavata* **sp. nov.** is characterised by gregarious, red brownish to dark red, oval-subglobose to globose perithecia that are formed on a basal stroma; (4–7-)8-spored cylindrical asci; ellipsoidal or rarely broadly ellipsoidal, uniseptate, smooth or finely verruculose ascospores; clavate, aseptate microconidia and absence of macroconidia. *Geejayessia sinica* **sp. nov.** is characterised by red to bright red, pyriform, subglobose to globose, perithecia on a basal stroma, collapsing laterally when dry; subcylindrical to clavate asci with a rounded apex; ellipsoidal, uniseptate ascospores; and falcate, multiseptate macroconidia with an arcuate tip. Morphological distinctions of the new species from the related fungi are discussed. This is the first report of *Geejayessia* from Asia.

Keywords

Cosmospora-like fungi, Nectriaceae, Systematic, Taxonomy

Introduction

Some fusarium-like species having gregarious, multicoloured, broadly ampulliform short-necked or broadly ellipsoidal perithecia were previously placed in *Cosmospora* Rabenh. and *Nectria* (Fr.) Fr. (Booth 1959; Samuels and Rogerson 1984; Nirenberg and Samuels 2000) until the genus *Geejayessia* Schroers, Gräfenhan & Seifert, typified by *G. cicatricum* (Berk.) Schroers, was introduced (Schroers et al. 2011). The genus is characterised

by prosenchymatous stromata erumpent through substrates, caespitose, broadly pyriform, pale orange, brownish to reddish-orange, bright red to black perithecia, reacting to potassium hydroxide (KOH) and lactic acid (LA); cylindrical or clavate asci with eight ascospores; broadly ellipsoidal to ellipsoidal ascospores that are uniseptate, slightly constricted at the septum, hyaline or pale brown to yellowish-brown, smooth or verruculose at maturity; and multiseptate, slightly curved macroconidia with conspicuous pedicellate foot cell (Schroers et al. 2011; Lombard et al. 2015). Members of *Geejayessia* exhibit host specificity and mainly occur on *Buxus* spp., *Celtis occidentalis* and *Staphylea trifolia* and were reported only from Europe, North America and Oceania (Samuels and Rogerson 1984; Nirenberg and Samuels 2000; Schroers et al. 2011).

In our examinations of nectriaceous collections from central China, two cosmosporalike fungi were encountered. Judging by perithecial gross morphology, anatomic structures and culture characteristics, they represented two previously undescribed species of *Geejayessia*. Their taxonomic placements were confirmed by multigene phylogenetic analyses. Distinctions between the new species and their closely related fungi are discussed.

Materials and methods

Sampling and morphological studies

Specimens were collected from Shennongjia National Nature Reserve and Longyuwan National Forest Park and were deposited in the Herbarium Mycologicum Academiae Sinicae (**HMAS**). Methods used by Luo and Zhuang (2010) and Schroers et al. (2011) were generally followed for morphological observations. The test for colour changes of the perithecial wall was made with 3% KOH and 100% LA. To observe internal and microscopic characteristics of the perithecial wall, longitudinal sections through ascomata were made with a freezing microtome (YD-1508-III, Jinhua, China) at a thickness of 6–8 μm. Microscopic examinations and measurements were taken from longitudinal sections and squash mounts in lactophenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Photographs were taken with a Leica DFC450 digital camera (Wetzlar, Germany) attached to a Leica M125 stereomicroscope (Milton Keynes, UK) for gross morphology and a Zeiss AxioCam MRc 5 digital camera (Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Göttingen, Germany) for anatomical structures. Measurements of individual structures were based on 30 units, except when otherwise noted. Cultures were obtained by single ascospore isolation from fresh ascomata. To determine colony features, isolates were grown on cornmeal dextrose agar [CMD, 4% (w/v) cornmeal + 2% (w/v) dextrose + 2% (w/v) agar], potato dextrose agar [PDA, 20% (w/v) potato + 2% (w/v) dextrose + 2% (w/v) agar] and synthetic nutrient-poor agar (SNA; Nirenberg 1976) in 90 mm plastic dishes at 25 °C for 7 d. For the observation of conidiophores, macroconidia and microconidia, cultures were grown on SNA at 25 °C with alternating periods of light/darkness (12 h/12 h). Colony growth rates were measured after 7 d.

Table 1. List of species, herbarium/strain numbers and GenBank accession numbers of materials used in this study.

Species	Herbarium/strain numbers	GenBank accession numbers		
		acl1	ITS	*rpb2*
Albonectria albosuccinea (Pat.) Rossman & Samuels	BBA 64502	HQ897837	HQ897788	HQ897699
A. rigidiuscula (Berk. & Broome) Rossman & Samuels	CBS 122570	HQ897896	HQ897815	HQ897760
Cyanonectria buxi (Fuckel) Schroers, Gräfenhan & Seifert	CBS 125554	HM626629	HM626660	HM626688
C. cyanostoma (Sacc. & Flageolet) Samuels & P. Chaverri	CBS 101734	HQ897895	FJ474076	HQ897759
Dialonectria episphaeria (Tode) Cooke	CBS 125494	HQ897892	HQ897811	HQ897756
D. ullevolea Seifert & Gräfenhan	CBS 125493	HQ897918	KM231821	HQ897782
Fusarium sambucinum Fuckel	CBS 14695	KM231015	KM231813	KM232381
F. sublunatum Reinking	BBA 62431	HM897916	HQ897830	HQ897780
Fusicolla acetilerea (Tubaki, C. Booth & T. Harada) Gräfenhan & Seifert	BBA 63789	HQ897839	HQ897790	HQ897701
F. matuoi (Hosoya & Tubaki) Gräfenhan & Seifert	CBS 58178	HQ897858	KM231822	HQ897720
Geejayessia atrofusca (Schwein.) Schroers & Gräfenhan	CBS 125505	HM626628	HM626659	HM626682
G. celtidicola Gräfenhan & Schroers	CBS 125502	HM626625	HM626657	HM626685
G. cicatricum (Berk.) Schroers	CBS 125552	HQ728171	HQ728145	HQ728153
G. desmazieri (De Not. & Becc.) Schroers	CBS 125507	HM626633	HM626651	HM626675
G. clavata Z.Q. Zeng & W.Y. Zhuang	HMAS 248725	**KY873305**[a]	**KY873307**	**KY873309**
G. sinica Z.Q. Zeng & W.Y. Zhuang	HMA S248726	**KY873306**	**KY873308**	**KY873310**
G. zealandica (Cooke) Schroers	CBS 11193	HM626626	HM626658	HM626684
Macroconia leptosphaeriae (Niessl) Gräfenhan & Schroers	CBS 100001	HQ897891	HQ897810	HQ897755
M. papilionacearum (Seaver) Gräfenhan & Seifert	CBS 125495	HQ897912	HQ897826	HQ897776
Microcera coccophila Desm.	CBS 31034	HQ897843	HQ897794	HQ897705
M. diploa (Berk. & M.A. Curtis) Gräfenhan & Seifert	BBA 62173	HQ897899	HQ897817	HQ897763
Nalanthamala psidii (Sawada & Kuros.) Schroers & M.J. Wingf.	CBS 116952	KM231073	AY864836	KM232401
Neocosmospora ramosa (Bat. & H. Maia) L. Lombard & Crous	CBS 50963	KM231004	KM231802	KM232369
N. vasinfecta E.F. Sm.	CBS 32554	KM231005	KM231803	KM232370
Stylonectria applanata Höhn.	CBS 125489	HQ897875	HQ897805	HQ897739
S. purtonii (Grev.) Gräfenhan	DAOM 235818	HQ897919	HQ897831	HQ897783
Thyronectria concentrica (Mont. & Fr.) Voglmayr & Jaklitsch	CBS 47469	KM231080	KM231835	KM232408

[a] Numbers in bold indicate the newly provided sequences.

DNA extraction, PCR amplification and sequencing

The genomic DNA was extracted from fresh mycelium following the methods of Wang and Zhuang (2004). Three primer pairs, acl1-230up/acl1-1220low (Gräfenhan et al. 2011), ITS5/ITS4 (White et al. 1990) and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify the sequences or partial sequences of the larger subunit of the ATP citrate lyase (ACL1), the internal transcribed spacers with the 5.8S nuclear ribosomal DNA (ITS) and the second largest subunit of the RNA polymerase II (RPB2), respectively. PCR reactions were performed on an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, California, USA), based on the procedures detailed in Gräfenhan et al. (2011), White et al. (1990) and Liu et al. (1999). DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences).

Sequence alignment and phylogenetic analyses

Newly generated sequences and those retrieved from GenBank are listed in Table 1. *Nalanthamala psidii* (Sawada & Kuros.) Schroers & M.J. Wingf. and *Thyronectria concentrica* (Mont. & Fr.) Voglmayr & Jaklitsch were used as outgroup taxa. Sequences were assembled, aligned and the primer sequences were trimmed with BioEdit 7.0.5 (Hall 1999) and converted to NEXUS files by ClustalX 1.8 (Thompson et al. 1997). The partition homogeneity test of ACL1, ITS and RPB2 regions was performed with PAUP 4.0b10 (Swofford 2002). To confirm the phylogenetic positions of the new species, sequences of ACL1, ITS and RPB2 were combined and analysed with Bayesian Inference (BI) and Maximum Parsimony (MP) analyses. The MP analysis was performed with PAUP 4.0b10 (Swofford 2002) using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. Topological confidence of resulted trees was tested by maximum parsimony bootstrap proportion (MPBP) with 1000 replications, each with 10 replicates of random addition of taxa. The BI analysis was conducted by MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo algorithm. Nucleotide substitution models were determined by MrModeltest 2.3 (Nylander 2004). GTR+I+G was shown to be the best-fit model for the combined sequences in the BI analysis. Four Markov chains were run simultaneously for 1,000,000 generations with the trees sampled every 100 generations. A 50% majority rule consensus tree was computed after excluding the first 2500 trees as 'burn-in'. Bayesian inference posterior probability (BIPP) was determined from the remaining trees. Trees were examined in TreeView 1.6.6 (Page 1996). BIPP greater than 90% and MPBP greater than 50% are shown at the nodes.

Results

Sequence comparison and phylogenetic inference

The ACL1, ITS and RPB2 sequences of 25 taxa belonging to 10 genera having fusarium-like asexual states were analysed through the methods of BI and MP. The PHT (P = 0.01) indicated that the individual partitions were not highly incongruent (Cunningham 1997); the three loci were thus combined for phylogenetic analyses. The combined datasets include 2258 characters, of which 1085 were constant, 173 variable and parsimony-uninformative and 1000 parsimony-informative. The MP analysis resulted in a single most parsimonious tree (tree length = 4885, CI = 0.4491, HI = 0.5509, RI = 0.5638, RCI = 0.2532). The final matrix was deposited in TreeBASE with accession No. S20853. The BI tree generated is shown (Figure 1). The topology of the BI tree is similar to that of the MP tree. The 25 investigated species were grouped together (BIPP/MPBP = 100%/96%) and further segregated into two main clades (Figure 1). Species of *Geejayessia* clustered into one clade together

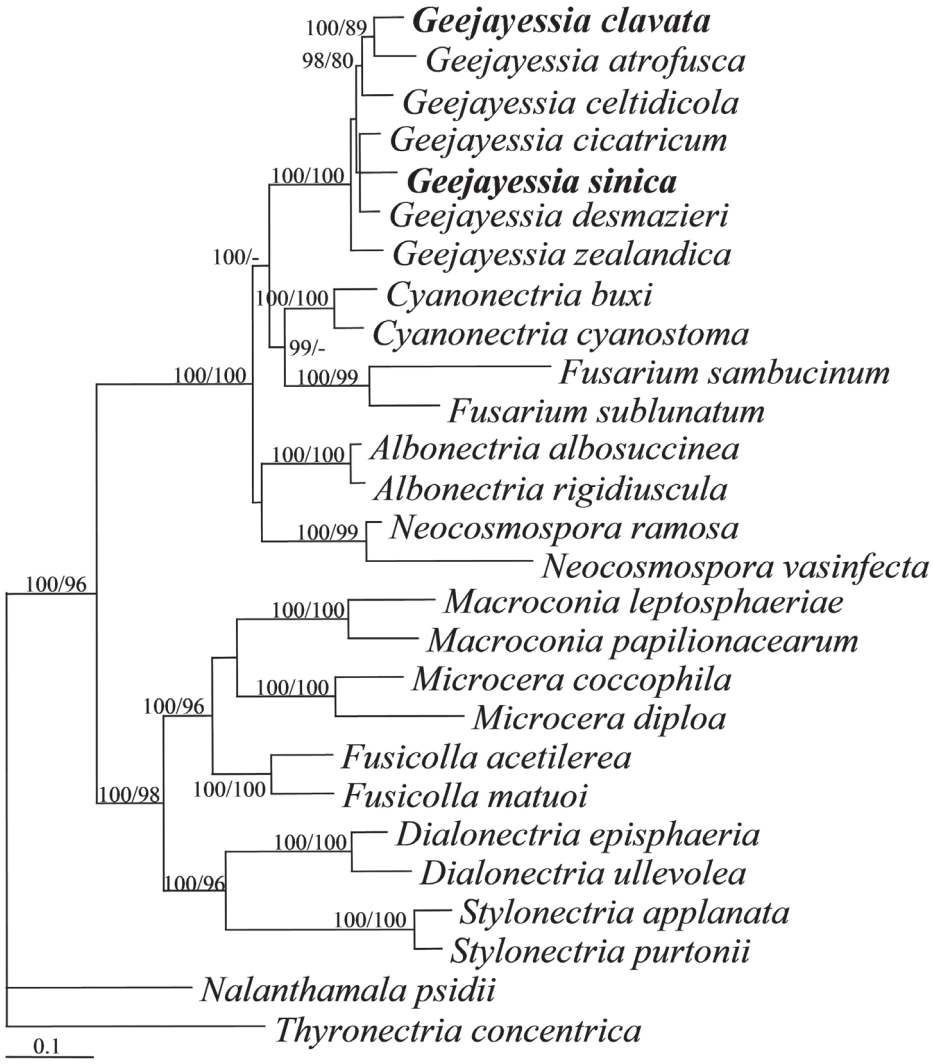

Figure 1. A Bayesian Inference trees inferred from the combined ACL1, ITS and RPB2 sequences. BIPP (left) above 90% and MPBP (right) above 50% are indicated at nodes.

with *Albonectria* Rossman & Samuels, *Cyanonectria* Samuels & P. Chaverri, *Fusarium* and *Neocosmospora* E.F. Sm. (BIPP/MPBP = 100%/100%) and those of *Dialonectria* (Sacc.) Cooke, *Fusicolla* Bonord., *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schroers, *Microcera* Desm. and *Stylonectria* Höhn. formed another clade (BIPP/MPBP = 100%/98%). HMAS 248725, HMAS 248726 and other representatives of *Geejayessia* formed a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed their taxonomic positions in the genus.

Taxonomy

***Geejayessia clavata* Z.Q. Zeng & W.Y. Zhuang, sp. nov.**
Fungal Names: FN570429
Figures 2, 3

Holotype. CHINA, Henan Province, Longyuwan, 33°40'45"N, 111°46'26"E, alt. 1500 m, on bark of *Buxus* sp., 17 September 2013, H.D. Zheng, Z.Q. Zeng & Z.X. Zhu 8728 (holotype: HMAS 275654), dried ex-type culture HMAS 248725.

Etymology. The specific epithet refers to the clavate microconidia.

Description. Mycelium not visible around ascomata or on host. Ascomata perithecial, crowded in group of 5 to 40, on basal stroma, oval, subglobose to globose,

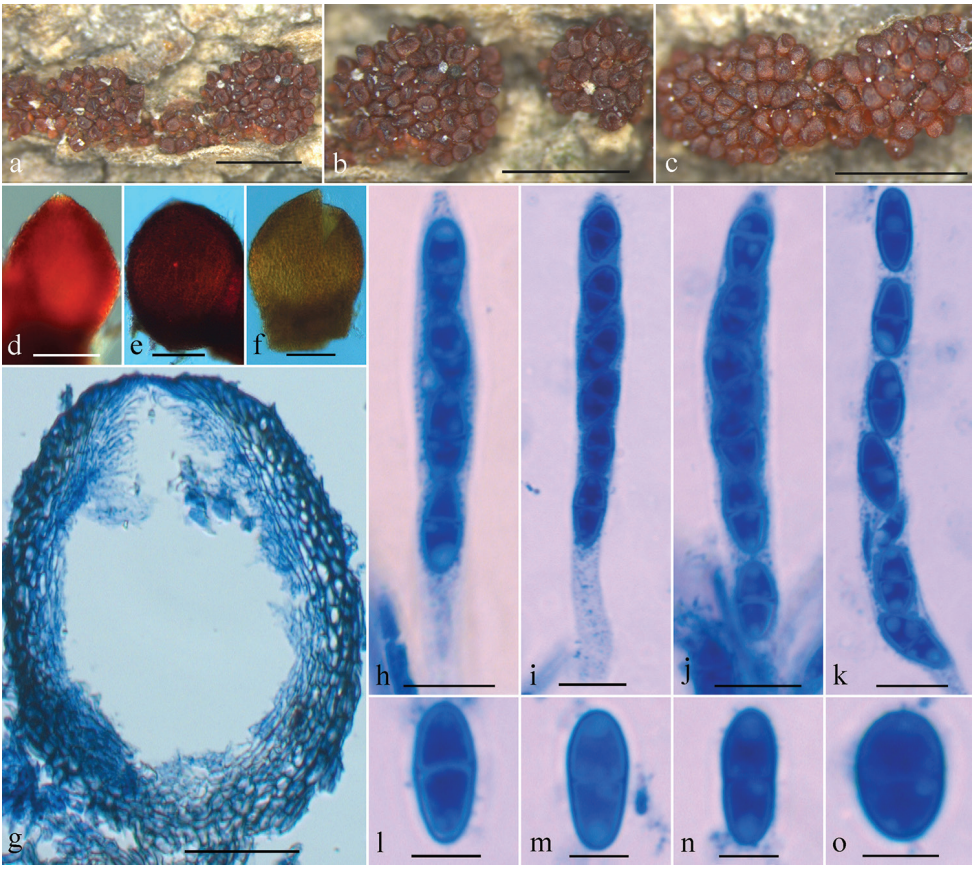

Figure 2. *Geejayessia clavata* sexual state (holotype, HMAS 275654): **a–c** Ascomata on natural substrate **d–f** colour of perithecium in water (**d**), 3% KOH (**e**) and 100% lactic acid (**f**) **g** median section through perithecium **h–k** Asci with ascospores **l–o** Ascospores. Scale bars: 1 mm (**a–c**); 100 µm (**d–f**); 50 µm (**g**); 10 µm (**h–k**), 5 µm (**l–o**).

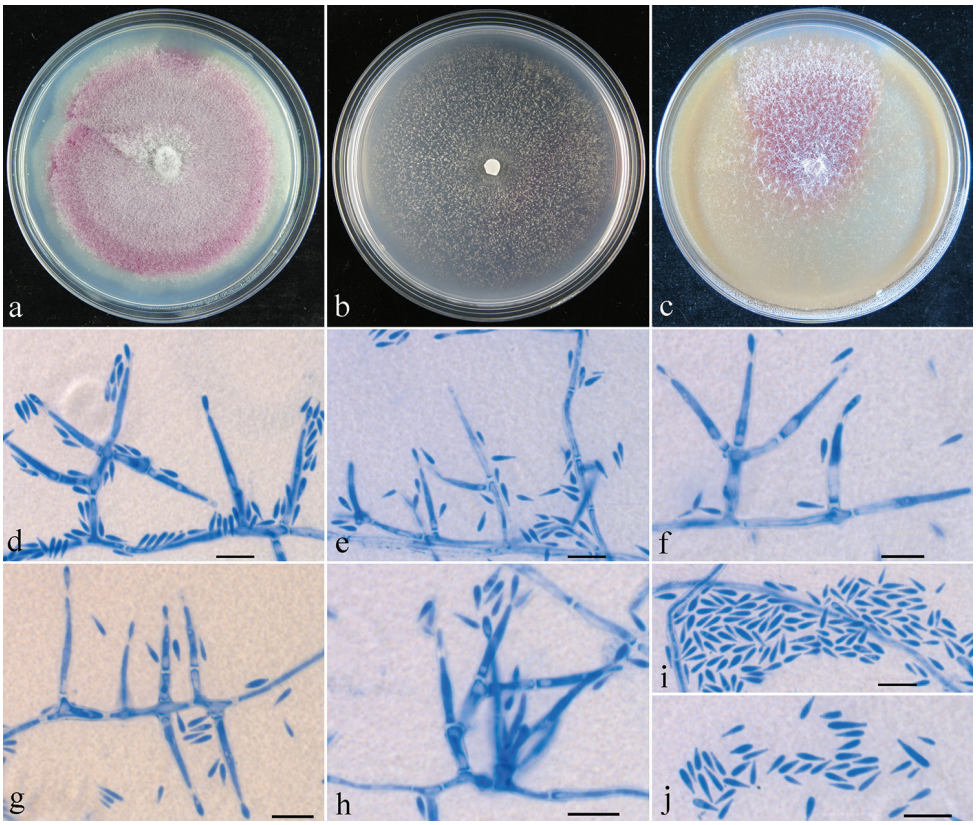

Figure 3. *Geejayessia clavata* asexual state (HMAS 248725): **a–c** colony on PDA (**a**) SNA (**b**) and CMD (**c**) **d–j** conidiophores, phialides and/or microconidia on SNA. Scale bar: 10 µm (**d–j**).

smooth, bright red when fresh, red brownish to dark red when dry, with a darker red ostiolar region, turning purple red in KOH and orange yellow in LA, 128–175 × 206–255 µm (n = 17). Perithecial wall consisting of a single layer, 15–25 µm thick, cells forming textura prismatica, 2–12 × 2–6 µm, walls 1–1.2 µm thick. Asci cylindrical, with a rounded apex, (4–7-)8-spored, 55–75 × 5–9 µm. Ascospores ellipsoidal to broadly ellipsoidal, equally 2-celled, slightly constricted at septum, smooth or finely verruculose, hyaline or pale brown, obliquely uniseriate in ascus often with ends overlapping, 7.5–12 × 4.5–5.5 µm.

Culture characteristics. Colony on PDA 48 mm diam. after 7 d at 25 °C, surface cottony, aerial mycelium white, producing vinaceous pigment in medium. Colony on SNA 30 mm diam. after 7 d at 25 °C, surface slightly floccose, with sparse whitish aerial mycelium. Colony on CMD 56 mm diam. after 7 d at 25 °C, surface floccose, with sparse whitish aerial mycelium, producing vinaceous pigment in medium. Conidiophores with short simple branches. Conidiogenous cells monophialidic, cylindrical, tapering toward the tip, 12–63 × 1.5–3.5 µm. Conidia clavate, not in chains, hyaline, aseptate, 4–7 × 0.8–2 µm (n = 60). Macroconidia and chlamydospores not observed.

Notes. Attempts were made to obtain macroconidia of the fungus in culture, but failed. Although the falcate macroconidia are lacking, the major phenotypic features of the fungus, such as occurrence on bark of *Buxus* sp., perithecia broadly ampulliform with a short neck, asci cylindrical with a rounded apex, ellipsoidal ascospores uniseptate and conidiophores monophialidic, fit well with the generic concept of *Geejayessia*. The molecular data confirm the taxonomic placement and indicate its close relationship with *G. atrofusca* (Figure 1, BIPP/MPBP = 100%/89%). *Geejayessia atrofusca* differs significantly in dark brown to black ascomata that do not change colour in KOH or LA, wider asci [(7.5-)9.8-13.3(-15) µm wide] and longer ascospores [(10-)11.2-14.2(-17.0) µm long]. Its microconidia are oblong to slightly curved and falcate but not clavate and are longer and wider (Samuels and Rogerson 1984).

***Geejayessia sinica* Z.Q. Zeng & W.Y. Zhuang, sp. nov.**
Fungal Names: FN570430
Figures 4, 5

Type. CHINA, Hubei Province, Shennongjia, 31°29'17"N, 110°20'58"E, alt. 2800 m, on bark of *Buxus* sp., 15 September 2014, Z.Q. Zeng, H.D. Zheng, W.T. Qin & K. Chen 9606 (holotype: HMAS 254520), dried ex-type culture HMAS 248726.

Etymology. Specific epithet refers to the type locality China.

Description. Mycelium not visible around ascomata or on host. Ascomata perithecial, solitary or in groups of 5 to 40, with a basal stroma, pyriform or subglobose to globose, smooth, collapsing laterally when dry, red to bright red with a dark red ostiolar region, turning dark purple red in KOH and light yellow in LA, 255–343 × 176–314 µm (n = 14). Perithecial wall of a single layer, 18–38 µm thick, of textura prismatica, cells 8–23 × 2–6 µm, walls 1.2–1.5 µm thick. Asci subcylindrical to clavate, with a rounded apex, 6(–8)-spored, 88–123 × 7–10(–12.5) µm. Ascospores ellipsoidal, hyaline or pale brown, smooth or finely warted, bicellular, slightly constricted at septum, obliquely uniseriate, 10–18(–20) × 5–7.5 µm.

Culture characteristics. Colony on PDA 42 mm diam. after 7 d at 25 °C, surface cottony, with whitish aerial mycelium, forming concentric rings, with pale vinaceous pigment produced in medium. Colony on SNA 26 mm diam. after 7 d at 25 °C, surface slightly velvet, with sparse whitish aerial mycelium. Colony on CMD 40 mm diam. after 7 d at 25 °C, surface radial, slightly floccose, with sparse whitish aerial mycelium. Conidiophores with short simple branches. Conidiogenous cells monophialidic, cylindrical, slightly tapering toward the tip, indefinite in length. Macroconidia falcate, with an arcuate tip and a pedicellate foot cell, hyaline, (3–4–)5-septate, 3-septate: 30–53 × 4–5 µm, 4-septate: 50–60 × 4.5–5.2 µm, 5-septate: 53–80 × 4.6–5.3 µm. Microconidia and chlamydospores not observed.

Notes. *Geejayessia sinica* is phylogenetically related to and morphologically similar to *G. cicatricum* and *G. desmazieri* in perithecial gross morphology, subcylindrical to

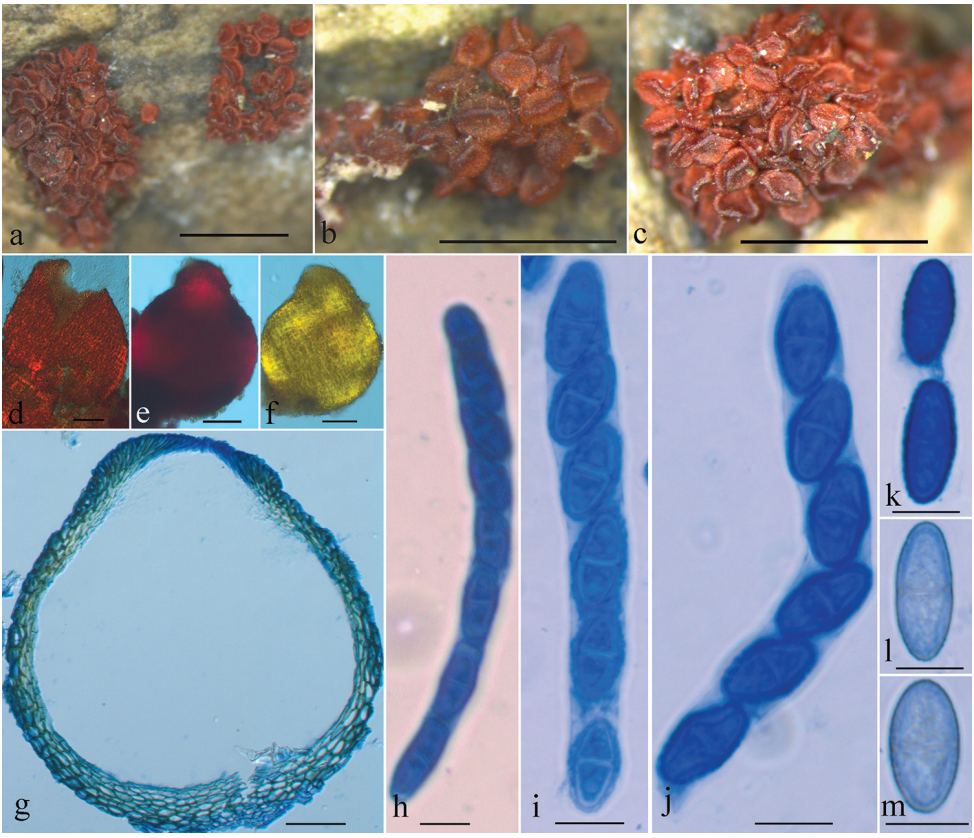

Figure 4. *Geejayessia sinica* sexual state (holotype, HMAS 254520): **a–c** ascomata on natural substrate **d–f** colour of perithecium in water (**d**), 3% KOH (**e**) and 100% lactic acid (**f**) **g** median section through perithecium **h–j** asci with ascospores **k–m** ascospores. Scale bars: 1 mm (**a–c**); 100 µm (**d–f**); 50 µm (**g**); 10 µm (**h–m**).

clavate asci, ellipsoidal to broadly ellipsoidal, uniseptate ascospores, falcate macroconidia (Schroers et al. 2011). *Geejayessia cicatricum* differs from *G. sinica* in having smaller perithecia (160–260 × 125–250 µm) and ascospores [(9.5–)11.5–13(–14.5) × (4.5–)5.0–6(–6.5) µm], thinner perithecial wall [(12–)13.5–18(–21) µm thick), shorter asci [(65.5–)73–92.5(–103) µm long), macroconidia with more septa [(2–)5–7(–8)] and slow growth on PDA (15–20 mm diam. after 7 d at 25 °C) (Schroers et al. 2011). *Geejayessia desmazieri* is distinguished by shorter asci [(75.5–)85(–100) µm long], smaller ascospores [(9.5–)11–12.5(–15) × (4.5–)5.5–6(–7) µm] and slow growth on PDA (20 mm diam. after 7 d at 25 °C) (Schroers et al. 2011). The ITS sequence of *G. sinica* differs from that of the other two species by 29 bp and 29 bp divergences in total length of 521 bp. The protein-encoding gene sequences of *G. sinica* differ from those of *G. cicatricum* (*G. desmazieri*) by 59 (66) bp differences of 815 bp long ACL1 fragment and 34 (35) bp differences of the 672 bp long RPB2 region.

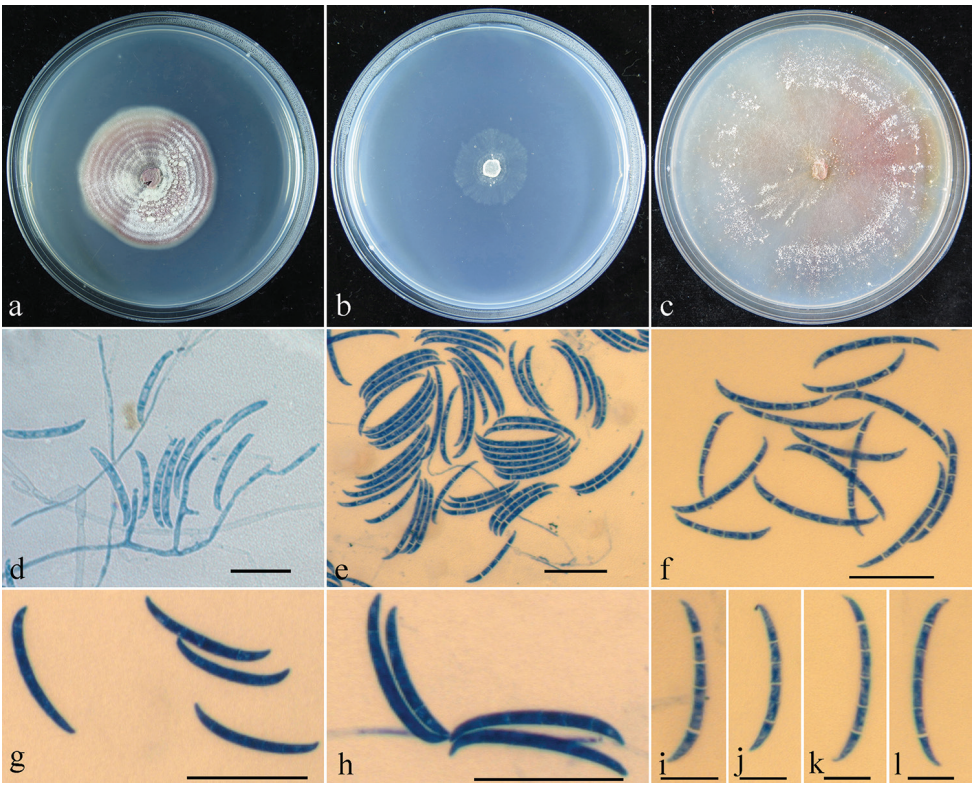

Figure 5. *Geejayessia sinica* asexual state (HMAS 248726): **a–c** colony on PDA (**a**), SNA (**b**) and CMD (**c**) **d** conidiophores, conidiogenous cells and macroconidia on SNA **e–l** Macroconidia on SNA. Scale bars: 50 μm (**d–h**); 10 μm (**i–l**).

Discussion

Schroers et al. (2011) recognised five species of *Geejayessia*. *Geejayessia montana* Lechat & J. Fourn was recently described and its placement was supported by morphological characteristics of both sexual and asexual states, as well as analysis of ITS sequences (Lechat and Fournier 2017). Meanwhile, a new combination, *G. hispanica* (Lechat & Priou) Lechat & J. Fourn was proposed based on the ITS sequence of *Geejayessia* sp. BRFM 1015 (GenBank accession no. JX082350) (Lechat and Fournier 2017). However, '*Geejayessia hispanica*' grows on *Phoenix canariensis* rather than *Buxus*, *Celtis* or *Staphylea*, which deviates from the original generic concept of the genus (Schroers et al. 2011). This fungus was treated as *Cosmospora hispanica* Lechat & Priou in the present study. *Cosmospora matuoi* Hosoya & Tubaki was also combined with *Geejayessia* as *G. matuoi* (Hosoya & Tubaki) Lechat & Rossman (Lechat and Rossman 2017). Nevertheless, Gräfenhan et al. (2011) and Lombard et al. (2015) treated *Cosmospora matuoi* as a member of *Fusicolla*, which is followed in this study. To clarify the taxonomic positions of '*G. hispanica*' and '*G. matuoi*', more evidence is certainly required.

According to the International Code of Nomenclature for algae, fungi and plants (McNeill et al. 2012), the name *Fusarium* is accepted as the correct generic name for fungi with *Gibberella* Sacc. sexual states (Rossman et al. 2013). The asexual states of other genera are marked as fusarium-like (Lombard et al. 2015). In the present study, the phylogeny, based on analyses of the combined ACL1, ITS and RPB2 sequences, recognised nine clades amongst the investigated taxa which are in accordance with the genera *Albonectria*, *Cyanonectria*, *Dialonectria*, *Fusicolla*, *Geejayessia*, *Macroconia*, *Microcera*, *Neocosmospora* and *Stylonectria*. This result is basically consistent with that by Schroers et al. (2011).

Joining the two new species to the *Geejayessia* clade, the tree topology (Figure 1) remains basically the same as that revealed by Schroers et al. (2011). Our result showed *G. clavata* and *G. atrofusca* both forming microconidia in culture, grouped together with relatively high statistical supports (Figure 1, BIPP/MPBP = 100%/89%). *Geejayessia sinica*, *G. cicatricum* and *G. desmazieri*, as sister-groups, are poorly supported (BIPP/MPBP less than 50%).

Host specificity has been shown in some fungi of Nectriaceae; for example, *Thyronectria aurigera* (Berk. & Ravenel) Jaklitsch & Voglmayr occurs only on Oleaceae, *T. berolinensis* (Sacc.) Seaver on *Ribes* and *T. aquifolii* (Fr.) Jaklitsch & Voglmayr on *Ilex aquifolium* (Jaklitsch and Voglmayr 2014; Zeng and Zhuang 2016). Species of *Geejayessia* are also host-specific. As known currently, *G. clavata*, *G. sinica*, *G. cicatricum* and *G. desmazieri* occur only on *Buxus* spp., *G. celtidicola* only on *Celtis occidentalis* and *G. atrofusca* only on *Staphylea trifolia* (Schroers et al. 2011).

The genus *Geejayessia* was previously known from Europe, North America and Oceania (Samuels and Rogerson 1984; Nirenberg and Samuels 2000; Schroers et al. 2011). The new species discovered from central China extends the distribution of the genus to Asia.

Acknowledgements

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RESEARCH ARTICLE

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A new *Gymnopus* species with rhizomorphs and its record as nesting material by birds (Tyrannidae) in the subtropical cloud forest from eastern Mexico

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Abstract

A new species of *Gymnopus* is described on the basis of collections from the subtropical cloud forest of eastern Mexico. Macro- and micromorphological characters, in combination with ITS sequences obtained from fruit body tissues, were used for its taxonomic circumscription. Basidiomata of this species were found growing scattered on fallen twigs of *Quercus* and also developing abundant long, black, wiry rhizomorphs. The authors discovered that these latter are used as part of nesting material by *Myonectes oleaginous* (Tyrannidae) inhabiting the subtropical cloud forest studied. A macro- and microscopical description as well as a discussion and illustrations are provided. A new combination in *Gymnopus* is proposed for *Marasmius westii*, a synonym of *Marasmius brevipes*.

Keywords

Marasmioid fungi, Neotropical fungi, nesting biology, Omphalotaceae

Introduction

The Santuario de Bosque de Niebla (SBN) is a secondary-growth subtropical cloud forest, persisting as the main peri-urban natural forested area (ca. 30 ha) at southwest Xalapa City, Veracruz (east coast of Mexico). Part of it was a shade-grown coffee plan-

tation abandoned several years ago and nowadays, the SBN (formerly called Parque Ecológico Francisco Javier Clavijero) is a forest ecosystem whose canopy is dominated mostly by trees of *Quercus*, *Carpinus*, *Clethra*, *Oreopanax*, *Ostrya* and *Turpinia*, amongst others. It is an area under conservation and protection by the Instituto de Ecología A.C. and the forest is functioning as an important refuge and reservoir of biological diversity. Permanent systematic field observations carried out on the site are allowing us to document the macrofungal community with special attention to the diversity and ecology of mushrooms (agarics, boletes and milk caps) and it has given us the opportunity to discover new or unusual species of different taxonomic groups, for example *Crepidotus*, *Crinipellis*, *Hygrocybe*, *Lactarius* and *Lepiota* (Bandala and Montoya 2004; Bandala et al. 2006, 2008, 2012, 2016; Montoya and Bandala 2004, 2005, 2008; Montoya et al. 2005).

In the present study, specimens of a marasmioid species producing small basidiomata and abundant black, wiry rhizomorphs were found growing scattered on fallen twigs of *Quercus*. Macro- and microscopical features of basidiomata (hyaline basidiospores, pileipellis non-gelatinous of repent hyphae with diverticulate terminal elements; glabrous, central stipe with homogeneous trama of unbranched hyphae; well-developed rhizomorphs) suggested that our samples relate to members of *Marasmius* sect. *Androsacei* Kühner (Desjardin 1987; Desjardin and Petersen 1989). With the advance of molecular systematics on this and other taxonomic groups of marasmioid or even gymnopoid fungi, evidence has been obtained by different authors to recognise that several species earlier placed in sections within the genera *Marasmius* Fr., *Marasmiellus* Murrill and *Micromphale* Gray have phylogenetic relationships with members of *Gymnopus* (Pers.) Gray (Moncalvo et al. 2002; Wilson and Desjardin 2005; Mata et al. 2007; Petersen and Hughes 2016; Tkalčec and Mešić 2013; Antonín and Noordeloos 2010). Members of section *Androsacei* within *Gymnopus*, for example, show close relationships with the species of *Micromphale* sect. *Perforantia* Singer and sect. *Rhizomorphigena* Singer (Moncalvo et al. 2002; Mata et al. 2004; Wilson and Desjardin 2005¸ Mata et al. 2007; Petersen and Hughes 2016).

A phylogeny, based on ITS sequences obtained here from basidiomata and rhizomorphs collected in the study area, including sequences (downloaded from GenBank: https://www.ncbi.nlm.nih.gov/genbank/) of related marasmioid/gymnopoid fungi, revealed indeed, the phylogenetic relationships of the Mexican species within *Gymnopus.* The macro- and micro-morphological features depicted in this fungus, as well as its distinct position in the phylogenetic analysis, allowed its recognition as a new species which is proposed here. A description accompanied of photographs of basidiomata, illustrations of microscopic features, the displayed phylogeny on the basis of ITS sequences and a taxonomic discussion are provided in this article. During the course of samplings of the *Gymnopus* species studied, we discovered that the long, wiry black rhizomorphs occur in fallen twigs or entangled in aerial branches in the low canopy level, where they are available for use as nesting material by bird species of the Tyrannidae that inhabit the forest under study, which is also discussed.

Materials and methods

Sampling and morphological study

Between May 2016 and June 2017, weekly explorations were conducted in the Santuario del Bosque de Niebla, Instituto de Ecología, A.C., at Xalapa. Fresh basidiomata and their rhizomorphs were gathered on fallen twigs of *Quercus*. Some rhizomorphs were also collected from aerial tree branches at, or a little higher, than breast height and others directly from bird nests hanging from branches of a tree of *Turpinia insignis* (H.B. & K.) Tul. Descriptions of macroscopic characters are based on fresh collections which were photographed and their colours recorded following Kornerup and Wanscher (1967) and Munsell (1994). Microscopic observations were made on dried material mounted in potassium hydroxide (KOH) 3% and stained with 1% Congo red or analszed in Melzer´s solution (Largent et al. 1977). Thirty-five basidiospores per collection were measured in length and width, following the protocol of Bandala et al. (2012). Symbols $\bar{x}_m$ and $\bar{q}_m$ in descriptions refer to the range of mean values per collection (n = 4 collections) of length and width and length/width ratio of basidiospores in side view, respectively. Line drawings were made using a drawing tube. Collections are part of XAL herbarium (Thiers 2018).

DNA extraction, PCR amplification and sequencing

The extraction of genomic DNA of basidiomes and rhizomorphs was performed using the DNA kit extraction Exgene Plant SV mini (GeneAll Biotechnology, Co). PCR was performed to amplify the ITS (Internal Transcribed Spacer) using primers ITS1F, ITS5/ITS4, (White et al. 1990; Gardes and Bruns 1993). PCR conditions: (i) initial denaturation at 95 °C for 5 min; (ii) 35 cycles of 30 sec at 95 °C, 30 sec at 55 °C and 40 sec at 72 °C; and (iii) a 5 min final elongation at 72 °C. Amplified PCR products were sequenced (Macrogen Inc., Seoul, Korea) using a Genetic Analyzer 3730XL (Applied Biosystems). Once sequences were assembled and edited, they were deposited at GenBank database (Benson et al. 2017) with accession numbers indicated in Fig. 1.

Phylogenetic methods

A dataset, using PhyDE v.0.9971 (Müller et al. 2010), was constructed with the sequences obtained in this study together with related sequences retrieved from GenBank database (http://www.ncbi.nlm.nih.gov) identified with the aid of BLAST tool. The dataset was complemented with other available ITS sequences of *Gymnopus* species at GenBank (Fig. 1), representing the sections *Androsacei*, *Gymnopus*, *Levipedes* (Fr.) Halling, *Perforantia* and *Rhizomorphigena* (after Antonín et al. 2014; Petersen and Hughes 2016). The evolutionary model that best fitted the data and a phylogenetic analysis, un-

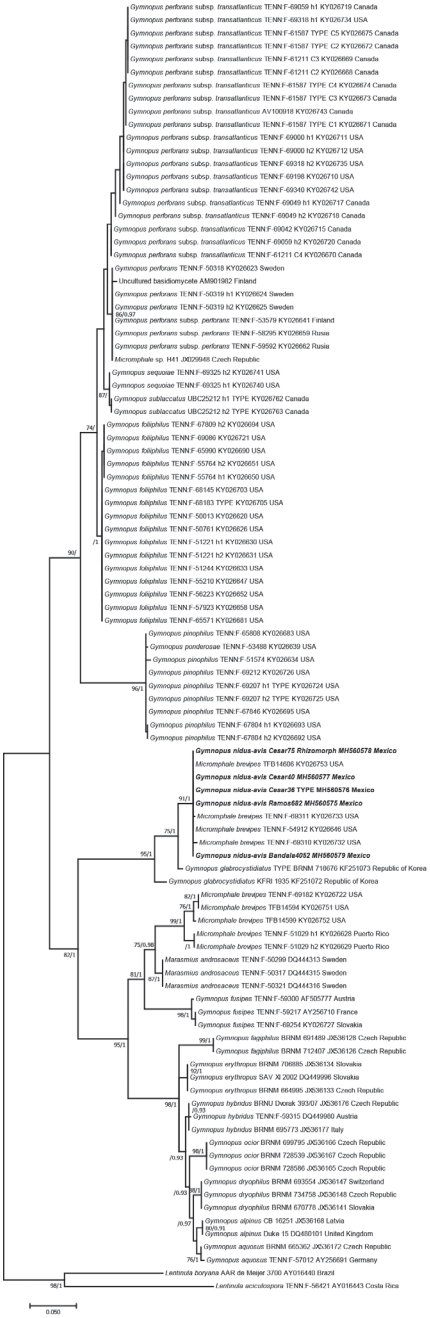

Figure 1. Phylogenetic relationships within *Gymnopus* species inferred from the ITS sequence dataset by maximum likelihood method (ML). Tree with the highest log likelihood (-3619.93). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The bootstrap values and Bayesian posterior probabilities (obtained after Bayesian inference) are indicated on the tree branches (BS/BPP). Sequences obtained in this study are in bold.

der maximum likelihood (ML) (500 bootstrap replications) were achieved with MEGA 7.0 (Kumar et al. 2016), while a phylogenetic analysis under Bayesian Inference (BI) with MrBayes v 3.2.6 (Ronquist et al. 2012; Montoya et al. 2014). *Lentinula* species were included as outgroup taxa (Fig. 1; alignment in TreeBASE 22984). Resulting phylogenetic trees were displayed using Mega 7.0 and FigTree v1.4.3 (Rambaut 2016), respectively. Only bootstrap values (BS) of ≥70% and Bayesian posterior probabilities (BPP) of ≥0.90 were considered and indicated on the tree branches (BS/BPP) of Fig. 1.

Results

We recovered four fresh collections of basidiomata from which four ITS sequences were generated, including one from a rhizomorph (Fig. 1). In the inferred molecular phylogeny, that included 95 sequences of marasmioid/gymnopoid taxa worldwide (Fig. 1), the five generated sequences of the Mexican *Gymnopus* species clustered in a strongly supported and isolated clade (91/1.0), sister to *G. glabrocystidiatus* Antonín, R. Ryoo & K.H. Ka known from the Republic of Korea (Antonín et al. 2014). Both species in the analysis appear together in a well-supported branch (95/1.0), separated from other sequences (after Petersen and Hughes 2016) supporting phenotypically lookalike marasmioid species as *Micromphale brevipes* (Berk. & Ravenel) Singer traditionally placed in sect. *Rhizomorphigena* (clade with high support 99/1.0) or *Gymnopus androsaceus* (L.) J.L. Mata & R.H. Petersen from sect. *Androsacei* (clade with high support 87/1.0) (Fig. 1), which probably suggests that Mexican and Korean taxa belong to a different section or, in concordance with other authors, the resultant clades reflect the relationships amongst closely related taxa of *G. androsaceus* complex (Wilson and Desjardin 2005; Mata et al. 2007; Hughes and Petersen 2016). Taking into account both the results of the phylogenetic analysis and the distinctive set of morphological features that the Mexican *Gymnopus* specimens possess (see description below), we concluded that they represent a new *Gymnopus* species which inhabits the subtropical cloud forest from eastern Mexico and it is proposed here. In the discussion below, we comment on the specimens supporting the sequences recorded as "*Micromphale brevipes*", some of which appear in the phylogeny nested with the Mexican taxon, while others clustered in a separate clade (Fig. 1).

Gymnopus nidus-avis **César, Bandala & Montoya, sp. nov.**
MycoBank: MB827326
Figs 2–4

Holotype. MEXICO. Veracruz: Municipality of Xalapa, Santuario del Bosque de Nieblа, Instituto de Ecología A.C., 1343 m a.s.l., gregarious, on fallen twigs of *Quercus*, 20 April 2016, Cesar 36 (XAL).

Diagnosis. Pileus pale brown to brown. Lamellae adnexed, distant, very pale brown. Basidiospores ellipsoid to subcylindrical. Basidia 2–4-spored, narrowly clavate.

Figure 2. *Gymnopus nidus-avis*: basidiomata (César 36, holotype). Scale bar: 4 mm

Cheilocystidia 20–39 × 3–8 µm, irregularly cylindrical, with constrictions and small lateral appendages. Pileipellis hyphae with colourless incrustations; terminal elements appendiculate. Pileus and lamellar tissues clampless.

Gene sequences ex-holotype. MH560576 (ITS).

Etymology. Referring to the use of rhizomorphs as nesting material by birds.

Basidiomata marcescent. *Pileus* 1–7 mm diam., convex to plano-convex, usually somewhat depressed over the disc and occasionally slightly infundibuliform with age, some developing a weak umbo, smooth or weakly rugulose, subtly striate when young becoming somewhat sulcate and then wavy towards the margin, this latter slightly decurved, surface dry, minutely granulose under lens, matt, pale brown (7.5 YR 7/4–6; 10YR 7/4) to brown (7.5 YR 5/6; 10 YR 4/4); context thin (up 1 mm thick), soft, whitish. *Lamellae* adnexed, distant (7–17), very pale brown (2.5 Y 8/2), narrow to moderately broad (up to 1 mm broad), sometimes forked, lamellulae of two different lengths, rarely weakly intervenose with age, margin entire. *Stipe* 1–12 × 0.2–0.3 mm, central, sometimes only slightly eccentric, cylindrical or tapered towards the base,

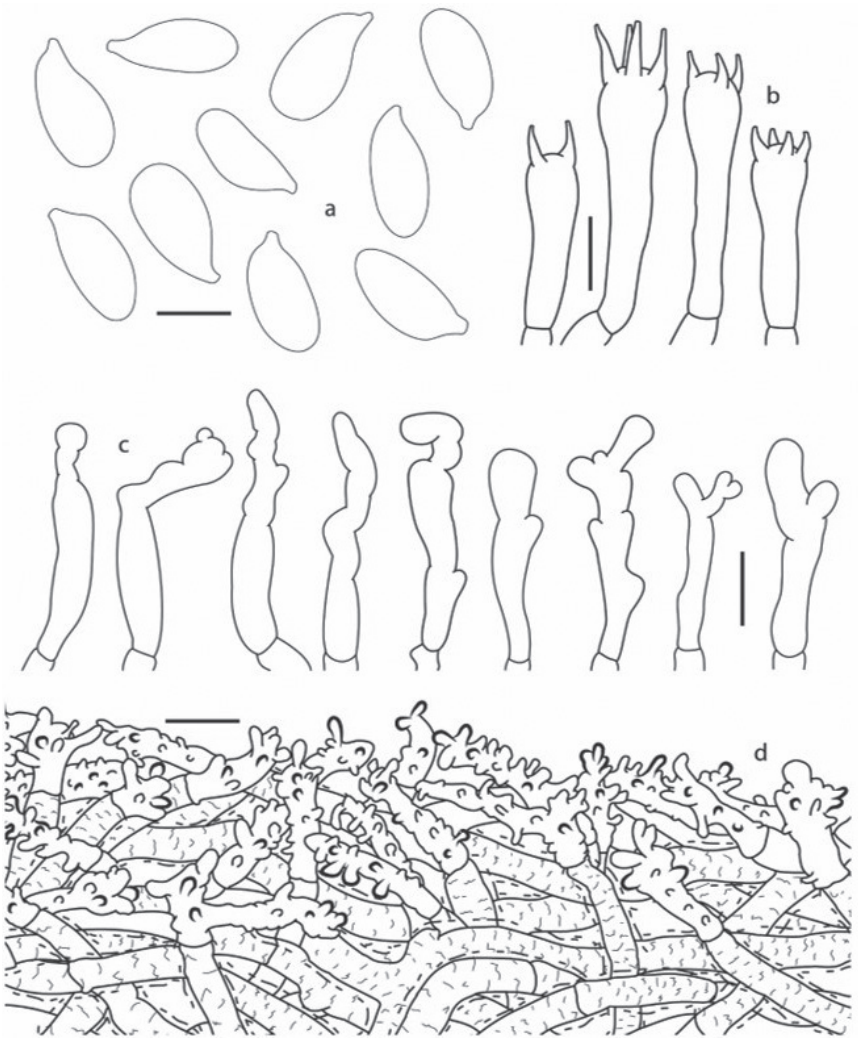

Figure 3. *Gymnopus nidus-avis*: **a** Spores **b** Basidia **c** Cheilocystidia **d** Pileipellis (holotype). Scale bars: 5 μm (**a**); 10 μm (**b–d**).

straight, often curved, solid, glabrous, very finely striate (under lens), reddish-brown at the apex (2.5 YR 4/6), dark brown to black below (10YR 2/1, 7.5YR 2.5/2), insititious, at times erumpent, arising either from the substratum or from rhizomorphs; context light brown (2.5Y 6/4). *Rhizomorphs* up to 500 × 1 mm, simple, black, wiry, abundant. Odour and taste not distinctive.

Basidiospores 7–10 × 3–5.5 μm, $\bar{x}_m$ = 8.3–9.1 × 3.7–4; $\bar{q}_m$ = 2–2.4 (n = 4), ellipsoid to subcylindrical, somewhat lacrymoid with a weak suprahilar depression, hyaline, inamyloid, thin-walled. *Basidia* 20–41 × 5–10 μm, 2–4-spored, clavate to narrowly clavate, hyaline, inamyloid, thin-walled, clampless. *Cheilocystidia* 20–39 × 3–8 μm, irregularly cylindrical to narrowly-claviform, simple or usually bifurcate, with small

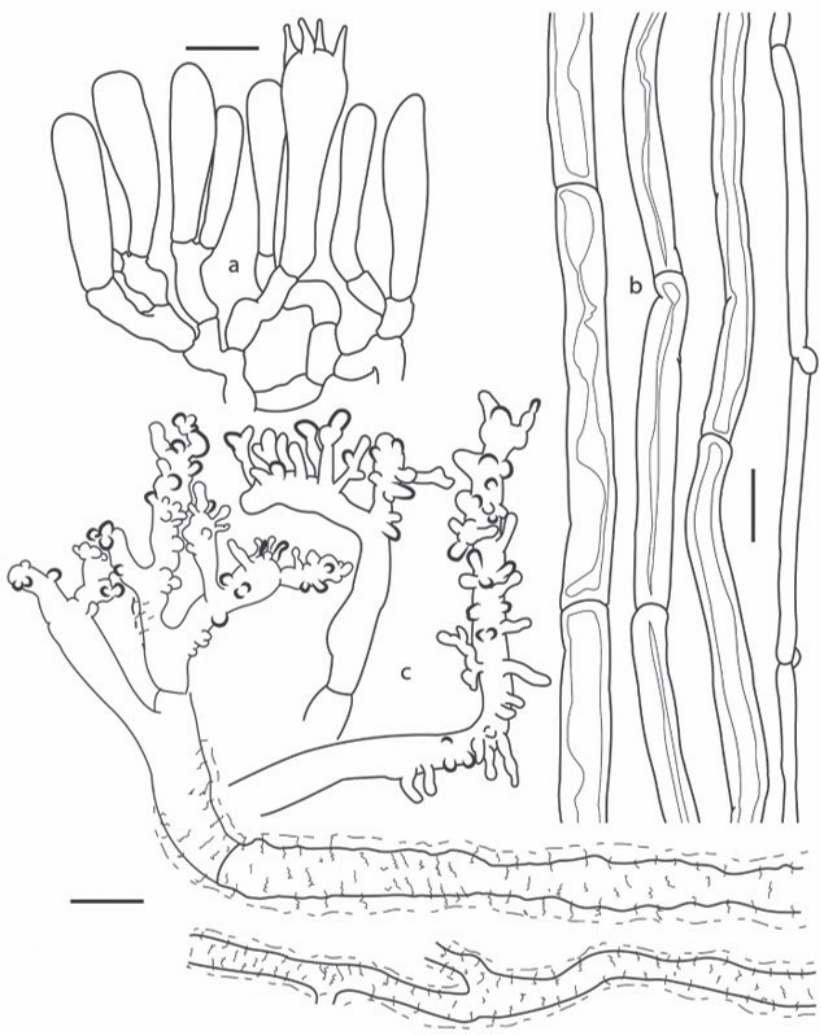

Figure 4. *Gymnopus nidus-avis*: **a** Hymenial trama elements **b** Thick- and thin-walled hyphae from stipe medullary tissue **c** Hyphae and terminal elements of pileipellis (holotype). Scale bars: 10 µm (**a–c**).

lateral appendages and constrictions, moderately abundant, shortly projected beyond the hymenium level, hyaline, inamyloid, thin-walled, clampless. *Pilleipellis* composed of compactly interwoven, cylindrical, non-gelatinised, thin-walled, clampless, hyaline hyphae, 4–8 µm diam., irregularly covered by colourless, refractive incrustations of fine or moderately broad, discontinuous lines, arranged in a more or less irregularly transversal pattern, bearing repent or slightly erect, hyaline, slightly dextrinoid terminal elements which are irregularly cylindrical, with numerous appendages or with short to moderately large lateral outgrowths, thin-walled or the apices often thick-walled, with a morphology similar to a Rameales-structure. *Pileus trama* hyphae interwoven,

4–6 µm diam., cylindrical, often bifurcate, thin-walled, hyaline, weakly dextrinoid, smooth, often intermixed, some covered with colourless refractive encrusting material. *Hymenophoral trama* regular to subregular, with cylindrical, thin-walled, hyaline, inamyloid to weakly dextrinoid, clampless hyphae 3–5 µm diam. *Stipitipellis* composed of repent, cylindrical, thick-walled, heavily dark brown pigment-encrusted, clampless hyphae 5–6 µm diam., dextrinoid; with scattered, hyaline or brown-pigmented, diverticulate terminal elements 4–19 (–21) × 3–4 (–5) µm, thin-walled. *Stipe trama* hyphae more or less parallel, composed by cylindrical or more or less ventricose hyphae, 4–15 (–20) µm diam., thick-walled (1–5 µm thick), smooth or with colourless encrusting material, towards the central medulla they appear intermixed with hyaline, smooth, cylindrical hyphae, 3–6 µm diam., thin or slightly thick-walled (<1 µm thick), occasionally clamped. *Clamp connections* absent in pileus and lamellar tissues, present in the slender, medullary hyphae of stipe.

Habitat. In subtropical cloud forest, scattered or gregarious on fallen twigs of *Quercus*, often the basidiomes arising directly from the wiry, black rhizomorphs and these latter at times are entangled, hanging from aerial branches.

Additional specimens examined. MEXICO. Veracruz, Municipality of Xalapa, Santuario del Bosque de Niebla, Instituto de Ecología A.C., 1343 m a.s.l., 18 May 2006, Bandala 4052; 7 July 2016, César 41; 10 Aug 2016, Ramos 682 (all at XAL).

Discussion

Amongst the species that produce tiny, marcescent basidiomes and long, black, wiry rhizomorphs, *Gymnopus nidus-avis* can be recognised by the colour of pileus and lamellae, these latter adnexed and distant, size and shape of basidiospores and cheilocystidia, 2–4-spored basidia, pilleipellis hyphae bearing colourless, refractive encrusting material, with appendiculate terminal elements (similar to a Rameales-structure) and with the pileus hyphae and lamellar trama (hymenial elements included) lacking clamp connections. Interestingly, the presence of clamp connections exclusively is confined to the slender, thin-walled, hyphae of stipe trama, even mycelia obtained from tissues in axenic culture did not present clamped septa. The Mexican species is genetically close to the Korean *G. glabrocystidiatus*, with which it shares morphological features as the filiform stipe, pileipellis composed of encrusted, diverticulate hyphae and clampless hyphae. Basidomata of *G. glabrocystidiatus*, however, are slender and longer (pileus 4–8 mm; stipe 15–40 × 0.5 mm) lacking rhizomorphs, grow on needles of *Abies* and have broadly clavate or pyriform cheilocystida, 2-spored basidia and terminal elements of the pileipellis with irregular coralloid shape or broom-like (Antonín et al. 2014).

The new species is macro-morphologically similar to *Marasmius brevipes* Berkeley & Ravenel (*Micromphale*, Singer, in Dennis 1953), a species occurring in southern USA, growing also on *Quercus* sticks, with other known records from Alaska, Marti-

nique, Puerto Rico and Trinidad (Dennis 1953; Pegler 1983; Desjardin and Petersen 1989; Petersen and Hughes 2016). The differences between both species are very subtle in the pileus and lamellae colours, being in *M. brevipes* brown or dark reddish-brown, with a slightly darker disc and brownish-grey to light brown or pale pinkish-cinnamon colours, respectively. However, the pileus of *M. brevipes* is more markedly striate, even sulcate, plane at centre (i.e. without evidence of umbo), besides having adnate lamellae and shorter stipe (1–2.5 × <0.5 mm; 1–4 × 1 mm or 4–7 × 0.2–0.4 mm) which is often eccentric (Dennis 1951, 1953; Pegler 1983; Desjardin and Petersen 1989). Microscopically *M. brevipes* is a very distinctive species that may be readily recognised by the heavily brown pigment-encrusted pileipellis elements, with short, coralloid terminal hyphae, thick-walled, hyaline or pale brownish, smooth or weakly encrusted, inamyloid hyphae of pileus and lamellar trama, clamp connections common on all tissues and amygdaliform and less cylindrical basidiospores (Dennis 1951, 1953; Desjardin and Petersen 1989), in contrast with those of *Gymnopus nidus-avis*.

Results of the phylogenetic analysis (Fig. 1) suggest that *G. nidus-avis* and *Marasmius brevipes*, both with small, tiny basidiomata and long black rhizomorphs, to some extent could be easily confused. Several sequences treated by Petersen and Hughes (2016) under "*Micromphale brevipes*" were included in the present study (Fig. 1), three of them grouping in the same clade together with the sequences of the Mexican specimens. Staff at TENN Herbarium confirmed to us that two of these specimens (TENN 54912 and 69310) have clampless hyphae in pileus and lamellar tramae, hence these specimens are interpreted here to be contaxic with the Mexican species and not with the type specimen of *Marasmius brevipes* which possesses clamp connections on all tissues, including the basidia, cheilocystidia and pileipellis elements, as described by Desjardin and Petersen (1989) and in the type study of *Marasmius brevipes* by Desjardin (1989). Other sequences labelled also under "*Micromphale brevipes*", in the phylogeny inferred (Fig. 1) appeared in a separate clade with high support (99/1.0). They belong to samples TENN 51029 and 69182 which have clamp connections in pileus and lamellar trama, suggesting that some of them could be contaxic with the type specimen of *Marasmius brevipes* representing the *Rhizomorphigena* section (Hughes and Petersen 2016).

Marasmius brevipes is a species accepted and validly published (Berkeley and Curtis 1853; Desjardin and Petersen 1989). If the species is recognised to belong to the group of marasmioid species phylogenetically close to *Gymnopus* sect. *Androsacei* (Kühner) Antonín & Noordel., as suggested by the analyses obtained by Hughes and Petersen (2016) and here (Fig. 1), we note that the species has not been transferred to *Gymnopus*. The name *Gymnopus brevipes* (Bull.) Gray, however, is occupied by an accepted synonym for *Melanoleuca brevipes* (Bull.) Pat. (Index Fungorum; Mycobank 486476). An alternative name is that of the synonym, *Marasmius westii* Murr., following a type study by Desjardin (1989) that documented the presence of clamp connections (see also Desjardin and Petersen 1989 and Hesler 1959). The new combination for that marasmioid species seems to be pertinent (Arts. 6, 41) and the following is proposed:

***Gymnopus westii* (Murrill) César, Bandala & Montoya, comb. nov.**
Mycobank: MB828158

Basionym. *Marasmius westii* Murrill, Proc. Florida Acad. Sci. 7:110. 1945.

Syn.: *Marasmius brevipes* Berk. & Ravenel, in Berkeley and Curtis, Ann. Mag. Nat. Hist., Ser. 2 12: 426. 1853.

=*Micromphale brevipes* (Berk. & Ravenel) Singer, in Dennis, Kew Bull. 8: 42. 1953.

Not *Agaricus brevipes* Bull., Herb. Fr. 11: tab. 521. 1791 (*Gymnopus*, Gray, Nat. Arr. Brit. Pl. 1: 609.1821; *Melanoleuca*, Pat., Essai Tax. Hyménomyc.: 158, 1900.).

Reports of marasmioid fungi as nesting material for Passeriformes have been referred in several works as filaments, rhizomorphs or horse-hair fungi and recorded from the Nearctic and the Neotropical regions (Sick 1957; Mc Farland and Rimmer 1996; Aubrecht et al. 2013). These fungal materials have been identified as *Marasmius androsaceus*, *M. brevipes*, *M. crinis-equi* F. Muell. ex Kalchbr., *M. nigrobrunneus* (Pat.) Sacc. and *M.* sp. Fungal material from *Marasmius* sp. and *Crinipellis* sp. was recorded in Mexico as being associated with nests of birds in a tropical forest from Tabasco in the south of Mexico (Gómez et al. 2014). In the present study, one of the sequences (MH560578), included in the obtained phylogeny (Fig. 1), belongs to a rhizomorph of *Gymnopus nidus-avis* re-collected in a nest of *Myonectes oleaginous* Lichtenstein. This *Gymnopus* species represents a new species in the list of marasmioid taxa found interacting with birds.

All the basidiomes collected in the present study were found on fallen twigs in the low canopy level but it is possible that fructifications occur also on rhizomorphs at the top of the trees, where these latter are found and used by birds. Previous reports have suggested that bird efforts of picking this inconspicuous material is rewarded with the high tensile strength, reduced water uptake and antimicrobial properties of the rhizomorphs, which consequently protect the offspring (Aubrecht et al. 2013; Freymann 2007). Preliminary results, based on various sequences obtained from rhizomorphs gathered in different nests of bird species found in the study site, suggest the presence of an important diversity of marasmioid rhizomorph-forming species in the cloud forest studied. It is interesting to note also that we could evidence the presence of nests of wasps of *Polybia rejecta* Fabricius, near one of *Myonectes oleagineus* built with rhizomorphs of *Gymnopus*. It coincides with the observations made by Joyce (1993) in Costa Rica regarding the presence of nests of that wasp species near *Tolmomyas sulphurescens* and *Cacicus* spp. nests. These latter authors concluded that such association could reduce predation, remarking the importance of the fungal rhizomorphs in this complex ecological interaction.

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Six new species and reports of *Hydnum* (Cantharellales) from eastern North America

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Abstract

Five species of *Hydnum* have been generally recognized from eastern North America based on morphological recognition: *H. albidum*, *H. albomagnum*, *H. repandum* and varieties, *H. rufescens*, and *H. umbilicatum*. Other unique North American species, such as *H. caespitosum* and *H. washingtonianum*, are either illegitimately named or considered synonymous with European taxa. Here, seventeen phylogenetic species of *Hydnum* are detected from eastern North America based on a molecular phylogenetic survey of ITS sequences from herbarium collections and GenBank data, including environmental sequences. Based on current distribution results, sixteen of these species appear endemic to North America. Of these, six species are described as new: *H. alboaurantiacum*, *H. cuspidatum*, *H. ferruginescens*, *H. subconnatum*, *H. subtilior*, and *H. vagabundum*. Geographic range extensions and taxonomic notes are provided for five additional species recently described as new from eastern North America. A new name, *H. geminum*, is proposed for *H. caespitosum* Banning ex Peck, non Valenti. Overall, species of *Hydnum* are best recognized by a combination of morphological and molecular phylogenetic analyses. Taxonomic descriptions are provided for seventeen species, including epitype designations for *H. albidum*, *H. albomagnum*, and *H. umbilicatum*, taxa described more than 100 years ago, and molecular annotation of the isotype of *H. washingtonianum*. Photographs and a key to eastern North American *Hydnum* species are presented.

Keywords

Basidiomycota, Agaricomycetes, Hydnaceae, ectomycorrhizal fungi, taxonomy, systematics, type studies

Introduction

Hydnum L: Fr (=*Dentinum* Gray) is a genus of ectomycorrhizal (ECM) mushroom-forming fungi found primarily in temperate forests of Asia, Australia, Europe, and North America. Until recently, only twelve species of *Hydnum* were commonly accepted worldwide. Initial phylogenetic studies of European *Hydnum* revealed higher than expected taxonomic diversity in the genus, with thirteen molecularly recognized clades masquerading under four morphologically-defined species names (Grebenc et al. 2009), which have since been described as new species (Olariaga et al. 2012, Vizzini et al. 2013, Niskanen et al. 2018). Following a global survey of diversity in the genus which estimated 31 species worldwide based on molecular phylogenetic analysis (Feng et al. 2016), additional taxonomic work in Europe and North America has raised the global species count to 34 (Buyck et al. 2017, Niskanen et al. 2018), which is estimated to be less than half of the total number of *Hydnum* species (Niskanen et al. 2018). This previously overlooked diversity may be due to morphological stasis in evolution of basidiome morphology and lack of attention to regional characterization of the *Hydnum* flora in locations outside Europe.

Hydnum, in its original Linnean concept, contained all species of mushroom-forming fungi with a spinose hymenophore (e.g., Fries 1821). Indeed, over 900 names have been attributed to the genus per Index Fungorum (www.indexfungorum.org). However, molecular phylogenetic analysis showed that the spinose hymenophore has evolved independently many times in distantly related taxa (Hibbett et al. 1997). As a consequence, most species formerly contained in *Hydnum* have been moved to other genera. Species of *Hydnum* in the contemporary sense (Donk 1956) and typified by *H. repandum* L.:Fr. are united by their smooth hyaline basidiospores, white to orange basidiomes, and stichic basidia, in which the meiotic spindle is vertically oriented (Donk 1933, Maas Geesteranus 1971, Restivo and Petersen 1976, Pine et al. 1999). Ecologically, *Hydnum* form ECM associations with a variety of vascular plant species including members of Pinaceae (Agerer et. al 1996), Myrtaceae (McNabb 1971), Fagales (McNabb 1971, Feng et. al 2016, Niskanen et al. 2018), Salicaceae (Niskanen et al. 2018), Malvaceae (Niskanen et al. 2018), and Dipterocarpaceae (Lee et al. 2002). The genus is distributed mostly in temperate areas, with a few reports from tropical and subtropical forests in southeast Asia (Lee et al. 2002, Feng et al. 2016) and the neotropics (Garibay-Orijel et al. 2006, Sarmiento and Fontecha 2013, Feng et al. 2016, Niskanen et al. 2018).

Previous analysis of global *Hydnum* diversity revealed the presence of six distinct clades of *Hydnum* in eastern North America, only one of which also occurs on another continent (Feng et al. 2016). Currently, five species have been described from eastern North America: *H. albidum* Peck, *H. albomagnum* Banker, *H. aerostatisporum* Buyck, D.P. Lewis & V. Hofst., *H. caespitosum* Banning ex Peck (*non* Valenti), and *H. umbilicatum* Peck. Banker (1906) and Harrison and Grund (1987) recognized six species of *Hydnum* from across North America in their taxonomic treatments. Of those species that occur in eastern North America, four were described over one hundred years ago, and the application of those names has not been clarified in light of molecular phylogenetic analyses. Here, we resolve those species names by taxonomic revision of type

specimens and DNA sequencing of contemporary collections, as well as document *Hydnum* species diversity and distribution in eastern North America. Seventeen species are treated here, of which six are described as new. A taxonomic key to species from eastern North America is included.

Methods

Dried specimens of *Hydnum* were obtained from TENN, CORT, NYS, NY, WTU, and CSU. Herbarium abbreviations follow Thiers [continuously updated]. Additional collections were borrowed from the personal herbarium of Michael Kuo (Charleston, Illinois). Fresh *Hydnum* specimens were collected from localities in the eastern United States (North Carolina, Tennessee, Georgia, Florida, Virginia). Color documentation of fresh material follows Kornerup and Wanscher (1967; e.g., 5A2), Munsell Soil Color Charts (1954; e.g., 10YR 4/7), or Ridgway (1912; e.g., "Ochraceous-Tawny"). Macroscopic descriptions were taken from fresh material. In some instances, 5% KOH and 10% $FeSO_4$ were applied to pilei to test for macrochemical reactions.

Microscopic features were examined on a Nikon Eclipse 80i microscope from dried material rehydrated in 5% KOH and stained with Congo red or phloxine. Measurements and photographs were taken using a Nikon DS-Fi1 camera and Nikon NIS Elements 3.1 software. Basidiospores were measured from spore prints where available or spine tissue, and *Q* (quotient of basidiospore length to width) was calculated for each spore. The number of spores measured for each species is represented as n=total number/number of specimens (e.g., n=20/3). Measurements in excess of two standard deviations are denoted in parentheses and averages in italics.

DNA extractions were performed using two methods. Fresh or dried material less than five years old was extracted using an Extract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, MO, USA). Older dried specimens were extracted using an HP Fungal DNA Extraction Kit (Omega Bio-Tek, Norcross, Georgia, USA). For specimens >50 years old, 10–20 mg ground tissue was incubated in extraction buffer at 65 °C for 72 hours prior to the first extraction step. The extraction was performed in a laminar flow hood to minimize contamination.

Primers ITS1F and ITS4 (Gardes and Bruns 1993, White et al. 1990) were used to amplify and sequence the nuclear rDNA internal transcribed spacer 1, 5.8S rRNA, and internal transcribed spacer 2 (hereafter, ITS). For older materials, we amplified and sequenced the two spacer regions separately following Ammirati et al. (2007) using primers ITS1F/ITS2 and 5.8SR/ITS4. Sequencing was performed on an Applied Biosystems 3730 Genetic Analyzer at the University of Tennessee Genomics Core. Sequence reads were assembled using Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan, USA).

GenBank sequences labeled as *Hydnum*, as well as closely matching environmental sequences, were downloaded. Sequences were visualized in AliView 1.20 (Larsson 2014) and aligned using MUSCLE 3.8.31 (Edgar 2004). Minor adjustments were made manually to the alignment. The GTR+I+GAMMA substitution model was se-

lected as the best-fit model for Bayesian inference (BI) analysis in PartitionFinder 2.1.1 (Lanfear et al. 2016). BI analyses were performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003); the global analysis ran for 10 million generations with sampling every 1000th generation. Following global BI tree visualization, clades that did not contain sequences from eastern North America were pruned to form a second tree figure for easier graphical representation. Maximum Likelihood (ML) analysis was performed on the pruned dataset in RAxML 8.2.8 (Stamatakis 2014) using 1000 bootstraps under a GTRGAMMA model of nucleotide substitution following the RAxML user manual recommendation. BI analysis of the pruned data set ran for 5 million generations with sampling every 500th generation.

The resulting phylogenies were visualized in FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Mislabeled sequences were omitted, as well as *Hydnum* from the southern hemisphere due to high levels of sequence divergence (Feng et al. 2016). *Sistotrema alboluteum* was chosen as an outgroup based on Feng et al. (2016). DNA alignments and tree files are available at TreeBase (submission 22888).

Species are recognized here as monophyletic groups that differ in morphology, ecology, and/or geographic distribution.

Results

119 ITS sequences were produced for this study (Suppl. material 1). For the BI analyses, the average standard deviation of split frequencies reached below 0.01 after 4 million generations (global phylogeny) and 2 million generations (pruned phylogeny), so the first 40% of sampled trees were discarded as the burn-in for each analysis. Posterior probabilities (PP) for each analysis were calculated from 12002 samples from two independent runs for both the global and pruned analyses. In each case the potential scale reduction factor (PSRF) convergence diagnostic reached a value of 1.0 for all parameters, indicative of sufficient sample size.

The global phylogeny contained 397 sequences and 61 species-level clades (Suppl. material 2). Species-level clades containing sequences from eastern United States and Canada are colored red in the global circle tree (Fig. 1). PP ≥ 0.95 for nodes of these clades are denoted with an asterisk. The pruned version of the global tree, including taxon tip labeling information, is shown in Fig. 2. Four major clades of North American *Hydnum* were recovered, each with ML bootstrap support ≥ 70%. These are labeled by subgenus: *Alba*, *Pallida*, *Hydnum*, *Rufescentia* (Niskanen et al. 2018). A sister group relationship was recovered between subg. *Hydnum* and *Rufescentia*. In subg. *Alba* we recovered three monophyletic lineages that originate from eastern North America and correspond to phylogenetic species: *H. alboaurantiacum* sp. nov., *H. albidum*, and *H. albomagnum*. Subg. *Pallida* is represented by a single phylogenetic species from eastern North America: *H. subtilior* sp. nov. In subg. *Hydnum* three phylogenetic species from eastern North America were detected: *H. vagabundum* sp. nov., *H. washingtonianum*, and *H. subolympicum*. Subg. *Rufescentia*, centered around *H. rufescens*, contains the

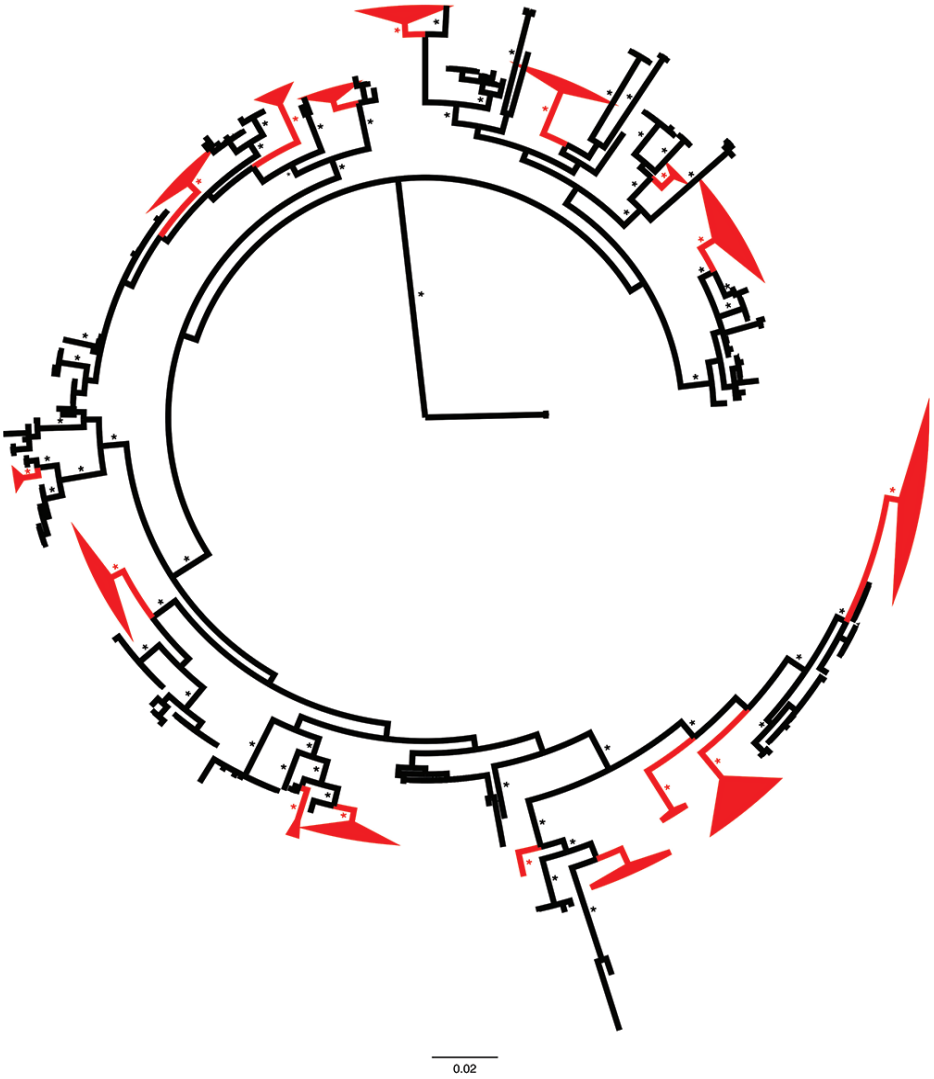

Figure 1. Global BI phylogeny of *Hydnum*. Species-level clades containing sequences from eastern United States and Canada are colored red. Posterior probabilities ≥ 0.95 are denoted with asterisks.

largest number of eastern North American phylogenetic species, including *H. ferrugi-nescens* sp. nov., *H. aerostatisporum*, *H. canadense*, *H. mulsicolor*, *Hydnum* sp. AS30, *H. subconnatum* sp. nov., *H. quebecense*, *H. cuspidatum* sp. nov., and *H. umbilicatum*.

We were unable to produce ITS sequences from the holotypes of the following species: *H. albidum*, *H. albomagnum*, *H. umbilicatum* and *H. caespitosum*. As a result, we designated epitypes for three of those species – *H. umbilicatum* 10651TJB (CORT 012241), *H. albidum* 10640TJB (CORT 012029), and *H. albomagnum* RAS231 (TENN 073062), which are represented by ITS data. Each designated epitype was

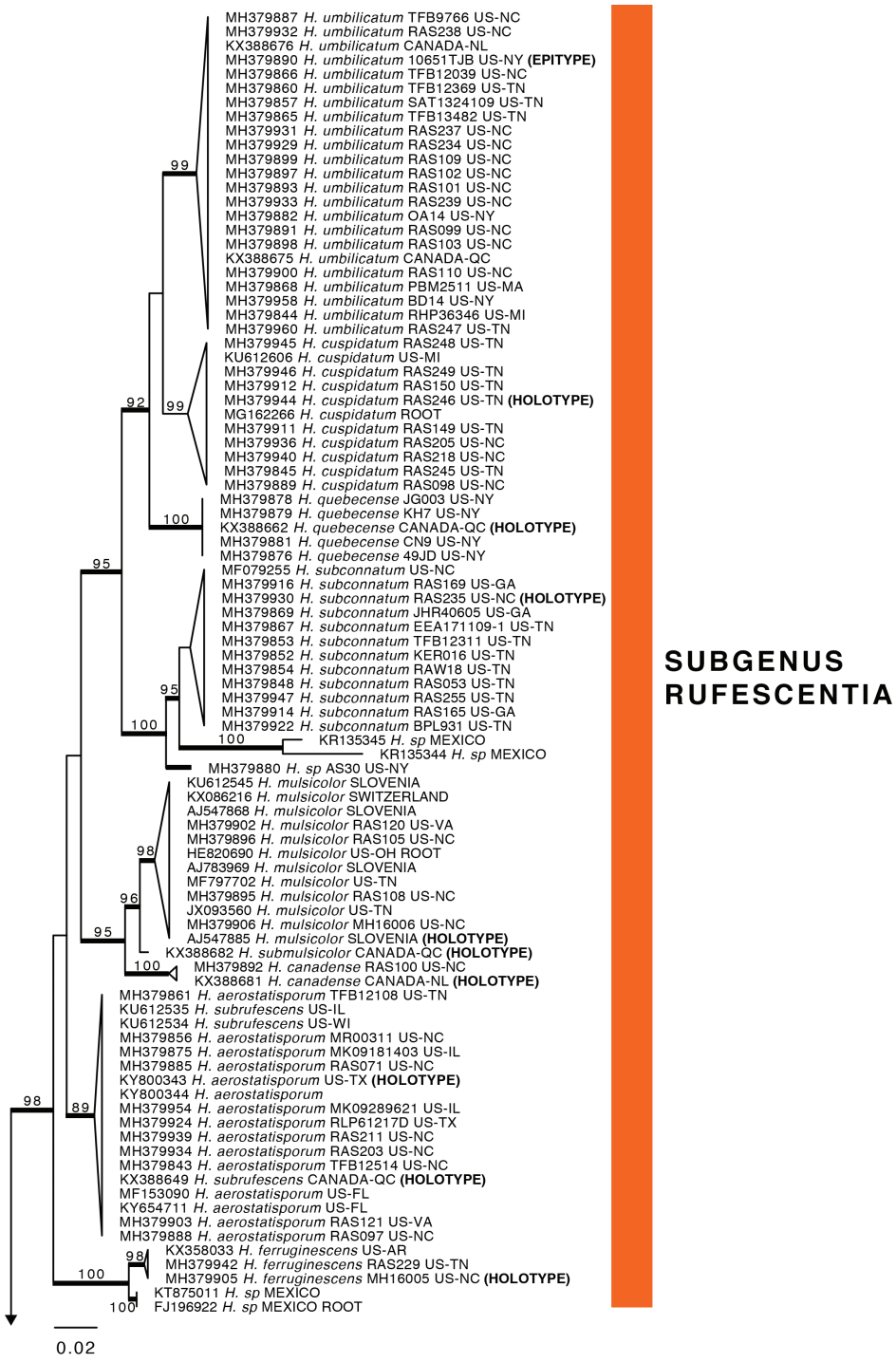

Figure 2. ML pruned phylogeny of North American species of *Hydnum*. Bootstrap support values ≥70% are shown above branches. Posterior probabilities ≥0.95 are shown with branches in bold.

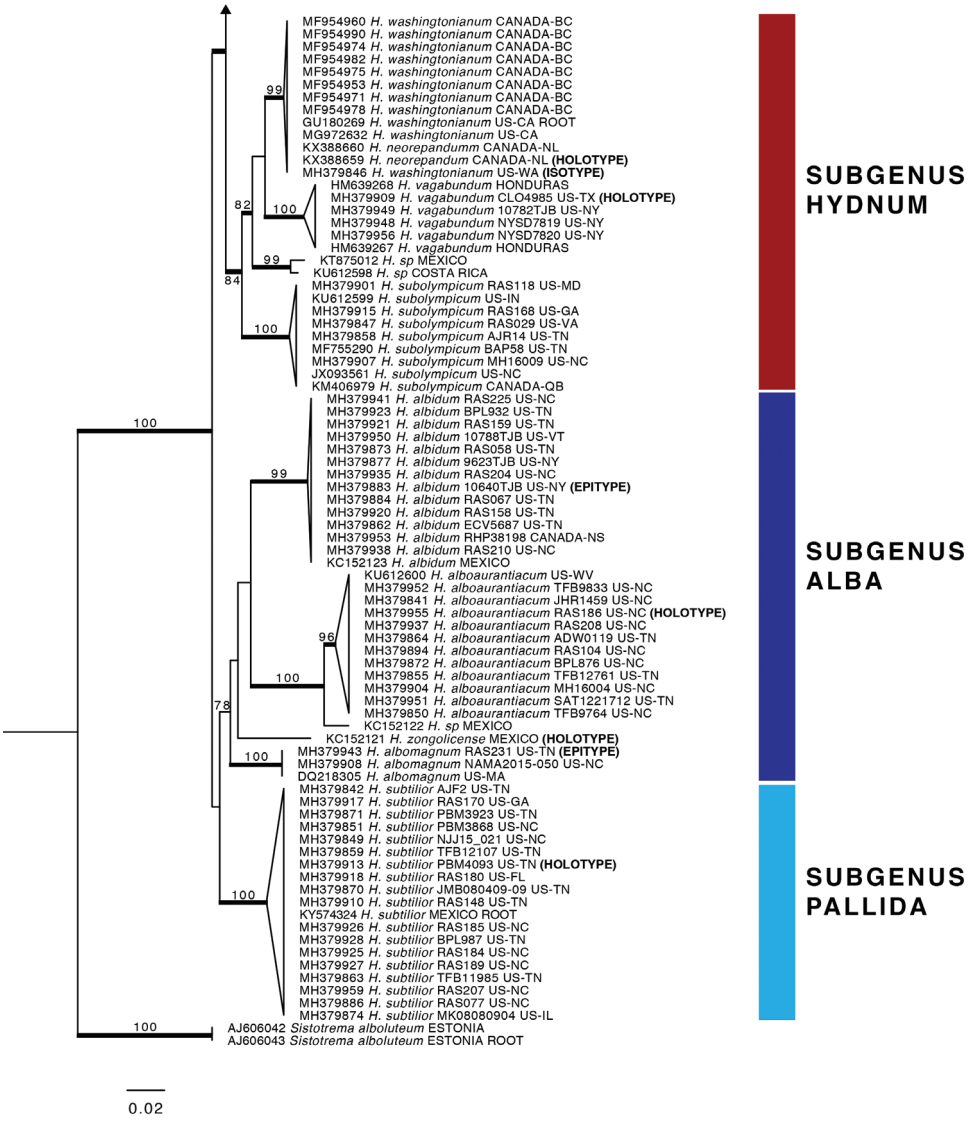

Figure 2. Continued.

chosen based on morphological consistency and geographic proximity to sites containing holotypes. We were able to sequence partial ITS regions from two historical Peck collections labeled *H. albidum*, but based on morphology those specimens represent a separate, larger, white species that we describe here as *H. vagabundum*.

A total of sixteen species-level monophyletic groups from eastern North America were recovered, representing ten described and six undescribed species. One additional species, *H. geminum*, was not represented by any modern collections, and thus its

phylogenetic position is unconfirmed. Below, taxonomic descriptions are presented for eastern North American species by subgenus. Of the recovered species, only one eastern North American *Hydnum*, *H. mulsicolor*, also occurs outside North America. This result is consistent with recent work that showed eastern North American clades of *Hydnum* are largely endemic (Feng et al. 2016, Niskanen et al. 2018).

None of the species studied display a clear preference for tree host genus. Many occupy a wide geographic range within eastern North America, with three species (*H. albidum*, *H. subtilior*, *H. vagabundum*) also found in Central America. One species, *H. washingtonianum*, occurs in both eastern and western North America.

Basidia in *Hydnum* were consistently suburniform, often undulating, and tapered to a narrow pedicel. Basidium shape was not considered a diagnostic trait and thus omitted from taxonomic descriptions below. Basidiospores of *Hydnum* were inamyloid and acyanophilous (Hall and Stuntz 1971). Morphological variation across *Hydnum* was low compared to other genera, with few variable microscopic features. As a consequence, species varied by differences in basidiospore size and shape, number of sterigmata, and pileipellis elements. Several taxa in subg. *Rufescentia* were nearly morphologically indistinct from one another and could be identified in the field only by a combination of morphology and distribution/habitat data. Even so, ITS sequencing is necessary to confidently identify those species.

Taxonomy

Hydnum subg. *Alba* Niskanen & Liimat., Mycologia 110: in press (2018)

Hydnum albidum **Peck, Bulletin of the New York State Museum 1(2): 10 (1887)**
MycoBank Epitypification: MBT381859
GenBank: MH379883
Figs 3A, B, 6A

= *Hydnum repandum* var. *albidum* (Peck) Bres., Iconographia Mycologica 21: 1045 (1932)
= *Dentinum albidum* (Peck) Snell, Mycologia 37: 51 (1945)
= *Hydnum repandum* f. *albidum* (Peck) Nikol., Flora Plantarum Cryptogamarum URSS. Fungi. Familia Hydnaceae 6(2): 306 (1961)

Type. UNITED STATES. New York: Rensselaer County, Sandlake, ground in thin woods, Jul *ca*. 1886, C.H. Peck (holotype: NYS-F-134). **Epitype.** UNITED STATES. New York: Cortland County, Kennedy State Forest, Scutt Road (42.4685; -76.1656), on humus in forest with *Quercus rubra*, *Fagus*, *Acer*, 550 m, 30 Jul 2014, T.J. Baroni 10640TJB (CORT 012029, epitype here designated).

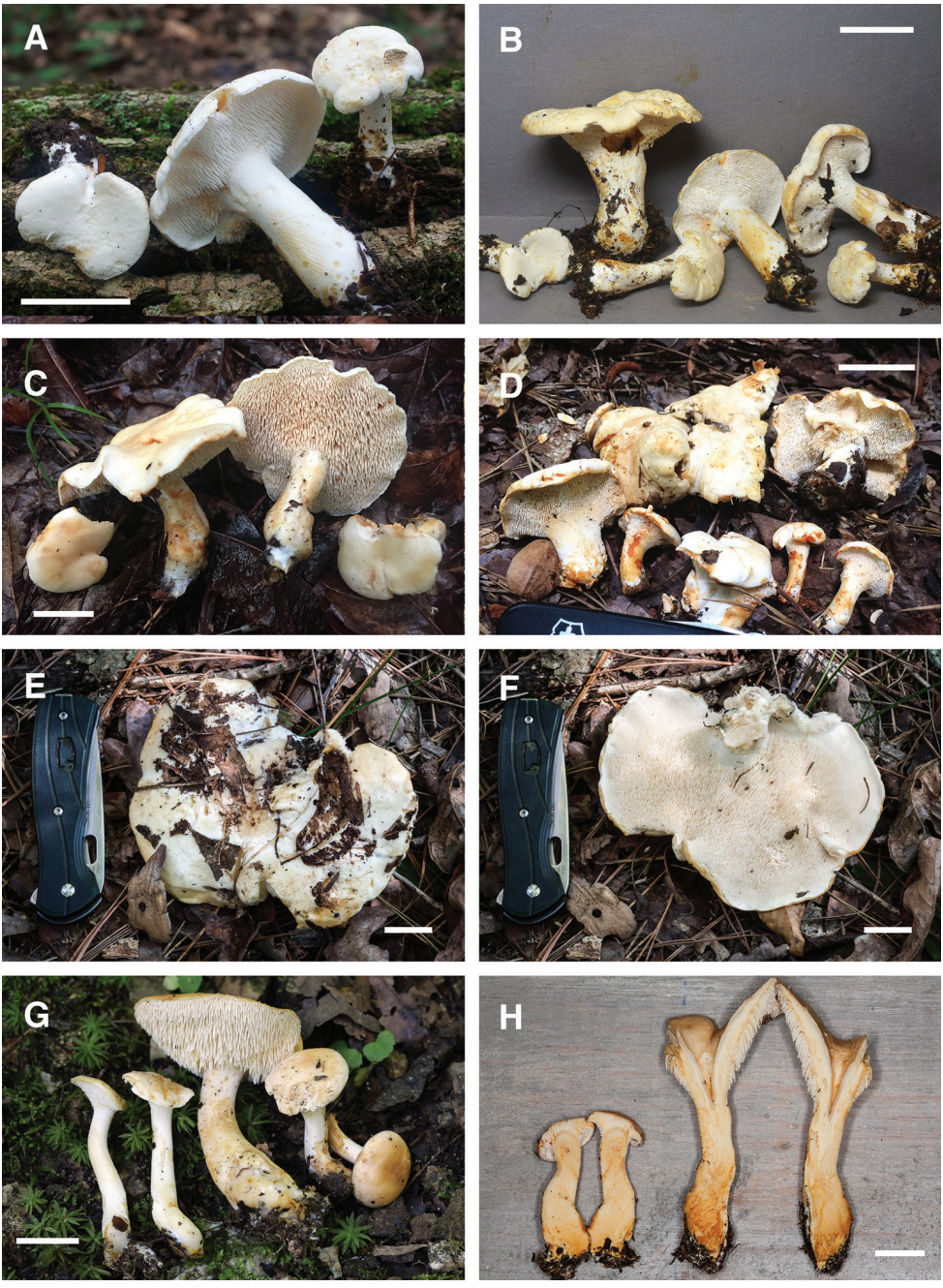

Figure 3. Basidiomes of *Hydnum* species. **A, B** *H. albidum* 10640TJB (CORT 012029, epitype, photo T.J. Baroni) **C** *H. alboaurantiacum* RAS186 (TENN 073053, holotype, photo R.A. Swenie) **D** *H. alboaurantiacum* BPL876 (TENN 073003, photo B.P. Looney) **E, F** *H. albomagnum* RAS231 (TENN 073062, epitype, photo R.A. Swenie) **G** *H. subtilior* PBM4093 (TENN 073034, holotype, photo P.B. Matheny) **H** *H. subtilior* showing characteristic staining of context RAS180 (TENN 073050, photo R.A. Swenie). Scale bar: 20 mm.

Description. Pileus 15–50 mm wide, round to reniform, convex to plano-convex or uplifted, disc sometimes shallowly depressed; surface glabrous, sometimes irregularly bumpy or mottled in appearance, bright white becoming cream or cream-peach (10YR 8/4), no reaction to KOH; margin entire and incurved when young, undulating in age. Spines 1–6 mm long, easily rubbing off, subdecurrent to decurrent, white to cream white (10YR 8/3). Stipe 15–45 × 5–15 mm, central or eccentric, equal to slightly enlarged or bulbous at the base, then tapering into ground, concolorous with the pileus, staining orange-ochre (5A4–5B7 or "Yellow Ochre"). Basal mycelium white when present. Context white to pale cream, staining slowly orange (5A6) after five mins. Odor mild at first, then pleasantly fruity like apricots when stored in foil. Taste mild, pleasant, or occasionally peppery.

Basidiospores 4.5–*5.2*–6 µm × 3–*4*–4.5(5) µm, *Q*=(1.05)1.07–*1.33*–1.58(1.74) (n=72/5), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 28–36(40) × 6–7(8) µm with 5–6(7) sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–6 µm wide. Clamp connections present.

Distribution. Eastern Canada and U.S. and central Mexico – Nova Scotia, Vermont, New York (type), Tennessee, North Carolina. Also Veracruz, Mexico (GenBank KC152123).

Ecology. In hardwood and mixed woods with *Betula*, *Quercus*, *Fagus*, *Tsuga*, *Pinus*, *Abies*. June to early September.

Other specimens examined. CANADA. Nova Scotia: Victoria County, Cape North, Grey Glen Brook, Farm lot, with *Abies*, *Betula*, 80 m, 8 Sep 1973, R.H. Petersen TFB38198 (TENN 038198). UNITED STATES. New York: Cortland Co., Kennedy Forest, Scutt Hill Road, on humus under *Quercus*, *Fagus*, *Acer*, 550 m, 10 Aug 2003, T.J. Baroni 9623TJB (CORT 014489). North Carolina: Great Smoky Mountains National Park, Heintooga Round Bottom Road, top of road on embankment with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS204 (TENN 071752). Great Smoky Mountains National Park, Heintooga Round Bottom Rd., on embankment with *Betula*, *Quercus*, *Tsuga*, 1525 m, 17 Aug 2017, R.A. Swenie RAS210 (TENN 073173). Tennessee: Great Smoky Mountains National Park, Ogle Place Nature Trail, near stream in soil among leaf litter with *Tsuga*, *Betula*, 670 m, 5 Jun 2016, R.A. Swenie RAS058 (TENN 072000). Great Smoky Mountains National Park, Schoolhouse Gap Trail, on embankment with *Quercus*, *Pinus*, *Betula*, 550 m, 8 Jul 2017, R.A. Swenie RAS158 (TENN 073041). Vermont: Windham County, Stratton Mountain Resort area, 650 m, 28 Jul 2017, T.J. Baroni 10788TJB (CORT 014475).

Discussion. *Hydnum albidum* was originally described from New York by Peck (1887) and produces small white to cream-colored basidiomes with small subglobose to broadly elliptic basidiospores. In the protologue Peck distinguishes *H. albidum* from *H. repandum* by the smaller basidiomes and spores, as well as white coloration. In a later description Peck (1897) added that *H. albidum* is an edible but uncommon species "uniformly colored in all its parts". Of the two small white species of *Hydnum* that occur in eastern North America (Fig. 3A-D), both *H. albidum* and *H. alboaurantiacum* have

similarly small subglobose basdiospores (Fig. 6A–B). However, *H. alboaurantiacum* quickly stains bright orange within minutes wherever handled, while *H. albidum* stains much less vividly brown-orange, sometimes only hours after handling. In addition, *H. alboaurantiacum* is only known from the southeastern US. While we were unable to successfully sequence DNA from the holotype of *H. albidum*, several collections from the region of the type locality are consistent with the morphology of the holotype of *H. albidum*, and one of these is designated as an epitype.

In addition to the holotype, there are several other historical collections made by Peck to which he applied the name *H. albidum*. Based on basidiospore measurements alone, it is clear three of the eight collections have much larger spores than *H. albidum*. We successfully sequenced partial ITS from two of those three collections, which matched modern specimens from Texas, New York, and Honduras belonging to a species more closely related to *H. repandum* (see discussion of *H. vagabundum*).

Hydnum repandum var. *album* (Quél.) Rea is a European variety, the name of which has been widely applied in North America (Coker and Beers 1951, Smith et al. 1981, Harrison and Grund 1987, Roody 2003). The description of *H. repandum* var. *album* by Roody (2003) appears to refer to *H. subtilior*, while displaying a photo of what is perhaps *H. albidum*. However, the spores of *H. albidum* are smaller than the 7–8.5 × 5.5–7 µm listed by Roody (2003), Harrison and Grund (1987), Smith et al. (1981), and Coker and Beers (1951). Thus, the American concept of *H. repandum* var. *album* is best interpreted as *H. subtilior*, described below.

***Hydnum alboaurantiacum* Swenie & Matheny, sp. nov.**
MycoBank No: MB825492
GenBank: MH379955
Figs 3C, D, 6B

Diagnosis. Most similar to *Hydnum albidum* but differs from it by the slightly stouter basidiomes that stain bright orange within minutes of handling. Differs from *H. subtilior* and *H. vesterholtii* by smaller basidiospores.

Type. UNITED STATES. North Carolina: Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail (35.5634; -83.3092), scattered under *Betula*, *Fagus*, with *Tsuga* nearby, 28 Jul 2017, R.A. Swenie RAS186 (holotype: TENN 073053).

Etymology. *alboaurantiacum* (L.) white-orange, referring to the coloration of the basidiomes, which stain bright orange.

Description. Pileus 20–70 mm wide, irregularly round, convex, becoming shallowly convex to depressed, occasionally umbilicate; margin thin, wavy to lobed, incurved becoming decurved; surface matt, glabrous, pale to cream white (“Pale Ochraceous Buff”), quickly bruising orange (“Zinc Orange” or “Xanthine Orange”, 6A6-8). Spines 1–7 mm long, brittle in mass and breaking easily, adnate to subdecurrent, white to cream-orange (“Pale Ochraceous Buff” to “Light Ochraceous Buff”, 4A2–5A3). Stipe

17–50 × 6–21 mm, central or eccentric, terete or clavate, concolorous with the pileus, easily bruising orange (5A2). Context thin, firm, cream white, staining orange ("Xanthine Orange" to "Mars Yellow", 6A8 to 5B6–B7), especially in young specimens at base of stipe within five minutes when cut in half. Odor mild or sweet and fruity. Taste mild.

Basidiospores 4–*4.8*–6(7) µm × 3–*3.9*–5(6) µm, *Q*=1.00–*1.25*–1.52(1.54) (n=44/6), globose to ellipsoid, smooth, hyaline in KOH. Basidia 36–42 × 4.5–7 µm with 5–7 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–5 µm wide. Clamp connections present.

Distribution. Southeastern U.S. – North Carolina (type), Tennessee, and West Virginia (GenBank KU612600).

Ecology. In mixed woods with *Quercus*, *Tsuga*, *Pinus*, *Betula*, *Liriodendron*, *Fagus*. May to August.

Other specimens examined. UNITED STATES. North Carolina: Great Smoky Mountains National Park, Deep Creek, Indian Creek, on soil in mixed woods, 610 m, 9 Jul 1974, J.H. Restivo JHR1459 (TENN 040599). Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail, scattered under *Betula*, *Fagus*, *Tsuga*, 700 m, 28 Jul 2017, R.A. Swenie RAS186 (TENN 073053). Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on embankment with *Quercus*, *Betula*, *Tsuga*, 1525 m, 17 Aug 2017, R.A. Swenie RAS208 (TENN 071751). Macon County, vicinity of Highlands, Glen Falls Trail, 1100 m, 14 Jul 2000, E.B. Lickey TFB9833 (TENN 058812). Transylvania County, Pisgah National Forest, Yellow Gap Road, 310 m, 18 Jul 2000, R.H. Petersen TFB9764 (TENN 058665). Duke Forest, scattered in mixed duff with *Quercus*, *Pinus*, 150 m, 25 May 2016, B.P. Looney BPL876 (TENN 073003). Blue Ridge Parkway, near mile marker 342, side of road on embankment, deciduous woodlot, 1225 m, 19 Aug 2016, J. Schieb RAS104 (TENN 073014). Buncombe County, Bent Creek Experimental Forest, near Boyd Branch Road, mossy acidic forest with *Quercus alba*, *Q. rubra*, *Liriodendron tulipifera*, *Betula lenta*, *Tsuga canadensis*, 670 m, 22 Aug 2016, M. Hopping MH16004 (TENN 073548). Tennessee: Great Smoky Mountains National Park, Cades Cove, Gregory Ridge Trail, 550 m, 18 Aug 2005, E.B. Lickey TFB12761 (TENN 061328). Great Smoky Mountains National Park, Maddron Bald Trail, on mossy soil with *Tsuga*, *Quercus*, *Fagus*, *Pinus*, 550 m, 4 Aug 2012, S.A. Trudell SAT1221712 (TENN 067355). Sevier County, University of Tennessee Biology Field Station, on mossy soil with *Quercus*, *Tsuga*, *Pinus*, 450 m, 27 Jul 2009, A.D. Wolfenbarger AW0119 (TENN 064272).

Discussion. *Hydnum alboaurantiacum* has probably been mistaken for the closely related *H. albidum* due to the initial pale white coloration and similar basidiospore size and shape. However, *H. alboaurantiacum* quickly stains bright rusty orange on all parts of the basidiomes where handled, whereas *H. albidum* slowly stains a lighter brown-orange hue. In addition, *H. alboaurantiacum* often displays a larger and more stout stature than *H. albidum*. The two species are readily distinguished as separate clades in Fig. 2. *Hydnum alboaurantiacum* is known only from the southeastern US and appears derived from a grade of Central American taxa including the recently described *H. zongolicense* (Niskanen et al. 2018).

Hydnum albomagnum **Banker, Bulletin of the Torrey Botanical Club 28(4): 207 (1901)**
MycoBank Epitypification: MBT381860
GenBank: MH379943
Figs 3E, F, 6C

= *Dentinum albomagnum* (Banker) Pouzar, Ceská Mykologie 10 (2): 76 (1956)

Type. UNITED STATES. Alabama: Lee County, Auburn, Dec 1896, F.S. Earle (holotype: NY 776138). **Epitype.** UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, Bandy Creek area (36.4920; -84.6950), solitary on soil with *Quercus*, *Pinus*, 450 m, 23 Sep 2017, RAS231 (TENN 073062, epitype here designated).

Description. Pileus 60–110 mm wide, irregularly round, irregularly convex to plano-convex or uplifted; surface dull, glabrous with adhering debris, uneven and sometimes pitted in places, cream white (4A4) with patches of cottony white, becoming light tan in age (10 YR 6/4); margin thin, wavy to slightly lobed, incurved when young then raised in age. Spines 1–6 mm long, brittle in mass, adnate to subdecurrent, white to cream white (4A3–5A3). Stipe 20–40 × 13–20 mm thick, central or eccentric, clavate, occasionally split in two towards the apex; surface smooth, concolorous with spines, if bruising then only very slightly an hour or more after handling ("Yellow-Ocher" to "Ochraceous-Tawny"). Context fleshy, white, unchanging when cut. Odor mild or slightly acidic at first, then pleasantly fruity like apricots when stored in foil. Taste mild.

Basidiospores 5.5–*6.2*–7 µm × 3–*3.8*–5 µm, *Q*=1.24–*1.66*–2.07(2.17) (n=45/3), ellipsoid to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 38–46 × 5–6 µm with 4–5(6) sterigmata. Pileipellis a tightly interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 2.5–5 µm wide. Clamp connections present.

Distribution. Eastern U.S. – Massachusetts, North Carolina, Tennessee (epitype), and Alabama (holotype).

Ecology. In hardwood and mixed woods with *Quercus*, *Pinus*. September to December.

Other specimens examined. UNITED STATES. Massachusetts: Worcester County, Rutland State Park, gregarious under litter layer along edge of road with *Quercus*, *Pinus strobus*, 260 m, 1 Nov 2003, P.B. Matheny PBM2512 (TENN 066858). North Carolina: Buncombe County, Black Mountain YMCA Blue Ridge Assembly, Wolfpit Loop, in leaf litter in broadleaf woods with *Quercus*, *Acer*, 900 m, 24 Sep 2015, B. Moerk NAMA 2015-050 (F C0305359F). Tennessee: Knox County, New Hopewell, on soil in mixed woods, 300 m, 2 Dec 1951, L.R. Hesler (TENN 020243).

Discussion. The original description of *Hydnum albomagnum* by Banker (1901) was based solely on dried material. As mentioned in the protologue, this species has a shorter stipe and overall stouter appearance than other *Hydnum*. In addition, mature basidiomes are much larger than other whitish small-spored species. *Hydnum albomagnum* also has more strongly ellipsoid spores (*Q* values averaging 1.66) than

other species. Historically, *H. albomagnum* has been less frequently collected than other pale species of *Hydnum*, perhaps because the basidiomes are often buried underneath layers of needle and leaf litter and thus overlooked. As basidiomes emerge from the ground, leaf litter remains stuck to the matt glabrous surface of the pileus, masking its appearance. This characteristic, along with the creamy white coloration, large basidiome size, and small ellipsoid spores, makes this one of the easier *Hydnum* species to identify. DNA sequencing of the type specimen of *H. albomagnum* was not successful; however, all modern collections reported under this species name have identical ITS sequences and match the morphology of the original species description. An epitype is chosen from Tennessee material, closest to the location of the holotype from Alabama.

Collections that are likely *H. alboaurantiacum* (TENN 041525) and *H. vagabundum* (TENN 003140) have been misidentified as *H. albomagnum*, perhaps due to the larger white appearance of those specimens. However, those collections differ from *H. albomagnum* in spore dimensions and lack litter debris on the pileus, which persists even after drying in *H. albomagnum*.

Hydnum subg. *Pallida* Niskanen & Liimat., Mycologia 110: in press (2018)

***Hydnum subtilior* Swenie & Matheny, sp. nov.**
MycoBank No: MB825493
GenBank: MH379913
Figs 3G, H, 6D

= *Hydnum repandum* var. *album* (Quél.) Rea sensu Am. auct.

Diagnosis. *Hydnum subtilior* is most closely related to European *H. vesterholtii* but differs from it based on ITS molecular data and geographic distribution in eastern North America.

Type. UNITED STATES. Tennessee: Anderson County, Norris Dam State Park, Clear Creek Trail (36.2124; -84.0681), scattered on soil along trail under *Fagus*, *Carya*, *Quercus*, 24 Jun 2017, P.B. Matheny PBM4093 (holotype: TENN 073034).

Etymology. *subtilior* (L.) finer, more slender, in reference to the slim basidiomes.

Description. Pileus 20–90 mm wide, round or occasionally reniform, convex becoming plano-convex to depressed, sometimes umbilicate; surface matt, glabrous, sometimes cracking into scales at the center, light cream yellow to cream orange buff ("Marguerite Yellow" to "Light Ochraceous Buff", 4A3–A5 to 5A2–A4), yellow with KOH, negative with FeSO4; margin thin, entire, incurved when young then decurved and sometimes wavy in age, staining rusty orange-brown ("Ochraceous-Orange" to "Mars Yellow", 6A5 to 5B6–B7). Spines 1–8 mm long, adnexed to decurrent, cream white to pale orange-cream (5A1–A2). Stipe 20–60 × 5–21 mm, central or eccentric, sometimes curving, equal or enlarging towards base, cream white or slightly lighter than pileus, staining rusty orange-brown (5B6-B8). Context spongy, cream white

to pale orange-cream, slowly staining orange (5A4–6) throughout after five minutes where cut. Odor mild or sweet. Taste mild or pleasant.

Basidiospores 7–*8*–9 µm × 5–*6.3*–7.5 µm, *Q*=1.07–*1.27*–1.52 (n=51/5), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 32–44 × 7–9 µm with 3–5(6) sterigmata. Pileipellis an interwoven cutis. Hyphae smooth, cylindrical, thin-walled, mostly 3–7 µm wide. Clamp connections present.

Distribution. Eastern U.S. – Illinois, North Carolina, Tennessee (type), Georgia, and Florida. Also Michoacán, Mexico (GenBank KY574324).

Ecology. In hardwoods under *Quercus*, *Carya*, *Fagus*, *Carpinus* or in mixed woods with these trees or *Betula* and conifers such as *Tsuga* or *Pinus* or less frequently *Picea*. June to August.

Other specimens examined. UNITED STATES. Florida: Alachua County, San Felasco Hammock Preserve State Park, Moonshine Sink Trail, in soil with deep layer of leaf litter, forest almost entirely *Carya*, 75 m, 23 Jul 2017, R.A. Swenie RAS180 (TENN 073050). Alachua County, Sweetwater Preserve off 16th Street entrance, mixed hardwood forest of *Quercus*, *Carya*, *Carpinus* and occasionally *Pinus*, 35 m, 6 Aug 2017, B. Kaminsky & G. LaPierre (FLAS 61253). Georgia: Putnam County, Rock Eagle 4-H Camp, with *Quercus*, *Pinus*, *Carpinus*, 200 m, 20 Jul 2017, R.A. Swenie RAS170 (TENN 073049). Illinois: Coles County, Lakeview Park, scattered under *Quercus alba*, *Carya*, 215 m, 8 Aug 2009, M. Kuo MK08080904. North Carolina: Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail to Mt. Sterling, on soil under *Tsuga*, 500 m, 9 Aug 2012, P.B. Matheny PBM3868 (TENN 067482). Great Smoky Mountains National Park, Smokemont, Bradley Fork Trail, scattered under *Betula*, *Fagus*, *Quercus*, *Tsuga*, 700 m, 28 Jul 2017, R.A. Swenie RAS184 (TENN 073051). Great Smoky Mountains National Park, Heintooga Round Bottom Road, solitary with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS207 (TENN 073057). Tennessee: Great Smoky Mountains National Park, Tremont, Middle Prong Trail, scattered singly in mixed woods next to river with *Tsuga*, *Betula*, 450 m, 14 Jul 2013, P.B. Matheny PBM3923 (TENN 071999). Great Smoky Mountains National Park, Cades Cove Road, 610 m, 31 Jul 2004, R.H. Petersen TFB12107 (TENN 060045). Great Smoky Mountains National Park, Elkmont, solitary under *Quercus*, *Tsuga*, 670 m, 4 Aug 2009, J.M. Birkebak JMB080409-09 (TENN 064273). Great Smoky Mountains National Park, Tremont Institute, Lagoon Trail, solitary under *Tsuga*, *Carpinus*, *Betula*, 450 m, 23 Jun 2017, R.A. Swenie RAS148 (TENN 073035). Great Smoky Mountains National Park, Greenbrier picnic area, solitary in riparian forest under *Tsuga*, *Betula*, 500 m, 28 Jul 2017, B.P. Looney BPL987 (TENN 073032). Anderson County, Norris Dam State Park, Clear Creek Trail, solitary in litter on slope under *Quercus*, *Carya*, *Fagus*, 275 m, 31 Aug 2009, A.J. Floden AJF2 (TENN 069607).

Discussion. *Hydnum subtilior* is a common species in the southeastern U.S. found in deciduous and mixed forests with a variety of tree associates, often in deep layers of leaf litter. Environmental sequencing has recovered this species from *Quercus* root tips in central Mexico (García-Guzmán et al. 2017). The stipe is usually longer than the diameter of the pileus, and the overall coloration can range from light cream-yellow to peach or tan. The best diagnostic features for this species are the coloration and often elongated stature in combination with broadly ellipsoid spores averaging 8 × 6.3 µm.

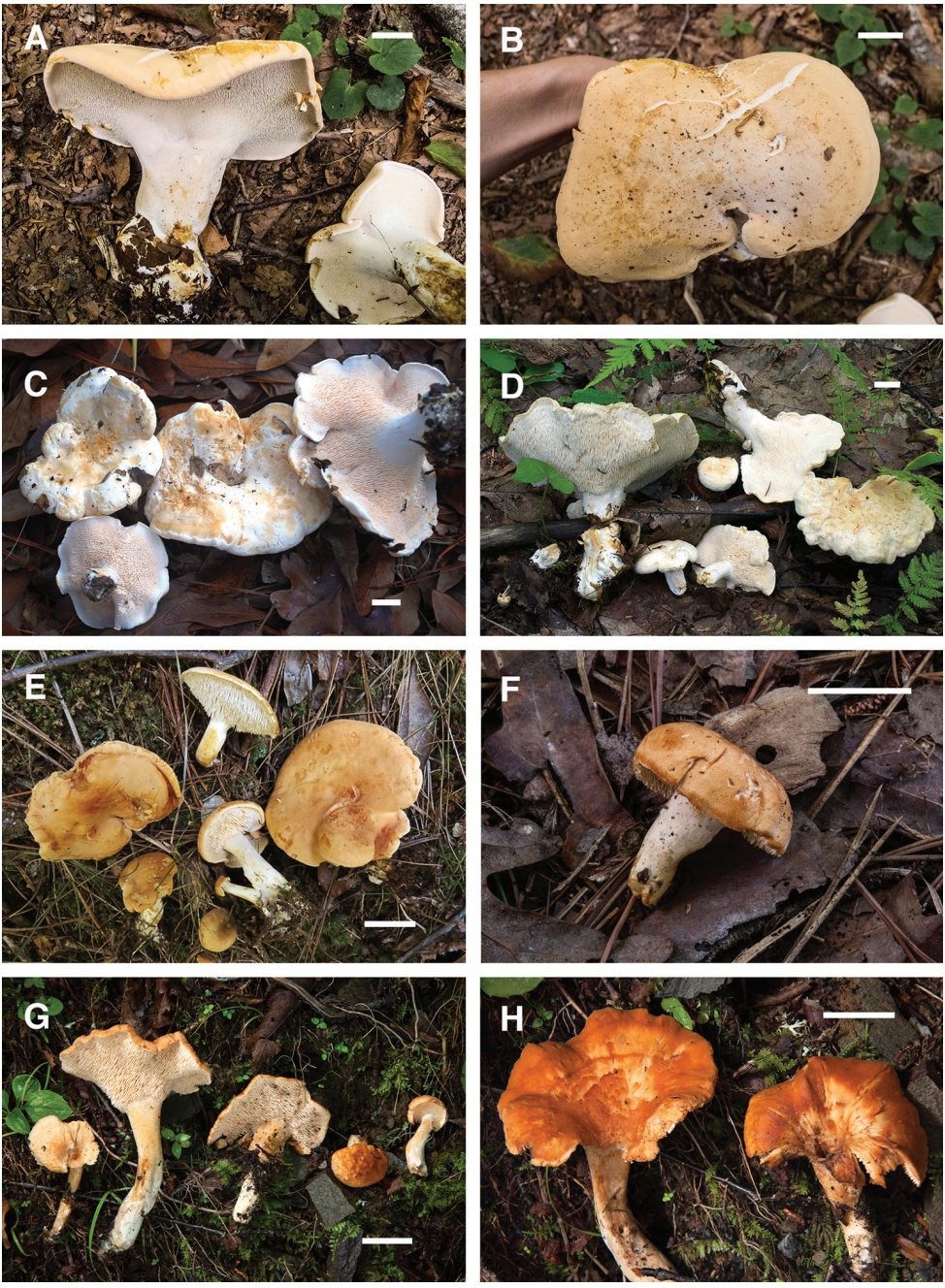

Figure 4. Basidiomes of *Hydnum* species. **A, B** *H. subolympicum* RAS029 (TENN 070845, photo R.A. Swenie) **C** *H. vagabundum* CLO4985 (CSU 01477, holotype, photo C.L. Ovrebo) **D** *H. vagabundum* 10782TJB (CORT 014461, photo T. J. Baroni) **E** *H. ferruginescens* MH16005 (TENN 073549, holotype, photo M. Hopping) **F** *H. ferruginescens* RAS229 (TENN 073061, photo R.A. Swenie) **G, H** *H. aerostatisporum* RAS071 (TENN 073001, photo R.A. Swenie). Scale bar: 20 mm.

In addition, the context of fresh basidiomes stains orange throughout within five minutes when cut in half (Fig. 3H).

Earlier authors (Coker and Beers 1951, Smith et al. 1981, Harrison and Grund 1987, Roody 2003) referred to this species as *H. repandum* var. *album*, a taxon originally described from Europe.

Hydnum subg. *Hydnum* L.

Hydnum subolympicum **Niskanen & Liimat., Mycologia 110: in press (2018)**
Figs 4A, B, 6E

= *Hydnum repandum* sensu Coker & Beers, 1951

Type. CANADA. Newfoundland and Labrador: Near Humber Village, trail to Barry's Lookout (48.9860; -57.7600), mature secondary growth of *Betula papyrifera* and *B. alleghaniensis*, also with *Cantharellus amethysteus*, 2 Sept 2012, A. Voitk 12.09.02. av12 (holotype DAOM744368, isotype K(M)249002).

Description. Pileus 80–130 mm wide, round, convex, becoming plano-convex; surface dry, glabrous, dull reddish-orange when young (5 YR 5/6) then cream to peach or dull orange in age (5A2-3), sometimes cracking in age to reveal white color of flesh; margin incurved and entire, becoming wavy and decurved, staining ochre to rusty brown very slowly after handling ("Yellow Ochre" to 5B8). Spines 1–7 mm long, close, subdecurrent, cream-yellow to pinkish cream. Stipe 30–100 × 20–40 mm, central or eccentric, tapering downwards to a slightly bulbous base, texture firm, smooth, white or off-white, staining orange cream to rusty orange, then yellow-brown when handled ("Mars Yellow", 10YR 5/8). Context white to cream, dry, firm, brittle, discoloration not observed. Odor mild or fruity and reminiscent of apricots. Taste mild or slowly bitter or peppery.

Basidiospores 6–*7.5*–9 µm × 5–*6.1*–7 µm, *Q*=1.07–*1.23*–1.46 (n=38/3), subglobose to broadly ellipsoid, smooth, hyaline in KOH. Basidia 36–42 × 6.5–8 µm with (3)4–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 µm wide. Clamp connections present.

Distribution. Eastern North America – Newfoundland and Labrador (type), Quebec (GenBank No. KM406979), Maryland, Virginia, North Carolina, Tennessee, and Georgia.

Ecology. In hardwood or mixed woods with *Quercus*, *Betula*. August to October.

Other specimens examined. UNITED STATES. Georgia: White County, Unicoi State Park, Unicoi to Helen Trail, hidden in soil embankment under roots, 460 m, 16 Jul 2017, R. Healy RAS168 (TENN 073047). Maryland: Harford County, Susquehanna State Park, 60 m, 9 Sep 2016, RAS118 (TENN 073021). North Carolina: Buncombe County, Bent Creek Experimental Forest, Boyd Branch, solitary in mature

bottomland forest including *Liriodendron tulipifera*, 670 m, 19 Sep 2016, M. Hopping MH16009 (TENN 073551). Tennessee: Great Smoky Mountains National Park, Schoolhouse Gap Trail, solitary in mixed woods under *Quercus*, 490 m, 26 Oct 2013, A.J. Ramsey AJR14 (TENN 073004). Virginia: Grayson County, Mount Rogers National Recreation Area, Mt. Rogers Trail, scattered along trail in mixed hardwood forest with *Betula*, *Quercus*, 1225 m, 17 Aug 2015, R.A. Swenie RAS029 (TENN 070845).

Discussion. This eastern North American species is phylogenetically allied with *H. repandum* and relatives in subgenus *Hydnum* along with *H. vagabundum* sp. nov. (described below) and *H. washingtonianum*. It can be distinguished from European *H. repandum* mainly by the different geographic distribution (eastern North America). This species differs from *H. vagabundum* by the smooth yellow-peach pileus that tends to crack in age and mostly non-lobate pileus margin. In comparison to *H. washingtonianum*, *H. subolympicum* produces smaller spores. Because of the shape and coloration, basidiomes of *H. subolympicum* in the field can resemble large chanterelles from above. Like many other species of *Hydnum*, basidiomes of this species often possess the sweet apricot-like odor that is characteristic of chanterelles. In our experience, this species is a choice edible. Coker and Beers (1951) likely referred to this taxon under the name *H. repandum*.

***Hydnum vagabundum* Swenie, Ovrebo & Matheny, sp. nov.**
MycoBank: MB825494
GenBank: MH379909
Figs 4B, C, 6F

Diagnosis. Closely related to *Hydnum subolympicum* but differs from it by the paler, more lobate pileus and ITS sequence divergence.

Type. UNITED STATES. Texas: Newton County, State Highway 87 and County Road 3062 (30.7080; -93.8270), scattered in soil under *Fagus*, *Pinus*, *Quercus*, 29 Dec 2011, C.L. Ovrebo CLO4985 (holotype: TENN 074443).

Etymology. *vagabundum* (L.), wandering, roving about, in reference to the broad distribution of this species in North America.

Description. Pileus 30–140 mm wide, round, convex, becoming plano-convex to broadly depressed; margin incurved and often lobed when young, then decurved or straight and wavy in age; surface matted tomentose or glabrous and pitted-grooved to bumpy in areas, off-white with pale pinkish buff tones (5A2), sometimes with slight ochre hues (5C4–C5), staining ochre where bruised. Spines 1–12 mm long, shortest near the pileus margin, adnate to subdecurrent, concolorous with the pileus or slightly darker pinkish-orange (5A3). Stipe 20–60 × 10–30 mm, central or eccentric, equal or with a swollen base, surface smooth or soft matted-tomentose, white or concolorous with the pileus, pinkish tan in areas, slowly staining ochre where bruised. Context solid, white, discoloring slight ochre (5C4–C5) where cut in half. Odor not distinctive. Taste mild or sweet-nutty, then slowly slightly acidulous.

Basidiospores 6.5–*7.4*–8.5 × 5–*6.1*–7.5 µm, *Q*=1.03–*1.22*–1.43(1.60) (n=97/4), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 39–57 × 8–10.5 µm with (3)4–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 3–7 µm wide. Clamp connections present.

Distribution. Eastern U.S. and Central America – New York, Texas (type), Honduras (GenBank HM639267–HM639268).

Ecology. In mixed woods with *Fagus*, *Pinus*, *Quercus*, *Tsuga*, *Picea*, *Betula*. May to December.

Other specimens examined. UNITED STATES. New York: Bolton, Sand Lake, August, C.H. Peck (NYS-D-7819). Rensselaer County, Burden Lake, 1 Sep, C.H. Peck (NYS-D-7820). Tompkins County, Hammond Hill State Forest, Red Man Run Rd., on humus in mixed woods with *Tsuga*, *Fagus*, *Picea, Betula alleghaniensis*, 520 m, 1 Sep 2017, T.J. Baroni 10782TJB (CORT 014461).

Discussion. *Hydnum vagabundum* is a large whitish species in subgenus Hydnum. Two collections of this species at NYS from the late 1800s and early 1900s were misidentified as *H. albidum* by Peck but feature distinctly larger basidiospores than the holotype of *H. albidum*. In addition, basidiomes of this species are much larger and fleshier than those of *H. albidum*. Partial ITS sequences obtained from Peck's specimens match two extant collections from New York and Texas, as well as GenBank sequences from western Honduras (HM639268, HM639267; Sarmiento and Fontecha 2013). In Honduras this species can be found in May and June, where it is a common edible (Sarmiento and Fontecha 2013). To date, *H. vagabundum* has the widest known range among the endemic eastern North American *Hydnum* species.

Peck's notes of what is described here as *H. vagabundum* indicate it as a "white, edible *Hydnum*". Another large whitish species, *H. albomagnum*, can be distinguished from *H. vagabundum* by the copious amount of leaf and needle debris that adheres to the pileus surface and smaller basidiospores.

Hydnum washingtonianum **Ellis & Everhart, Proc. Phila. Acad. 1894: 323 (1894)**

= *Hydnum neorepandum* Niskanen & Liimat., Mycologia 110: in press (2018)

Type. UNITED STATES. Washington: Kitsap County, Tracyton (47.6090; -122.6540), on ground in deep coniferous woods, 27 Dec 1893, A.M. Parker (holotype: NY 776185, isotype: WTU-F-14341).

Description. Pileus up to 40 mm wide, subplane, slightly depressed, thin, irregular; surface glabrous, "subviscose", wrinkled when dry, pale orange. Spines 3–5 mm long, terete, slender, acute, decurrent half way down the stipe, pale yellow but nearly white when fresh. Stipe up to 40 × 5–10 mm, subcylindrical, tapering slightly towards the base, central or slightly eccentric, pale orange. Context fleshy.

Basidiospores 7–*7.7*–8.5 µm × 6–*6.8*–7.5(8) µm, Q=1.04–*1.13*–1.22 (n=40/2), subglobose to broadly ellipsoid, smooth, thin-walled, hyaline in KOH. Basidia 31–41 × 7.5–8.5 µm with 4 sterigmata. Pileipellis hyphae not reviving. Clamp connections present.

Distribution. Western North America and eastern Canada – British Columbia, Washington (type), California (GenBank GU180269, MG972632), and Newfoundland and Labrador.

Ecology. On ground in coniferous woods. December.

Discussion. *Hydnum washingtonianum*, originally described from the Puget Sound region of Washington, is characterized by the pale orange pileus, yellowish decurrent spines, small globose basidiospores, and tough flesh. The species was considered synonymous with *H. repandum* by Maas Geesteranus (1964) and Hall and Stuntz (1971). However, we were able to produce a partial ITS sequence from the isotype (GenBank MH379846), which does not match European *H. repandum* sequences. Thus, we consider this species as an autonomous taxon with a mostly northern geographic distribution in North America. Phylogenetic analysis of the ITS sequence confirms this species from Washington, British Columbia, California, and Newfoundland and Labrador. *Hydnum washingtonianum* is associated with coniferous forests on both coasts, and one environmental sequence (GenBank GU180269) recovered this species on root tips of *Pinus muricata* in California.

Hydnum neorepandum, a recently described species from Newfoundland and Labrador (Niskanen et al. 2018), has an ITS sequence that differs by a single base pair from that of the isotype of *Hydnum washingtonianum*. The morphology of both protologues is also in agreement. Thus, we consider *H. neorepandum* a taxonomic synonym of *H. washingtonianum*.

Hydnum subg. *Rufescentia* Niskanen & Liimat., Mycologia 110: in press (2018)

***Hydnum ferruginescens* Swenie & Matheny, sp. nov.**
MycoBank: MB825495
GenBank: MH379905
Figs 3D, 5G

Diagnosis. Most closely related to the Eurasian *Hydnum magnorufescens* but differs from it by somewhat smaller basidiospores, ITS sequence divergence, and geographic distribution in the southeastern U.S.

Type. UNITED STATES. North Carolina: Buncombe County, Tanbark Ridge (35.6535; -82.4853), growing singly or conjoined in moss along trail with *Pinus strobus*, *Quercus prinus*, *Kalmia latifolia*, 915 m, 4 Sep 2016, M. Hopping MH16005 (holotype: TENN 073549).

Etymology. *ferruginescens* (L.), becoming ferruginous or rust-colored, in reference to the overall coloration of this species.

Description. Pileus 22–60 mm wide, round, convex, becoming depressed; margin incurved and entire when young, then irregularly lobed or degraded in age; surface dry,

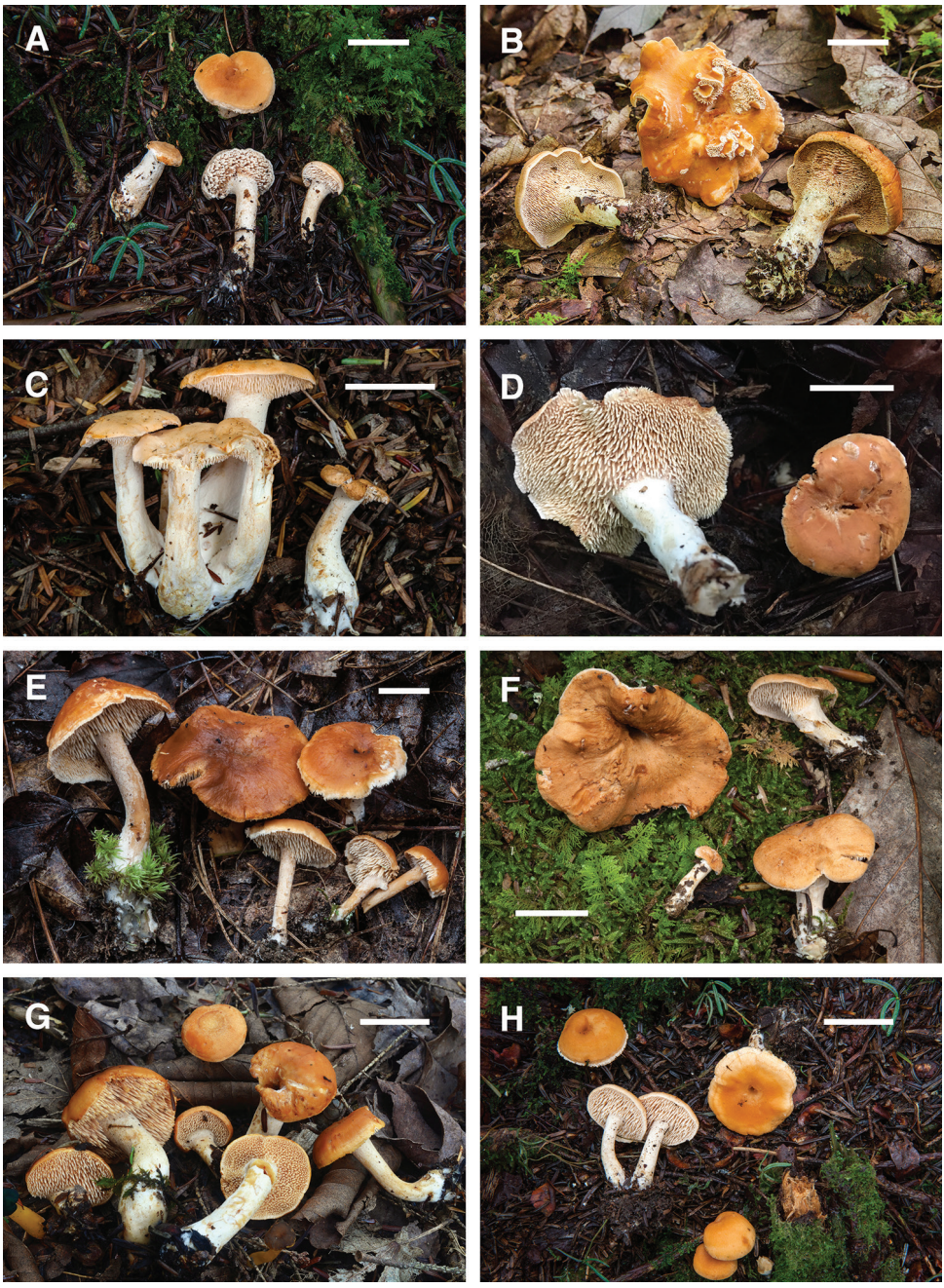

Figure 5. Basidiomes of *Hydnum* species. **A** *H. canadense* RAS100 (TENN 073010, photo R.A. Swenie) **B** *H. mulsicolor* RAS023 (TENN 070321, photo R.A. Swenie) **C** *H. subconnatum* RAS235 (TENN 073064, holotype, photo R.A. Swenie) **D** *H. subconnatum* RAS169 (TENN 073048, photo R.A. Swenie) **E** *H. cuspidatum* RAS246 (TENN 073086, holotype, photo R.A. Swenie) **F** *H. cuspidatum* RAS150 (TENN 073037, photo R.A. Swenie) **G** *H. umbilicatum* 10651TJB (CORT 012241, epitype, photo T.J. Baroni) **H** *H. umbilicatum* RAS101 (TENN 073011, photo R.A. Swenie). Scale bar: 20 mm.

glabrous, tawny to fulvous (5YR 5/8 to 6/8), discoloring slightly darker when handled. Spines 1–4 mm long, shorter near the margin, adnate to subdecurrent, white to cream (5A2–A4), bruising orange. Stipe 15–40 × 5–12 mm, central or eccentric, equal or slightly wider at apex, texture smooth, white or cream, lightly bruising orange (7.5 YR 7/8, 5A6-7); thick white mycelial mat sometimes present at base of stipe. Context white, unchanging after 5 minutes where cut in half. Odor not distinctive. Taste not distinctive or mildly fruity.

Basidiospores (5.5)6–*6.9*–8 µm × 5–*6.3*–7.5 µm, *Q*=1.01–*1.09*–1.22, (n=45/2), globose to subglobose, smooth, hyaline in KOH. Basidia 39–56 × 7.5–9 µm with (3)4–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 5–7 µm wide. Clamp connections present.

Distribution. Southeastern U.S. – North Carolina (type), Tennessee, Arkansas (GenBank KX358033).

Ecology. In mixed woods with *Quercus*, *Pinus*, *Carya*, *Tsuga*. September.

Other specimens examined. UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, West Bandy Trail, scattered along trail with *Quercus*, *Carya*, *Pinus*, *Tsuga*, 450 m, 23 Sep 2017, R.A. Swenie RAS229 (TENN 073061).

Discussion. *Hydnum ferruginescens* is known only from three occurrences in the southeastern U.S. This species is similar to *H. magnorufescens*, which has similarly sized basidiomes but slightly larger spores and is known from Europe and Asia.

Hydnum aerostatisporum Buyck, Lewis & V. Hofstetter, Crypt. Mycologie, 38: 101–146 (2017)

Figs 3E, F, 5H

= *Hydnum subrufescens* Niskanen & Liimat., Mycologia 110: in press (2018)

Type. UNITED STATES. Texas: Polk County, Big Thicket Natural Preserve, Big Sandy Creek Unit, Beaver Slide Trail (30.6150; -94.6700), 4 Jul 2014, Buyck 14.156 (PC0142475).

Description. Pileus (20)30–100 mm wide, irregularly round or sometimes reniform, convex to plano-convex, becoming funnel-shaped in age, sometimes with slit or umbilicus forming over stipe, surface dry, glabrous, subzonate when young, then cracking to coarsely scurfy in age, bright to medium brownish orange ("Xanthine Orange" to "Orange Rufous"), paler when young ("Salmon-Orange"), often cracking in age to reveal lighter color of context ("Pale Pinkish Buff"); margin incurved and entire when young, then wavy, irregular or degraded in age, discoloring slightly darker after handling. Spines 1–9 mm long, close, mostly awl-shaped but occasionally spathulate, adnate to subdecurrent, buff to peach ("Light Buff" to "Pinkish Buff"). Stipe 25–80 × (3)7–25 mm, central or eccentric, equal or slight bulbous at base in younger specimens, smooth, often with white hazy or cottony patches overlaid on surface, cream white to pale orange in younger basidiomes, then darker tan orange with age, discoloring very slightly

brownish orange when handled. Context cream to peach colored, firm, sometimes hollow with age, unchanging after 5 minutes when cut in half. Odor mild or sweet at first, then pleasantly fruity like apricots when stored in foil. Taste mild or weakly acrid.

Basidiospores *7*–*8.1*–8.5 × 6–*7*–8 µm, *Q*=1.01–*1.15*–1.33 (n=38/3), mostly broadly ellipsoid, smooth, hyaline in KOH. Basidia 40–47 × 8–10 µm with (2)3–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 µm wide. Clamp connections present.

Distribution. Eastern U.S. – Illinois, Texas (type), North Carolina, Tennessee, Virginia, and Florida.

Ecology. In hardwoods of *Quercus*, *Carya*, *Ulmus* or mixed woods including *Betula*, *Picea*, *Tsuga*. June to October.

Specimens examined. UNITED STATES. Florida: Alachua County, Gainesville, University of Florida Natural Area Teaching Laboratory, 30 m, 9 Sept 2016, A. I. Zuniga AIZ-021 (FLAS 60406). Alachua County, Gainesville, Possum Creek Park, in deep woods with *Carya* and some *Quercus*, soil rich and not sandy, 55 m, 9 Oct 2015, M.E. Smith MES1432 (FLAS 59996). Illinois: Coles County, Fox Ridge State Park, 230 m, 28 Sep 1996, M. Kuo MK09289621. Dewitt County, Weldon Springs State Recreation Area, gregarious under *Carya* with *Quercus alba*, *Quercus rubra*, *Ulmus* nearby, 215 m, 18 Aug 2014, M. Kuo MK09181403. North Carolina: Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on mossy bank with *Picea*, *Betula*, other hardwoods, 1580 m, 24 Jul 2016, R.A. Swenie RAS071 (TENN 073001). Great Smoky Mountains National Park, Heintooga Round Bottom Road, solitary on embankment with *Picea*, *Betula*, 1525 m, 17 Aug 2017, R.A. Swenie RAS211 (TENN 073174). Great Smoky Mountains National Park, Cataloochee, Big Fork Ridge Trail, 1100 m, 18 Jun 2005, E.B. Lickey TFB12514 (TENN 060681). McDowell County, near Little Switzerland, with *Tsuga*, ca. 1000 m, 19 Aug 2016, A. Funston RAS107 (TENN 073017). Tennessee: Great Smoky Mountains National Park, Cades Cove Road, 1 mile before Schoolhouse Gap Rd, 610 m, 31 Jul 2004, R.H. Petersen TFB12108 (TENN 060046). Great Smoky Mountains National Park, Tremont River Trail, solitary on slope in mossy area in mixed woods with *Tsuga* and hardwoods, 450 m, 4 Jul 2017, B. Teresi & K. Hucks RAS157 (TENN 073040). Texas: Polk County, Big Thicket National Preserve, Beaverslide Trail, on ground in mixed woods, 30 m, 12 Jun 2017, R.L. Pastorino RLP61217D (TENN 073547). Virginia: Shenandoah National Park, Hogback Mountain, 600 m, 9 Sept 2016, RAS121 (TENN 073024).

Discussion. *Hydnum aerostatisporum* is a commonly encountered species in the eastern U.S. It has been found primarily in hardwoods and mixed woods including conifers at high and low elevations on both sandy and non-sandy soils. The vibrant medium to dark orange pileus transitions from smooth in young specimens to conspicuously cracked and scurfy in age, often becoming funnel-shaped, occasionally with a hole or umbilicus. The stipe frequently becomes darker tan-orange in age, which is unusual in other medium-sized orange-pileate species of *Hydnum*. Basidiomes, particularly older specimens, often have patches of hazy white on the stipe surface.

Hydnum aerostatisporum was recently re-described as a new species from Quebec – *H. subrufescens* (Niskanen et al. 2018). The ITS sequence of the holotype of *H. subrufescens* differs from that of the holotype of the earlier described *H. aerostatisporum* by seven base pairs, but *H. subrufescens* does not form a well-supported monophyletic group in our phylogenetic analyses and recognition of *H. subrufescens* as a separate species would render *H. aerostatisporum* paraphyletic (Fig. 2). The morphology of both is consistent, including the similarly sized globose to subglobose spores. For these reasons, we consider *H. subrufescens* a taxonomic synonym of *H. aerostatisporum*.

Hydnum canadense Niskanen & Liimat., Mycologia 110: in press (2018)

Figs 3G, 5I

Type. CANADA. Newfoundland and Labrador: Near Grand Falls, south of the Exploits River, west of Hwy 360, south of Hwy 1, along a gravel road beside Moccasin Lake (48.9030; -55.5580), in conifer-dominated forest, 9 Sep 2009, K. Liimatainen & T. Niskanen 09-006 (holotype H7043727, isotype K(M)248978, isotype NY).

Description. Pileus 12–25 mm wide, irregularly round to slightly reniform, convex to plano-convex, surface dry, glabrous, orange ("Zinc Orange" to "Xanthine Orange"), sometimes cracking in age near central depression; margin incurved and entire or slightly degraded. Spines 1–3 mm long, adnate, cream-colored, at times thick and somewhat flattened. Stipe 15–35 × 5–8 mm, central or eccentric, equal or widening at base, firm, smooth, white to cream, lightly staining ochre to medium brownish orange ("Mars Yellow" to "Orange Rufous") where handled. Context not observed. Odor and taste mild.

Basidiospores 7–*8*–9(9.5) µm × 7–*7.6*–9 µm, *Q*=1.00–*1.05*–1.11, (n=38/1), globose to subglobose, smooth, hyaline in KOH. Basidia 38–46 × 7.5–9.5 µm with 3–5 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 5–7 µm wide. Clamp connections present.

Distribution. Eastern North America – Newfoundland and Labrador (type, GenBank KX388681) and North Carolina.

Ecology. In conifer forest with *Abies fraseri*. August to September.

Specimen examined. UNITED STATES. North Carolina: Yancey County, Mount Mitchell State Park, in fir forest, 2000 m, 19 Aug 2016, R.A. Swenie RAS100 (TENN 073010).

Discussion. *Hydnum canadense* is only known from high-elevation and higher latitude conifer forests in North Carolina and eastern Canada. The North Carolina collection was found among several basidiomes of *H. umbilicatum*. *Hydnum canadense* can be distinguished from *H. umbilicatum* by the 3–5 sterigmata (versus 2–4 sterigmata in *H. umbilicatum*) and ITS sequence divergence. *Hydnum canadense* differs from the closely related *H. mulsicolor* by the association with conifers and larger spore size. Another closely related species, *H. submulsicolor*, is morphologically indistinguishable from *H. canadense* according to Niskanen et al. (2018), and ITS sequencing is likely necessary to reliably differentiate it from *H. canadense*. The spores of the North Carolina collection of *H. canadense* reported here are more globose with a lower

average *Q* value than that of the Newfoundland and Labrador collections reported by Niskanen et al. (2018).

***Hydnum mulsicolor* Liimat. & Niskanen, Mycologia 110: in press (2018)**
Figs 3H, 5J

Type. SLOVENIA. Velike Lašče (45.8500; 14.6000): In forest of *Picea abies*, *Fagus sylvatica*, and *Corylus avellana*, GIS 1336 (holotype LJF1057).

Description. Pileus 30–45 mm wide, round, convex when young, becoming plano-convex to funnel-shaped; surface dry, glabrous or matted-tomentose, bright orange to tan ("Zinc Orange" to "Ochraceous-Tawny") becoming subzonate towards margin, sometimes distinctly umbilicate at center; margin incurved at first, becoming decurved, wavy, and lightening in color. Spines 1–7mm long, decurrent, pinkish cream with white tips. Stipe 25–45 × 5–8 mm, central or eccentric, equal or enlarging downwards, texture firm, smooth, with aborted spines at stipe apex and some texturing below, cream white, sometimes with small white cottony patches, staining orange when handled, a dense mat of basal mycelium present at base. Context not observed. Odor mild or pleasant. Taste not distinctive.

Basidiospores 6.5–*7.7*–8.5 µm × (5.5)6–*7.1*–8.5 µm, *Q*=1.00–*1.08*–1.19(1.24) (n=46/3), subglobose, smooth, hyaline in KOH. Basidia 52–60 × 7.5–9.5 µm with 3–4(5) sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 µm wide. Clamp connections present.

Distribution. Eastern U.S. and Central Europe – Ohio, Virginia, North Carolina, Tennessee (GenBank AJ547885, AJ547868, AJ783969), and Slovenia (type), Switzerland (GenBank KU612545, KX086216).

Ecology. In deciduous or mixed forests with *Quercus*. July to September.

Other specimens examined. UNITED STATES. North Carolina: Blue Ridge Parkway near Little Switzerland, deciduous woodlot with *Quercus*, 1100 m, 19 Aug 2016, M. Hopping RAS105 (TENN 073015). Blue Ridge Parkway near Little Switzerland, in mixed woods, 1100 m, 19 Aug 2016, D. Boes RAS108 (TENN 073018). Buncombe County, Tanbark Ridge, growing singly in mature acidic cove forest with *Quercus* and *Acer*, 915 m, 4 Sep 2016, M. Hopping MH16006 (TENN 073550). Tennessee: Great Smoky Mountains National Park, Cherokee Orchard, Bullhead Trail, on soil in mixed forest, 1340 m, 16 Jul 2015, R.A. Swenie RAS023 (TENN 070321). Virginia: Shenandoah National Park, milepost 21, 1000 m, 9 Sep 2016, RAS120 (TENN 073023).

Discussion. *Hydnum mulsicolor* is the only species of *Hydnum* in eastern North America that is also known to occur in Europe based on ITS phylogenetic analysis. The basidiomes are small to medium-sized with strongly decurrent spines, and the pileus color ranges from strikingly orange to tan. Prominent basal mycelium is also present as a dense mat or as distinct rhizomorphs at the stipe base. In the eastern US, *H. mulsicolor* is often found with *Quercus* in mixed or hardwood forests. It is closely related to *H. submulsicolor* and *H. canadense*, both of which are known only from coniferous forests in eastern North America and have slightly larger spores than *H. mulsicolor* (Niskanen et al. 2018).

***Hydnum quebecense* Niskanen & Liimat., Mycologia 110: in press (2018)**
Fig. 5K

Type. CANADA. Québec: Saint-Donat (46.3000; -74.2000), in conifer-dominated forest (*Tsuga*, *Abies*, *Picea*, *Betula*, and *Populus*), 5 Sep 2010, anonymous, T. Niskanen 10-064 (holotype H7043948, isotype K(M)248983, isotype NY).

Description. Pileus 2–20 mm wide, round or sometimes irregularly so, convex, apex sometimes depressed or umbilicate, surface dry, tomentose or velutinous, tan orange-brown to warm reddish-brown; margin incurved, becoming decurved and wavy. Spines 1–2 mm long, adnate when young, subdecurrent with age, cream-white to peach. Stipe 15–45 × 3–10 mm, central, equal to subclavate, glabrous to minutely velutinous, cream white to very light buff orange, staining buff orange when handled. Context solid, cream to tan. Odor and taste mild.

Basidiospores 8–*8.4*–9.5 µm × 7–*7.8*–9 µm, *Q*=1.00–*1.09*–1.28 (n=19/2), globose to subglobose, smooth, hyaline in KOH. Basidia 40–51 × 7–8 µm with 2–3 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–8 µm wide. Clamp connections present.

Distribution. Eastern U.S. and eastern Canada – New York, Quebec (type, GenBank KX388662).

Ecology. In moss, especially *Sphagnum*, under *Fagus* and *Picea*. July.

Other specimens examined. UNITED STATES. New York: Hamilton County, Raquette Lake, Long Point, Blue Mountain Trail, in moss under *Fagus grandifolia*, 550 m, 25 Jul 2001, J. D'Apice 49JD (CORT 7367). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum*, 550 m, 22 Jul 1997, J. Guardino JG003 (CORT 7330). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum*, 550 m, 23 Jul 1988, C. Nelson CN9 (CORT 7365). Hamilton County, Raquette Lake, Silver Beach Bog, in *Sphagnum* under *Picea*, 550 m, 26 July 1993, K. Hodge KH7 (CORT 7322).

Discussion. This species is closely related to *H. umbilicatum* but is characterized by the apparent habitat preference for *Sphagnum* bogs. The holotype from Quebec (KX388662) was described among *Sphagnum* in association with conifers and northern hardwoods (mainly *Picea*, but also *Tsuga*, *Abies*, *Betula*, *Populus*).

***Hydnum* sp. AS30**

Description. Pileus 20 mm wide, round, umbilicate, deep buff. Stipe less than 10mm long, central.

Basidiospores 6.5–*7.2*–8 µm × 6–*6.9*–7.5 µm, *Q*=1.00–*1.05*–1.1 (n=12/1), globose to subglobose, smooth, hyaline in KOH. Basidia 44–48 × 8–10.5 µm with (2) 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, hyaline, mostly 5–7 µm wide. Clamp connections present.

Distribution. Eastern U.S. – New York.

Ecology. In *Sphagnum*, under *Tsuga* and *Larix*. July.

Specimen examined. UNITED STATES. New York: Hamilton County, Raquette Lake, Silver Beach Bog, *Sphagnum* substrate under *Tsuga*, *Larix*, 550 m, 28 Jul 1982, A. Sabol AS30 (CORT 007356).

Discussion. This species is known only from a single basidiome collected in New York. The description is drawn from the original collection notes. It is closely related to *H. subconnatum* but differs from it by the smaller spore size, shorter stipe, and association with *Sphagnum*. It is recorded from the same locality and habitat as *H. quebecense* but differs from that species by ITS sequence and smaller spores. We refrain from describing the taxon as new until confirmed by additional collections and sequence data.

***Hydnum subconnatum* Swenie & Matheny, sp. nov.**
MycoBank: MB825496
GenBank: MH379930
Figs 4A, 5L

Diagnosis. Closely related to *Hydnum oregonense* but differs from it by ITS sequence divergence and geographic distribution. *Hydnum subconnatum* is known only from the southeastern U.S.

Type. UNITED STATES. North Carolina: Yancey County, Carolina Hemlocks Recreation Area, picnic area (35.8057; -82.2047), on soil growing in fused cluster and singly with *Tsuga carolinensis*, *Quercus*, *Liriodendron*, 840 m, 29 Sep 2017, R.A. Swenie RAS235 (holotype: TENN 073064).

Etymology. *subconnatum* (L.), born together, in reference to the fused stipe bases of multiple basidiomes.

Description. Pileus 10–75 mm wide, more or less round, broadly convex to plane, surface usually dry but sometimes slightly hygrophanous, glabrous, occasionally with shallow cracks or pits in age, sometimes umbilicate or with central depression, peach orange (6C8–6B7) to reddish-brown ("Cinnamon-Rufous"); margin incurved and entire, becoming eroded or split and sometimes wavy in age. Spines 1–8 mm long, shortest near margin, adnate to subdecurrent, white to pale orange (5A4, 6A3). Stipe 15–60 × 5–20(30) mm, central or eccentric, equal or widening to bulbous base, sometimes with up to four basidiomes fused together at base, texture smooth, white to dull tan, bruising orange-brown (6B8–7D7 or "Ochraceous-Buff"). Context fleshy, white to dull cream-brown, staining not observed. Odor mild or slightly sweet. Taste mild.

Basidiospores 8.5–*8.9*–10 µm × 7.5–*8.5*–9.5(10) µm, *Q*= 1.00–*1.05*–1.14(1.20) (n=34/3), globose to subglobose, smooth, hyaline in KOH. Basidia 48–61 × 8.5–10.5 µm with 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–6 µm wide. Clamp connections present.

Distribution. Southeastern U.S. – North Carolina (type), Tennessee, and Georgia.

Ecology. In mixed woods with *Quercus*, *Pinus*, *Tsuga*, *Fagus*, *Betula*, *Carya*, *Liriodendron*. July to December.

Other specimens examined. UNITED STATES. Georgia: Chatham County, Wormsloe Plantation, in duff with *Quercus*, *Pinus*, 8 m, 26 Dec 1976, J.H. Restivo JHR40605 (TENN 040605). White County, Unicoi State Park, Unicoi to Helen Trail, solitary beside trail with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, J.E. Uehling RAS165 (TENN 073026). White County, Unicoi State Park, Unicoi to Helen Trail, solitary beside trail with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, R.A. Swenie RAS169 (TENN 073048). Tennessee: Great Smoky Mountains National Park, under *Pinus*, *Tsuga*, *Quercus*, *Acer*, Meig's Creek Trail, 580 m, 30 Sep 2004, E.B. Lickey TFB12311 (TENN 060359). Great Smoky Mountains National Park, Cades Cove, scattered in mixed forest with *Fagus*, *Quercus*, *Carya*, *Pinus*, 520 m, 23 Nov 2013, R.A. Walter RAW18 (TENN 073005). Great Smoky Mountains National Park, Schoolhouse Gap Trail, scattered beneath *Pinus*, *Tsuga*, *Quercus*, *Betula*, 550 m, 26 Oct 2013, K.E. Rewcastle KER016 (TENN 073006). Great Smoky Mountains National Park, trail to Look Rock fire tower, some caespitose and forming a ring among *Quercus* litter on trail, 800 m, 17 Nov 2009, E.E. Austin EEA171109-1 (073744). Great Smoky Mountains National Park, Trillium Gap Trail, 9 Jul 2017, B.P. Looney BPL931 (TENN 073028). Big Ridge State Park, Ghost House Loop Trail, scattered to caespitose under *Quercus*, *Fagus*, *Carya*, *Pinus virginiana*, 320 m, 9 Nov 2015, R.A. Swenie RAS053 (TENN 070846).

Discussion. *Hydnum subconnatum* is known from the southeastern U.S. in a range of low elevation mixed forests such as oak-pine or hemlock-pine mixed with oak and beech. All known specimens are reported under 1000 m elevation. Basidiomes can occur in caespitose clusters with the stipe bases and two or more pilei fused together. The pileus coloration is highly variable, however, ranging from deep orange to pale peach and fading to tan towards the margin, making this a difficult species to distinguish at a glance. *Hydnum caespitosum* Banning ex Peck (non *H. caespitosum* Valenti), described from Maryland, occurs "at roots of trees and near old stumps" and is much paler in coloration, depicted by Banning in her painting as a yellowish species. Furthermore, our examination of the holotype of *H. caespitosum* revealed that basidiospores are much smaller in that species than in *Hydnum subconnatum*. The new name *H. geminum* is proposed below for *H. caespitosum* Banning ex Peck.

Specimens of *H. subconnatum* form a monophyletic group with support values <70% (ML) and <0.95 (BI). ITS sequence variation is relatively low (<1%) among sampled specimens of *H. subconnatum*, but the clade is highly dissimilar (8% sequence divergence) from Mexican taxa (Genbank KR135344-KR135345) that form a well-supported sister lineage.

Hydnum cuspidatum **Swenie & Matheny, sp. nov.**
MycoBank: MB825497
GenBank: MH379944
Figs 4B, 5M

Diagnosis. Closely related to *Hydnum umbilicatum* but differs from it by ITS sequence divergence as well as more elliptic basidiospores. Known so far in the southeastern and

upper midwest United States. Differs from *H. aerostatisporum* by the smaller basidiomes and slightly larger basidiospores.

Type. UNITED STATES. Tennessee: Big South Fork National River & Recreation Area, John Litton Farm Trail (36.4960; -84.6700), on soil with *Quercus*, *Tsuga*, *Pinus*, 425 m, 29 Oct 2017, R.A. Swenie RAS246 (holotype: TENN 073068).

Etymology. *cuspidatum* (L.), tapering to a fine, sharp point, in reference to the spines.

Description. Pileus (11)15–50 mm wide, round to oval or irregular and reniform, convex when young, becoming plane or depressed, margin incurved and entire, becoming irregularly wavy or degraded; surface glabrous, sometimes floccose-scaly or scabrous near the umbilicus, dull orange to deep orange-brown (5A6–6B7–6D8, "Tawny" to "Mikado Brown"), olive-brown with KOH, at times faded in color towards the margin. Spines 1–8 mm long, shorter near the margin, adnate, pale buff, cream-orange, or tan-orange (7.5YR 8/4–8/6 or 5A3–A5). Stipe 15–50 × 3–10(12) mm, central or eccentric, equal or enlarged towards base, sometimes curved, texture smooth, buff to peach-brown (5A2–A3 to 5B6–C6), sometimes with hazy thin white patches especially towards apex, staining only very slightly light brown (10YR 7/4–7/6 or 5A–B7); cottony white basal mycelium often present. Context often hollow, flesh white to cream. Odor not distinctive or sweet and fruity. Taste not distinctive.

Basidiospores (7)7.5–*8.5*–9.5(10.0) µm × 6–*7.2*–8.5 µm, *Q*=1.01–*1.18*–1.38(1.52) (n=99/6), subglobose to irregularly rounded-elliptic, smooth, thin-walled, hyaline in KOH. Basidia 39–56 × 7–9 µm with 3–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–7 µm wide. Clamp connections present.

Distribution. Eastern U.S. – Michigan (KU612606, MG162266), North Carolina, Tennessee (type), and Georgia.

Ecology. In deciduous or mixed woods with *Quercus*, *Pinus*, *Tsuga, Fagus, Betula, Carya, Carpinus, Picea*. June to October.

Other specimens examined. UNITED STATES. Georgia: White County, Unicoi State Park, Unicoi to Helen Trail, solitary with *Pinus* and mixed hardwoods, 460 m, 16 Jul 2017, R.A. Swenie RAS167 (TENN 073046). North Carolina: Blue Ridge Parkway near Little Switzerland, deciduous woodlot with *Quercus* and *Rhododendron maximum*, 1050 m, 19 Aug 2016, H. Hopping RAS106 (TENN 073016). McDowell County, Armstrong Creek, mixed woods, 450 m, 19 Aug 2016, J. Roberts RAS098 (TENN 073008). Great Smoky Mountains National Park, Big Creek Campground, under *Tsuga*, *Carpinus*, *Betula*, *Fagus*, *Quercus*, 525 m, 29 Jul 2017, B.P. Looney BPL989 (TENN 073033). Great Smoky Mountains National Park, Cataloochee Divide Trail, solitary with *Quercus*, *Betula*, *Tsuga*, 1525 m, 9 Sep 2017, RAS218 (TENN 073059). Great Smoky Mountains National Park, Balsam Mountain Road, 1525 m, 15 Aug 2005, E.B. Lickey TFB12725 (TENN 073033). Great Smoky Mountains National Park, Heintooga Round Bottom Road, scattered on embankment with *Betula*, *Picea*, 1525 m, 17 Aug 2017, R.A. Swenie RAS205 (TENN 073056). Tennessee: Great Smoky Mountains National Park, Tremont, Buckeye Trail, with *Tsuga*, *Betula*, 490 m, 23 Jun 2017, R.A. Swenie RAS151 (TENN 073038). Great Smoky Mountains National Park, Schoolhouse Gap Trail, scattered on embankment with *Quercus*, *Betula*, *Pinus*, 550 m, 18 Jul

2017, R.A. Swenie RAS160 (TENN 073043). Anderson County, Oak Ridge, UT Arboretum, on soil in forest with *Quercus*, *Carya*, 300 m, 26 Oct 2009, J. Heggan JRH102609-3 (TENN 071998).

Discussion. *Hydnum cuspidatum* is closely related to *H. umbilicatum* and is difficult to distinguish by morphology alone. As in *H. umbilicatum*, there is high variability in basidiome stature and color. The basidiospores of *H. cuspidatum* have a slightly higher *Q* value on average (1.18) than *H. umbilicatum* (1.06), otherwise phylogenetic analysis of ITS data is needed to distinguish the two species reliably. *Hydnum cuspidatum* occurs in deciduous or mixed forests in the midwest and southeastern U.S., where it can co-occur with *H. umbilicatum*.

The ITS sequences of *H. cuspidatum* have relatively high intraspecific variation (up to 3%) compared to other North American *Hydnum* species. The lack of distinguishing morphological or ecological features deters further differentiation into separate taxa at this time.

Hydnum umbilicatum **Peck, Ann. Rep. N.Y. St. Mus. 54: 953 (1902)**
MycoBank Epitypification: MBT381861
GenBank: MH379890
Figs 5C, D, 6N, O

Type. UNITED STATES. New York: Rensselaer County, Sandlake, ground in thin woods, September, ca. 1901, C.H. Peck (holotype: NYS-F-3258). **Epitype.** UNITED STATES. New York: Cortland County, Lime Hollow Nature Center Tunison Aquatic Lab (42.5578; -76.2486), on humus in wet, boggy area with *Tsuga*, *Betula alleghaniensis*, 27 Aug 2014, T.J. Baroni 10651TJB (CORT 012241, epitype here designated).

Description. Pileus 15–70 mm wide, round, conico-campanulate to irregularly convex, disc shallowly depressed to umbilicate; surface matt, glabrous or felty-fibrillose, orange-cream to orange-brown (5C6–8); margin entire and incurved when young to undulating in age, often paler in color than the rest of the pileus. Spines 1–8 mm long, aculeate, adnexed, fleshy pinkish to light orange (5A2–3). Stipe 20–80 × 4–15 mm, central or eccentric, equal to slightly enlarged downwards, glabrous or densely matted with fluffy fibrillose; white to peachy-pallid buff, staining ochre to medium brownish orange ("Mars Yellow" to "Orange Rufous"). Context white. Odor mild or pleasant. Taste mild, sometimes with nutty aftertaste.

Basidiospores 7.5–*8.4*–9.5 µm × 7–*8*–9 µm, *Q*=1.00–*1.06*–1.18 (n=97/5), globose to subglobose, smooth, thin-walled, hyaline in KOH. Basidia 43–52 × 7.5–10 µm with (1)2–4 sterigmata. Pileipellis an interwoven cutis, hyphae smooth, cylindrical, thin-walled, mostly 4–8 µm wide. Clamp connections present.

Distribution. Eastern North America – Michigan, Massachusetts, New York (type), Tennessee, North Carolina, Newfoundland and Labrador (GenBank KX388676), and Quebec (GenBank KX388675).

Ecology. In coniferous or mixed woods with *Tsuga*, *Pinus*, *Abies*, *Quercus*, *Betula*, *Fagus*. July to November.

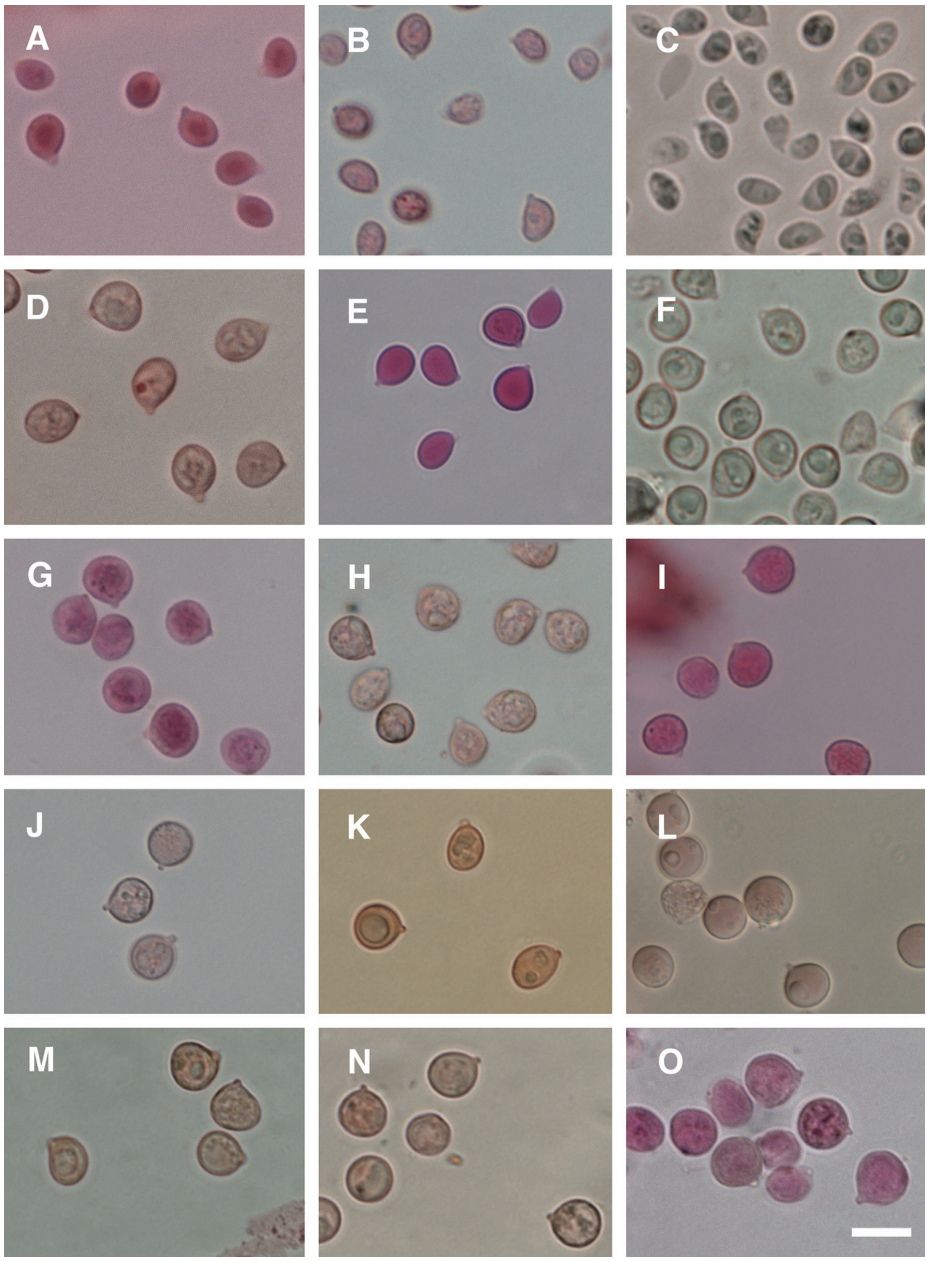

Figure 6. Basidiospores of *Hydnum* species. **A** *H. albidum* 9623TJB (CORT 014489) **B** *H. alboaurantiacum* TFB9833 (TENN 058812) **C** *H. albomagnum* PBM2512 (TENN 066858) **D** *H. subtilior* PBM3868 (TENN 067482) **E** *H. subolympicum* AJR14 (TENN 073004) **F** *H. vagabundum* 10782TJB (CORT 014461) **G** *H. ferruginescens* MH16005 (TENN 073549, holotype) **H** *H. aerostatisporum* MK9181403 **I** *H. canadense* RAS100 (TENN 073010) **J** *H. mulsicolor* MH16006 (TENN 073550) **K** *H. quebecense* JG003 (CORT 007330) **L** *H. subconnatum* RAS053 (TENN 070846) **M** *H. cuspidatum* BPL989 (TENN 073033) **N** *H. umbilicatum* 10651TJB (CORT 012241, epitype) **O** *H. umbilicatum* Peck (NYS-F-3258, holotype). Scale bar: 10 µm.

Other specimens examined. UNITED STATES. Massachusetts: Worcester County, Rutland State Park, in arcs scattered singly or gregarious with *Quercus*, *Pinus strobus*, 260 m, 1 Nov 2003, P.B. Matheny PBM2511 (TENN 066875). Michigan: Marquette County, Big Bay, Alder Creek, 240 m, 2 Sep 1971, R.H. Petersen TFB36346 (TENN 036346). New York: Tompkins County, Ridgewood Reserve, 305 m, 13 Sep 2007, O. Akinyemi OA14 (CORT 008175). Tompkins County, Eames Bog, 325 m, 22 Sep 2011, B. Demo BD14 (CORT 007319). North Carolina: Transylvania County, Pisgah National Forest, Yellow Gap Road, under *Quercus*, 850 m, 18 Jul 2000, Jason TFB9766 (TENN 058667). Blue Ridge Parkway near Little Switzerland, coniferous woodlot, 1000 m, 19 Aug 2016, N. Byers RAS103 (TENN 073013). Yancey County, Carolina Hemlocks Recreation Area, picnic area under *Tsuga carolinensis*, *Quercus*, possibly *Betula* or *Carpinus*, 840 m, 29 Sep 2017, R.A. Swenie RAS234 (TENN 058667). Yancey County, Mount Mitchell State Park, Balsam Mountain trail, under *Abies fraseri*, 1920 m, 19 Aug 2016, R.A. Swenie RAS099 (TENN 073009). Yancey County, Mount Mitchell State Park, Balsam Mountain trail, under *Abies fraseri*, 1920 m, 19 Aug 2016, R.A. Swenie RAS101 (TENN 073011). Yancey County, Mount Mitchell State Park, Balsam Mountain trail, under *Abies fraseri*, 1920 m, 29 Sep 2017, R.A. Swenie RAS238 (TENN 073066). McDowell County, Armstrong Creek trail, under *Quercus*, *Pinus*, *Liriodendron,* 450 m, 30 Sept 2017, R.A. Swenie RAS239 (TENN 073067). Great Smoky Mountains National Park, Big Creek, Baxter Creek Trail, with *Tsuga*, *Pinus*, *Quercus*, 936 m, 25 Aug 2004, E.B. Lickey TFB12039 (TENN 060288). Tennessee: Great Smoky Mountains National Park, Maddron Bald Trail, scattered on soil and hardwood leaf litter with *Tsuga*, *Quercus*, *Fagus*, *Pinus*, 575 m, 29 Aug 2013, S.A. Trudell SAT1324109 (TENN 068871). Great Smoky Mountains National Park, Greenbrier, Injun Creek Trail, under *Tsuga*, 450 m, 18 Nov 2004, E.B. Lickey TFB12369 (TENN 060445). Great Smoky Mountains National Park, Cosby, Gabes Mountain Trail, with *Tsuga* and mixed hardwoods, 685 m, 16 Oct 2006, E.B. Lickey TFB13482 (TENN 061745). Big South Fork National River & Recreation Area, John Litton Farm Trail, scattered at base of dead *Tsuga*, in mixed woods with *Tsuga*, *Quercus*, *Pinus*, 425 m, 29 Oct 2017, R.A. Swenie RAS247 (TENN 073181).

Discussion. *Hydnum umbilicatum* is widespread in eastern North America at low and high elevations, mostly in conifer-dominated forests or mixed woods including conifers. The macromorphology can vary dramatically among basidiomes with some specimens displaying the namesake umbilicate pileus while others do not. The presence of an umbilicus is not a unifying taxonomic feature as its presence has been observed in several distantly related clades of *Hydnum*. Peck (1901) included a color plate illustration with his description depicting basidiomes with thin, convex, umbilicate pilei and slender stipes that are slightly longer than the diameter of the pileus. Unfortunately, we were unable to obtain DNA sequences from the type collection. However, in comparison to other closely related clades, specimens of *H. umbilicatum* have slightly larger globose to subglobose basidiospores averaging 8.4 × 8 µm with average *Q* values below 1.08, which closely matches our spore measurements of the holotype. In addition, Peck mentioned that "sometimes a definite line separates the paler margin from the more highly colored center of the pileus", a trait that has been observed in several of the specimens of this species that cluster in a single ITS lineage.

Species from eastern North America – Incertae sedis

***Hydnum geminum* Swenie & Matheny, nom. nov.**
MycoBank MB825498
Fig. 7

≡ *Hydnum caespitosum* Banning ex Peck, Rep. N.Y. St. Mus. 44: 74 (1891), non Valenti (1868)

Type. UNITED STATES. Maryland: Carroll County, in clusters at the roots of trees and near old stumps, Aug-Sep, ca. 1880, M.E. Banning (holotype: NYS-F-3506).

Etymology. *geminum* (L.), twin, in reference to the clustered habit

Description. Pileus up to 40 mm wide, subconfluent, convex to expanded or subplane, subregular; surface appressed-fibrous, pale ochre, yellow, or dark flesh-colored. Spines short (<3 mm long), conical, acute, decurrent, pale ochre or light flesh color. Stipe up to 60 × 10 mm, united at the base, subcylindrical, subflexuous, floccose above, subglabrous below, whitish, staining yellow where bruised, solid. Context fleshy, white, turning yellow where cut. Taste mild.

Basidiospores 6–*6.4*–7 µm × 4.5–*5.2*–6 µm, *Q*=1.09–*1.24*–1.36 (n=12/1), broadly elliptic to subglobose, smooth, thin-walled, hyaline in KOH. Basidia not reviving, with 4–5 sterigmata. Pileipellis not observed. Clamp connections present.

Distribution. Eastern U.S. – Maryland (type).

Ecology. In clusters at the roots of trees and near old stumps, August to September.

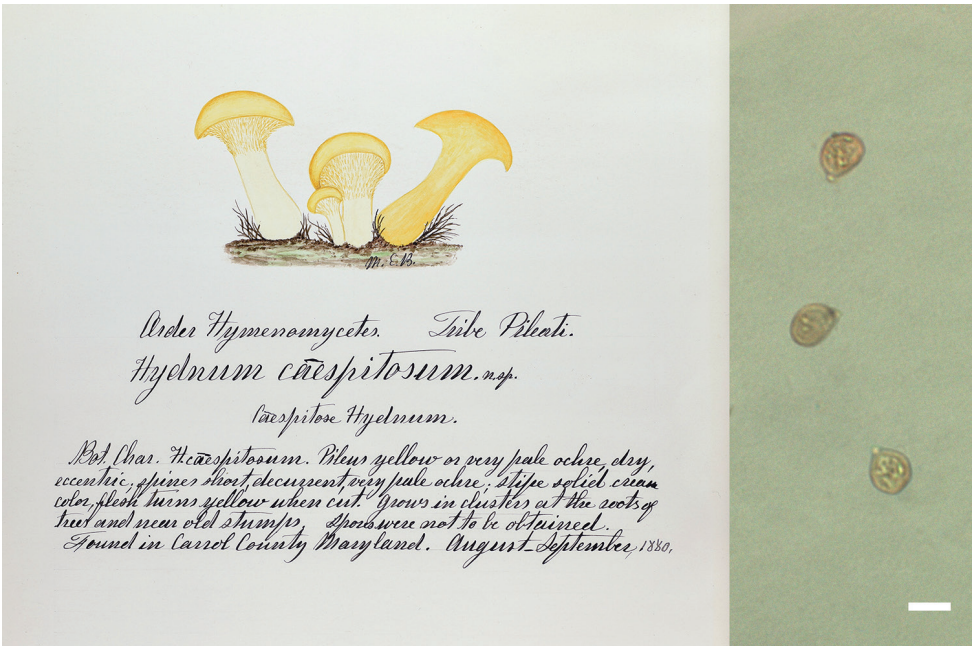

Figure 7. Holotype and basidiospores of *Hydnum caespitosum*. Scale bar: 5 µm.

Discussion. The new binomial *H. geminum* is introduced to replace the illegitimate name *H. caespitosum* Banning ex Peck, which is a later homonym of *H. caespitosum* Valenti. The gross morphological description here is reproduced from Banker (1906) after reformatting for style, and measurements appear to be based on dried specimens. Peck's protologue, Banning's painting, and Banker's notes depict a species best characterized by the overall yellowish color, short decurrent spines, flavescent or yellowing flesh, mild taste, and broadly elliptic to subglobose basidiospores that are mostly 6.5 × 5 µm in size. Although specimens of *H. subconnatum* (described above) may share the similar caespitose or clustered habit, it differs from *H. geminum* by the peach-orange to dark orange-brown pileus, longer non-decurrent spines, and a stipe that bruises orange-brown, not yellow. In addition, the basidiospores of *H. subconnatum* are larger than in *H. geminum* – 8.5–9.5 × 7.5–9 µm. Basidiomes of *H. subtilior* sometimes have an overall pale yellow tone and stain when bruised or cut in half, but basidiospores are smaller in *H. geminum*, and the overall basidiome stature of the holotype appears much stouter than in the generally slender *H. subtilior*.

Upon examining the holotype of *H. caespitosum*, we found that basidiomes had very short spines (<1 mm) with very few spores and basidia. This aligns with Peck's notes indicating he did not obtain spores and suggests the holotype consists of immature basidiomes.

We have not yet recorded *H. geminum* in eastern North America. Banker (1906) refers to a collection made by Earle from Connecticut now housed at NCU (NCU-F-0012251). We have not re-examined this collection, but Banker describes it as somewhat darker than the type.

Key to species of *Hydnum* in eastern North America

Note that the five species in couplet 17 are most reliably distinguished by phylogenetic analysis of ITS sequences.

1	Pileus white, cream, yellow, peach, pale orange, or light tan before handling	**2**
–	Pileus darker than above, orange to tawny brown before handling	**11**
2	Pileus mostly pale white to off-white or cream	**3**
–	Pileus mostly with tones of yellow, peach, pale orange, or light tan	**7**
3	Pileus small to medium-sized, <60 mm wide at maturity	**4**
–	Pileus larger than above, >60 mm wide at maturity	**5**
4	Basidiomes staining *bright orange* within two minutes of handling	***H. alboaurantiacum***
–	Basidiomes remaining white where handled or slowly staining orange-brown to ochre-brown after several minutes to hours	***H. albidum***
5	Pileus with adhering litter debris	***H. albomagnum***
–	Pileus free of adhering debris	**6**
6	Basidiomes staining bright orange where handled, spores <7 µm long	***H. alboaurantiacum***
–	Basidiomes not staining bright orange where handled, spores mostly >7 µm long	***H. vagabundum***

7 Stipe >20 mm wide.....***H. subolympicum***
– Stipe <20 mm wide.....**8**
8 Basidiospores mostly 4–6 × 3–5 µm.....***H. alboaurantiacum***
– Basidiospores larger than above, mostly 6–9 × 5–7.5 µm **9**
9 Basidiospores mostly 6–7 × 5–6 µm.....***H. geminum***
– Basidiospores mostly 7–9 × 5.5–7.5 µm.....**10**
10 Pileus light cream yellow to cream orange buff, known only from the southeastern U.S. and Mexico in deciduous or mixed woods.....***H. subtilior***
– Pileus pale orange, known only from eastern Canada and the western US in coniferous or mixed woods .....***H. washingtonianum***
11 Basidiomes caespitose .....***H. subconnatum***
– Basidiomes solitary or scattered.....**12**
12 Spines decurrent .....***H. mulsicolor***
– Spines subdecurrent or adnate **13**
13 Basidiospores mostly 5.5–7.5 × 5–7.5 µm.....***H. ferruginescens***
– Basidiospores larger than above, 6.5–10 × 6–9.5(10) µm **14**
14 In *Sphagnum* in conifer-dominated woods **15**
– On soil in deciduous, mixed, or coniferous woods **16**
15 Basidiospores 6.5–8 × 6–7.5 µm .....***H. sp. AS30***
– Basidiospores larger than above, 8–9.5 × 7–9 µm..... ***H. quebecense***
16 Basidiospores 8.5–10 × 7.5–9.5 µm, known only from the southeastern U.S. in mixed hardwoods under 900 m elevation .....***H. subconnatum***
– Basidiospores smaller than above, 7–9.5(10) × 6–9 µm, widespread or known only from northeastern North America in coniferous or mixed woods at various elevations.....**17**
17 Known from mixed and hardwoods at all elevations, common and widespread in eastern U.S. (Gulf coast, southeast, midwest)..***H. aerostatisporum***
– Known only from coniferous woods at low elevation in Quebec.....
..... ***H. submulsicolor***
– Known only from coniferous woods at low elevation in northeastern Canada and high elevation in southeastern U.S. ..... ***H. canadense***
– Known from coniferous, mixed forests, and hardwoods at various elevations in midwest and southeastern U.S. .....***H. cuspidatum***
– Known from coniferous and mixed woods at all elevations, common and widespread in eastern North America (southeast, northeast, midwest) .....
.....***H. umbilicatum***

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Supplementary material 1

Specimen data for sequences produced in this study

Authors: Rachel A. Swenie, Timothy J. Baroni, P. Brandon Matheny

Data type: specimen data

Link: https://doi.org/10.3897/mycokeys.42.27369.suppl1

Supplementary material 2

BI phylogeny of global species of *Hydnum* shown as a rectangular tree

Authors: Rachel A. Swenie, Timothy J. Baroni, P. Brandon Matheny

Data type: phylogeny data

Explanation note: Posterior probabilities are shown above branches.

Link: https://doi.org/10.3897/mycokeys.42.27369.suppl2

MycoKeys 42: 73–88 (2018)
doi: 10.3897/mycokeys.42.26118
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Detection of arbuscular mycorrhizal fungi associated with pecan (*Carya illinoinensis*) trees by molecular and morphological approaches

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Abstract

Arbuscular mycorrhizal (AM) fungal community associated with pecan (*Carya illinoinensis*) roots and rhizospheric soils was assessed by spore isolation and morphological characterisation and by pyrosequencing of AM molecular markers. The AM fungal community associated with pecan growing in the field, was always more diverse than that associated with pecan growing in containers. This was not observed when AM richness was studied, suggesting that soil disturbance by a reduction in host plant richness leads to a less equitable distribution of AM fungal species, in contrast to natural soils. The chosen primers (AMV4.5F/AMDGR) for pyrosequencing showed high AM fungal specificity. Based on 97% sequence similarity, 49 operational taxonomic units (MOTUs) were obtained and, amongst these, 41 MOTUs corresponded to the *Glomeromycota phylum*. The number of obtained AM sequences ranged from 2164, associated with field samples, to 5572 obtained from pecan trap pot culture samples, defining 30 and 29 MOTUs, respectively. Richness estimated by conventional species identification was 6 and 9 AM fungal species in soil and pot samples, respectively. *Claroideoglomus lamellosum*, *Funneliformis mosseae* and *Entrophospora infrequens* were the only taxa detected using both techniques. Predominant sequences in the pecan rhizosphere samples, such as *Rhizoglomus irregulare* and other less abundant (*Dominikia iranica*, *Dominikia indica*, *Sclerocystis sinuosa*, *Paraglomus laccatum*), were detected only by pyrosequencing. Detection of AM fungal species based on spore morphology, in combination with molecular approaches, provides a more comprehensive estimate of fungal community composition.

Keywords
Carya illinoinensis; arbuscular mycorrhizal fungi; spore traits; pyrosequencing; biodiversity.

Introduction

Carya illinoinensis (Wangenh.) K. Koch (pecan) is considered the most valuable nut-production species of North America and it is currently an expanding crop with great potential. In Argentina, pecan nut production is increasing and has been promoted by many agricultural producers. The existence of thousands of productive hectares between 1 to 50 years old has been estimated (Madero 2013). Since 2005, new suitable cultivars have been introduced, expanding nut production to different geographical regions of Argentina.

Pecan trees have been reported to be associated with ectomycorrhizal (ECM) fungi (Benucci et al. 2012; Bonito et al. 2011; Tarango et al. 2004). However, arbuscular mycorrhizal (AM) fungi were also observed (Brundrett et al. 1990; Muñoz-Márquez et al. 2009; Taber et al. 1982). Mycorrhizal associations in pecan trees may potentially be a key factor for successful acclimatisation of propagated plants in nurseries and in the successful transplanting of trees to field. At both stages, pecan plants are exposed to nutrient and water stressful conditions (Tarango et al. 2004).

The association amongst pecan trees and AM fungal species is still unknown for Argentinian cultivars. Generally, there is limited information concerning the establishment of AM associations with pecan in nature. Conventional (based on spores traits) and molecular methodologies are used for the characterisation of AM fungal communities. It was shown, by molecular analysis, that the ecosystem is more important for the determination of AM fungal community composition than the host species identity (Opik et al. 2006). Moreover, the second-generation sequencing technologies have been increasingly considered as useful tools for identifying AM fungi in environmental samples. In this context, recent metagenomic studies, based on second generation sequencing technologies, have provided new approaches to study microbial community composition in a wide range of environments (Unterseher et al. 2011). Nevertheless, it has been demonstrated that the new sequencing methods can only provide important information when combined with conventional studies on individual microorganisms (Baldrian et al. 2011, Colombo et al. 2014).

The objective of our study was to document the establishment of AM symbiosis in pecan trees growing in a non-native region. Our goal was to characterise, by conventional and molecular species identification, the AM fungal community in soils of an experimental pecan orchard. Complementarily, pecan seedlings were cultured in pots as 'trap-plants' to record AM fungal species composition when pecan is the only plant available. Finally, this work seeks to contribute to the existing knowledge of the potential of the pyrosequencing method for the study of AM fungal biodiversity.

Material and methods

Sampling and experimental design

Sampling was conducted in the *Estación Experimental Agropecuaria* (EEA) '*Delta del Paraná*' of *Instituto Nacional de Tecnología Agropecuaria* (INTA) in Buenos Aires province, Argentina. Soils of the EEA INTA *Delta* (34°10'S, 58°51'W) are in a deltaic plain with clear fluvial influence where nut production with pecan trees began 40 years ago. The climate is temperate humid with an annual average temperature of 17°C and an annual precipitation of 900–1000mm (INTA 1989). The soil texture was classified as silty clay loam, with pH 4.5, total C 4.48%, organic matter 9.96%, total N 0.3%, extractable P 0.29 meq.100 g^{-1}; K 0.47, Ca 9.8, Mg 5.6 and Na 0.47 meq.100 g^{-1}. The herbaceous vegetation that appeared under the trees canopy was periodically removed.

In August 2014, twenty pecan trees within the EEA-INTA *Delta del Paraná* were sampled, across an area of approximately 3 ha. Three soil cores of 250 ml (including pecan roots, AM spores and external mycelium) were taken under the canopy of each tree at 20 cm depth, placed in polypropylene bags and stored at 4 °C until processed. All soil cores (sub-samples) from the field were mixed to produce a single complex-sample (T0). This sample was subsequently divided into two parts destined for: i) the molecular and conventional characterisation of AM fungal community; and ii) the establishment of trap cultures in pots with pecan seedlings. Pecan seeds were also collected in the EEA-INTA *Delta del Paraná* during the autumn and stored at 4 °C until use. For stratification, seeds were soaked in tap water, placed in moist perlite and stored at 4 °C for 30 days.

In order to detect the AM fungi colonising pecan roots at sampling time, a microcosm assay was conducted in a greenhouse using the field sample (T0), as AM inoculum. In September, five 10 litre plastic containers were filled with a sterile mix of perlite-vermiculite (1:1) and 400 g of T0. Pecan seeds were surface-disinfected (immersed in 5% sodium hypochlorite solution for 20 min and rinsed with sterile water) and pre-germinated. One seed was placed in each pot. Pecan seedlings were grown under natural light and temperature, watered when necessary and irrigated with 50 ml of Hewitt (1966) nutritive solution without P (to avoid influencing mycorrhization) once a month during 11 months. At the end of the experiment, three sub-samples of the whole root system and rhizospheric soil were removed from each pecan plant and mixed to produce a complex-sample (T1) used for the molecular and morphological characterisation of AM fungal community.

A part of the pecan roots, collected from the field (23 subsamples) and from trap cultures (5 subsamples), were used to evaluate the AM fungal colonisation by observation under a binocular microscope (1000× magnification). Roots were stained by a modified Phillips and Hayman (1970) method: they were bleached with hydrogen peroxide, cleared with KOH (10% w/v, 15 min, 90 °C) and stained with trypan blue in lactic acid (0.02%, 10 min, 90 °C). Intraradical colonisation was quantified by

examination of 50 randomly selected root pieces (1 cm length). Frequency (*F%*) of mycorrhizal colonisation was calculated as the percentage of root segments containing hyphae, arbuscules or vesicles (Declerck et al. 2004). Photos were taken under an Olympus BX51 microscope coupled to an Infinity 1 digital camera.

AM fungal spore isolation and identification

Spores were recovered by successive wet sieving and decanting of soils and collected with a micropipette under a stereomicroscope. In order to ensure that all AM fungal species were sampled, a species accumulation curve was constructed in T0 and T1 as: number of new AM fungal species observed vs. weight of sampled soil. Five grammes of soil were added at each observation opportunity until no new species were found.

Spores characterisation was performed by mounting them in polyvinyl alcohol-lactic acid-glycerol (PVLG) and a mixture of PVLG-Melzer reagent and examining with a binocular microscope (1000× magnification). AM fungi were identified to species, whenever possible, based on morphological characters and subcellular structure of spores with the descriptions available at Professor Blaszkowski web page (http://www.zor.zut.edu.pl/Glomeromycota/) and at Błaszkowski (2012). Taxonomic assignment was performed according to the MycoBank database (http://www.mycobank.org/).

Relative abundance (RA%) of each AM species (calculated as: the number of spores of a particular AM species/the total number of identified AM spores) was plotted in rank-abundance diagrams; AM species were ranked from the most to the least abundant.

DNA extraction and pyrosequencing

Six metagenomic DNA isolations were carried out from T0 and six from T1 soil samples with the Mo Bio Power Soil DNA isolation kits (Mo Bio Laboratories, INC., Carlsbad, CA, USA) following manufacturer's protocol. Given the low DNA yields obtained for AM fungi and in order to increase the total AM fungal DNA isolated, all the AM spores and fine pecan roots from 100 g of T0 and 100 g of T1 soil samples (dry weight) were manually collected under a stereomicroscope for DNA isolation with the same commercial kit. All DNA samples from T0 were pooled together as well as those from T1. It has been previously demonstrated that composite samples could provide more accurate surveys than single samples due to the patchy distribution of AM fungi in soils (Xu et al. 2012).

Glomeromycota sequences of the small subunits (SSU) region of ribosomal DNA were amplified using the AMV4.5F and AMDGR primers. These primers were chosen because of their high AM fungal specificity (Lin et al. 2012; Lumini et al. 2009) with the aim of quantifying the AM fungal community by amplicon sequencing on a 454 Life Sciences Genome Sequencer FLX System (454 GS FLX) and Titanium chemistry (Roche Applied Science). Oligonucleotides were specifically designed for pyrosequencing with the 454 GS FLX Titanium. Amplicon Fusion Primers contain a directional 454 GS FLX Titanium Primer A or B sequence (in bold letters) which includes a four-

base library 'key' sequence (underlined) at the 5-prime portion of the oligonucleotide, in addition to the template-specific sequence at the 3-primer end. A Multiplex Identifier (MID) sequence or 'barcoding' was added to the reverse primer (in brackets) between the B primer and the template-specific sequences in order to sequence multiple samples in a single run. These sequences allowed automated software identification of each sample. Forward (Primer A – Key):

5'-**CGTATCGCCTCCCTCGCGCCA<u>TCAG</u>**AAGCTCGTAGTTGAATTTCG-3'

Reverse (Primer B – Key):

5'-**CTATGCGCCTTGCCAGCCCGC<u>TCAG</u>**(MID10bp)CCCAACTATCCCTATTAATCAT-3'.

PCR amplification was undertaken on a FastStart High Fidelity PCR system (Roche Applied Science, Mannheim, Germany) following the manufacturer's instructions. The PCR conditions were 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 57 °C for 45 s and 72 °C for 60 s and a final elongation step at 72 °C for 4 min. The reactions were purified and the pyrosequencing run was carried out on one quarter of the sequencing plate on a 454 GS FLX at the Instituto de Agrobiotecnología de Rosario (INDEAR) following the amplicon sequencing protocol provided by the manufacturer.

Pyrosequencing data analyses

Sequence data were quality controlled and de-noised with the ampliconnoise.py script of Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010). This script also eliminated chimeras. Sequences were clustered into molecular operational taxonomic units (MOTUs) using the pick_MOTUs.py script (QIIME) according to the 97% sequence similarity. This value was chosen in accordance with the conventional definition of microbial species (Konstantinidis et al. 2007). Non-AM fungal sequences were subsequently removed from the data sets (Lekberg et al. 2012) and molecular singletons were not considered in the analyses in order to avoid overestimation of species richness (Unterseher et al. 2011) The most abundant sequences from each MOTU were selected as representative and species identification was assigned comparing MOTUs sequences against the MaarjAM database (http://maarjam.botany.ut.ee/) and the GenBank database (http://www.ncbi.nlm.nih.gov). DNA similarity was performed using BLAST servers and only sequences with coverage and similarity values higher than 98% (resulting in e^- values close to zero) were considered. Representative MOTUs sequences, analysed in our study, have been deposited in The Sequence Read Archive (SRA) under the accession number: SRA058132.

Relative abundance (RA%) of each AM MOTUs (calculated as: number of sequences of an AM MOTU/the total number of identified MOTUs) was plotted in

rank-abundance diagrams; AM MOTUs were ranked from most to least commonly collected according to their abundance in the samples.

A rarefaction curve was constructed to assess sampling efficiency: the number of new AM fungal MOTUs observed vs. the number of sequences read. To calculate AM molecular richness within T0 and T1 samples, observed species (OS) and Chao 1 richness estimator were performed. Curve analyses were constructed with QIIME by randomly selecting a series of subsets from libraries in different sizes; this procedure was replicated by the programme ten times for each subset sample.

Results

Morphological biodiversity of AM fungi associated with pecan trees

After sampling 75 g of both pooled soils (T0 and T1), new AM fungal species were not observed (Fig.1a) indicating that all AM fungal species were already detected. Based on morphological taxonomic determinations, the AM fungal richness was 6 species in T0 and 9 in T1. Several differences in the AM community structure were found amongst field soil samples and one year old trap pot culture samples: rank-abundance species diagrams (Figs 2a,b) showed that two thirds of the identified spores at T0 belonged to *Claroideoglomus lamellosum* (Dalpé, Koske & Tews) C. Walker & A. Schüssler (37.5% of spores) and *Rhizoglomus microaggregatum* (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl (31%), followed by *Funneliformis coronatum* (Giovann.) C. Walker & A. Schüssler (12.5%). Spores of *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid., *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler and *Gigaspora margarita* W.N. Becker & I.R. Hall were also detected (6.25% for each species) in pecan rhizospheric soils. The number of isolated spores at T1 was nearly nine times higher than at T0. In these samples, *C. lamellosum* was also the dominant AM fungal species (49% of spores), followed by *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler (34%), which was not detected in T0 samples and *F. mosseae* (6%), *E. infrequens*, *Cetraspora pellucida* (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd., *R. microaggregatum*, *Diversispora eburnea* (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüssler, *Fuscutata rubra* (Stürmer & J.B. Morton) Oehl, F.A. Souza & Sieverd. and *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl were also identified with frequencies under 5%.

Molecular biodiversity of AM fungi associated with pecan trees

Based on 97% sequence similarity, a total of 49 MOTUs were obtained, 41 of them belonging to *Glomeromycota phylum*, proving the *Glomeromycota* specificity of the selected primers, since the majority of detected sequences corresponded to this phylum (98.5% and 94.8% in T0 and T1, respectively), followed by the *Basidiomycota* fungi *Piriformospora indica* (0.73% and 4.85% of sequences in T0 and T1, respectively) and

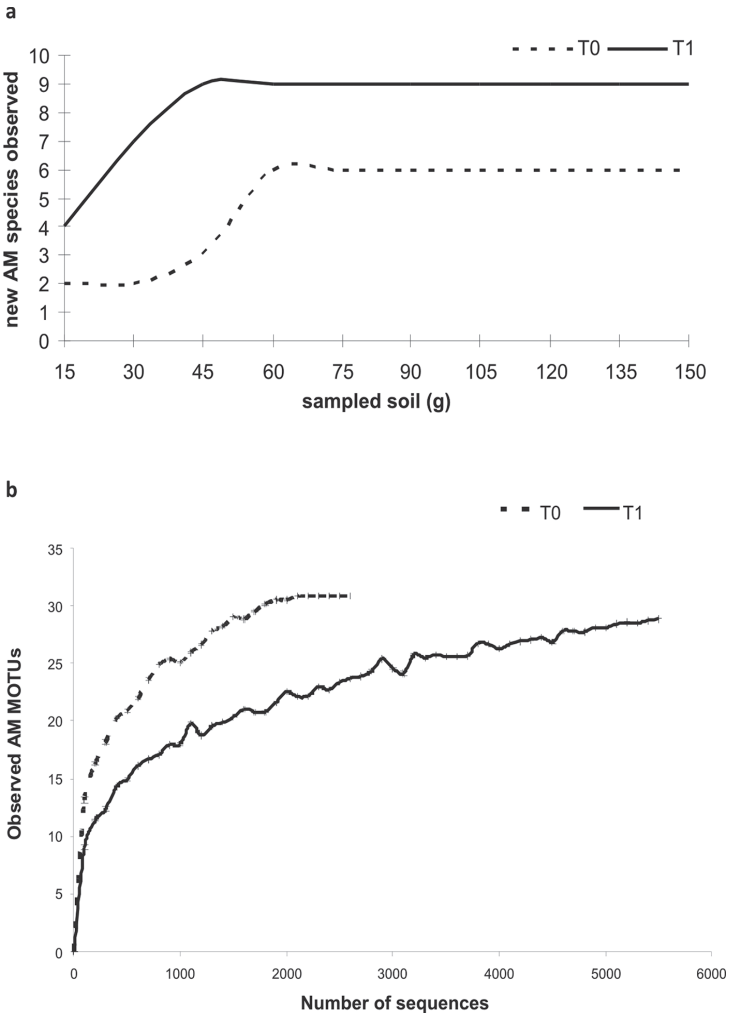

Figure 1. Morphological AM fungal species accumulation curves (**a**) and rarefaction curves (± errors, ten replicates for each subset) of observed AM MOTUs (**b**) detected on *C. illinoinensis* rhizosphere in T0 (field) and T1 (containers) samples.

Cyphellopsis anomala (0.034% of sequences in T1). Sequences of some *Chytridiomycetes* and other *Eukaryota* were also detected (Table 1).

A total of 2196 and 5876 sequences were obtained for T0 and T1, respectively (there were no chimera sequences). The number of *Glomeromycota* sequences was 7736 (average read length of 268±16bp). Number of sequences ranged from 2164 reads (TO), to 5572 reads (T1), defining 30 and 29 MOTUs, respectively (Table 1). The OS (Fig.1b) and Chao1 (Data not shown) rarefaction curves reached the plateau phase over 2000 (T0) and 5000 (T1) sampled sequences. The numbers of obtained and estimated MOTUs were close; AM richness appeared to be similar for T0 and T1 with both estimators: 30.8 and 28.9 (OS of T0 and T1) and 37.1 and 35.2 (Chao1 of T0 and T1).

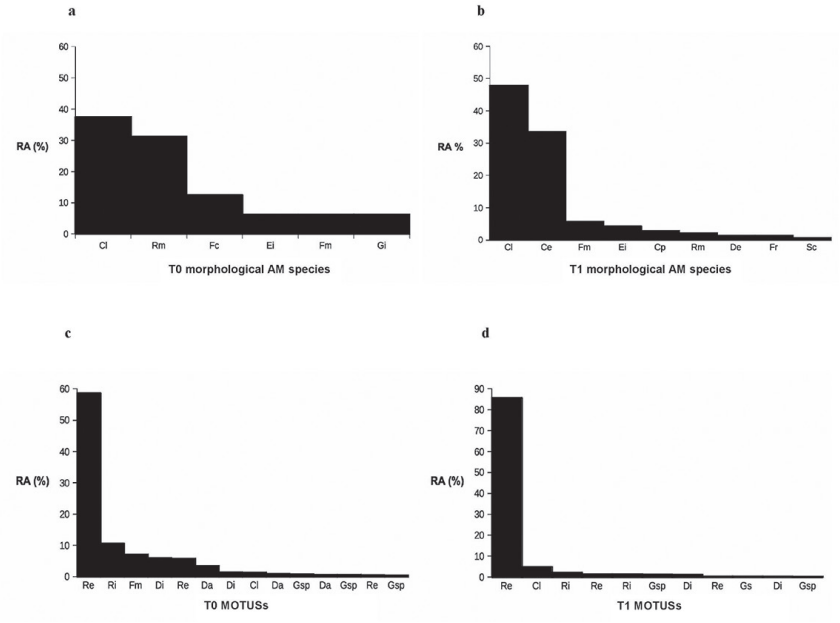

Figure 2. Rank-abundance diagrams of morphological AM fungal species (**a–b**) and rank-abundance diagrams of AM MOTUs (**c–d**) detected on *C. illinoinensis* rhizosphere in T0 (field) and T1 (containers) samples. RA: Relative abundance. Cl: *Claroideoglomus lamellosum*, Rm: *Rhizoglomus microaggregatum*, Fc: *Funneliformis coronatum*, Ei: *Entrophospora infrequens*, Fm: *Funneliformis mosseae*, Gi *Gigaspora margarita*, Ce: *Claroideoglomus etunicatum*, Cp: *Cetraspora pellucida*, De: *Diversispora eburnea*, Fr: *Fuscutata rubra*, Sc: *Septoglomus constrictum*, Re: *Rhizoglomus irregulare*, Ri: *Rhizoglomus intraradices*, Di: *Dominikia iranica*, Da: *Dominikia indica*, Gsp: *Glomus* sp.

Considering all the *Glomeromycota* genera identified, the proportion of *Rhizoglomus* sequences was always dominant in pecan rhizosphere (83% and 91% in T0 and T1, respectively). *Funneliformis* (7.2% and 0.16% in T0 and T1, respectively) and *Glomus* sequences (8.2% and 4% in T0 and T1, respectively) were more abundant in field samples but clearly diminished in pots. Particularly, *Claroideoglomus* had the opposite behaviour, as their sequences' abundances were lower at field (1.4%) than at pot (4.9%) level. A few sequences of *Paraglomus laccatum* (Błaszk.) Renker, Błaszk. & Buscot and *E. infrequens* (less than 0.05%) were also detected in both samples.

The rank-abundance species diagrams (Figs 2c,d) showed that, in T0, almost 60% of the total sequences belong to *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl and into the 95% of accumulated sequences, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, *F. mosseae*, *Dominikia iranica* (Błaszk., Kovács & Balázs) Błaszk., Chwat & Kovács, *Dominikia indica* (Błaszk., Wubet & Harikumar) Błaszk., G.S. Silva & Oehl and *C. lamellosum* were also detected. Meanwhile in T1, 90% of total sequences were of *R.*

Table 1. AM fungal MOTUs and non-target fungi sequence abundance.

Taxonomic assignation	MOTU no.	T0	T1
		Sequence abundance	
Diversisporales			
Entrophospora infrequens	1	0	2
Glomerales			
Glomus sp.	2	0	6
Glomus sp.	3	4	1
Glomus sp.	4	12	0
Glomus sp.	5	0	3
Glomus sp.	6	2	0
Glomus sp.	7	2	0
Glomus sp.	8	8	11
Glomus sp.	9	0	2
Glomus sp.	10	6	66
Glomus sp.	11	5	0
Glomus sp.	12	2	0
Glomus sp.	13	2	0
Glomus sp.	14	0	13
Glomus sp.	15	0	2
Glomus sp.	16	2	0
Glomus sp.	17	16	11
Glomus sp.	18	0	3
Claroideoglomus lamellosum	19	30	272
Funneliformis mosseae	20	154	9
Dominikia indica	21	75	62
D. indica	22	19	16
D. indica	23	0	2
D. indica	24	2	2
D. indica	25	2	0
D. indica	26	12	2
D. indica	27	2	0
D. indica	28	0	2
Sclerocystis sinuosa	29	0	2
S. sinuosa	30	4	17
S. sinuosa	31	0	2
Rhizoglomus irregulare	32	1	5
R. irregulare	33	11	17
R. irregulare	34	125	75
R. irregulare	35	1268	4772
Rhizoglomus intraradices	36	230	120
R. intraradices	37	2	74
Dominikia iranica	38	31	0
D. iranica	39	129	0
D. iranica	40	5	0
Paraglomerales			
Paraglomus laccatum	41	1	1
Total AM sequences		2164	5572
Total AM MOTUs		30	29

Taxonomic assignation	MOTU no.	T0	T1
		Sequence abundance	
Non-Target Fungi			
Basidiomycota			
Piriformospora indica	42	16	285
Cyphellopsis anomala	43	0	2
Chytridiomycetes	44	1	10
	45	2	0
	46	0	3
Other Eukaryota	47	10	0
	48	2	0
	49	1	4

irregulare and *C. lamellosum* was only detected in 95% of the accumulated sequences. The rest of sequences represented only 5%.

Most identified species were common to both soils, some were found only in field samples as *D. iranica*, while others only appeared (*E. infrequens*) or were much more abundant (*Sclerocystis sinuosa* Gerd. & B.K. Bakshi) in containers after one year of pecan culture.

Arbuscular mycorrhizal colonisation of pecan roots

At sampling time, the mycorrhization percentage of T0 natural pecan root was 17% (±6.8), while T1 roots showed 6.33 (±3.53), 28.33 (±15.9) and 29 (±3.6)% of mycorrhization, when observed at 60, 100 and 360 days of seedling growth, respectively.

Appressoria at entry points and intraradical longitudinal hyphae in the outer layers of the pecan root cortex were observed (Fig. 3a). Typical arbuscules were very frequent; however, the presence of vesicles was less commonly detected (Fig. 3b).

Discussion

Mycorrhization levels observed in pecan roots resemble those reported by Muñoz-Márquez et al. (2009), who registered values of AM fungal colonisation ranging from 13 to 32%. While most forest trees frequently form only one type of mycorrhizal association, ECM or AM (Mosse et al. 1981), some tree species exhibit both symbiosis (dos Santos et al. 2001), suggesting the existence of different niches or soil resources utilisation for these types of fungi in the same root system (Neville et al. 2002). Despite the apparent preference of pecan to associate with ECM in nature, we have demonstrated the AM fungal establishment in pecan roots when growing in natural soils and under greenhouse conditions. These observations are consistent with previous results (Muñoz-Márquez et al. 2009; Taber et al. 1982) reported in areas where pecan is native.

It has been proposed that EM colonisation promotes nutrient mobilisation in organic soils, while AM colonisation enhances the ability to exploit available phosphate in deeper mineral soils (Neville et al. 2002). This dual association in pecan roots could

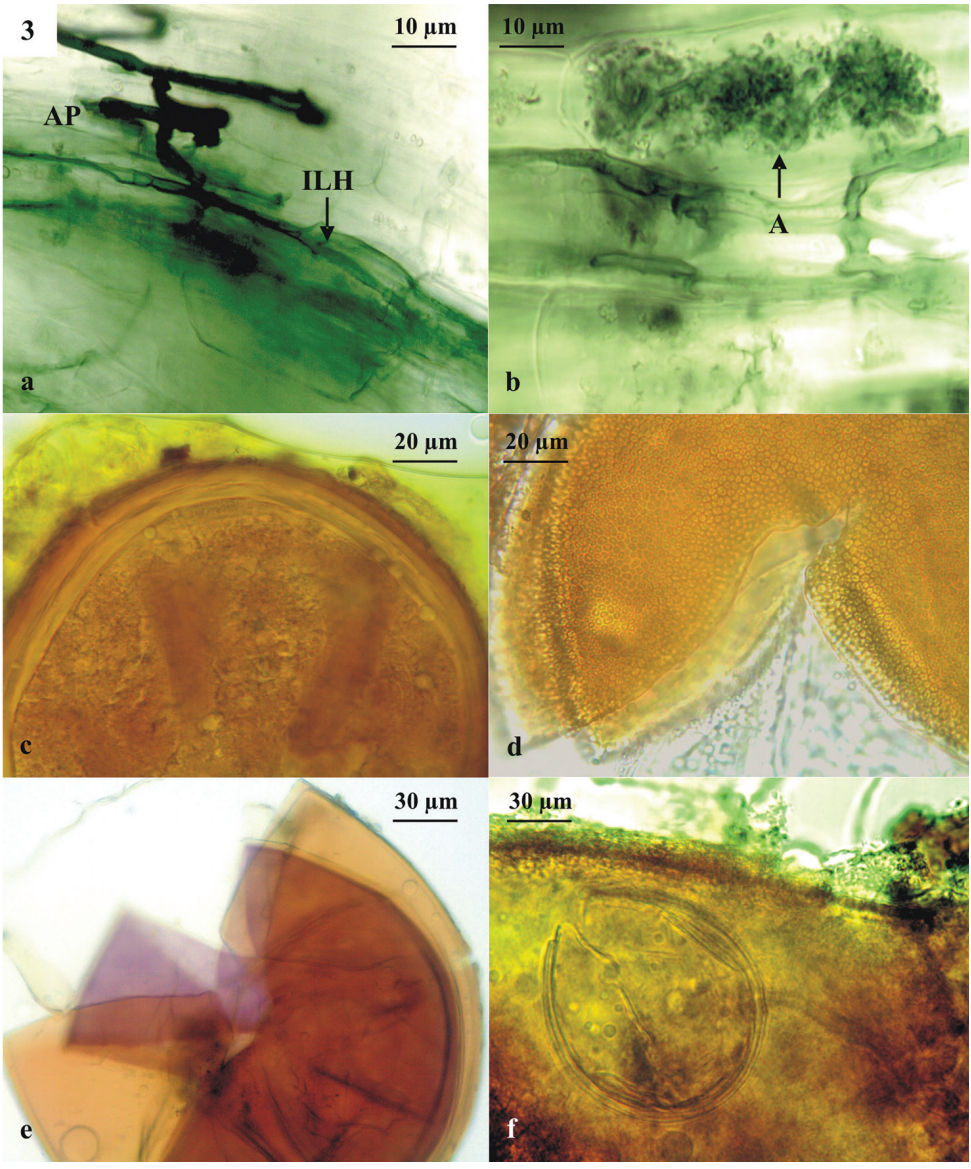

Figure 3. Arbuscular mycorrhizal intraradical colonisation in pecan roots (**a–b**). A: arbuscules, AP: appressoria, ILH: intraradical longitudinal hyphae. Spore of *Claroideoglomus lamellosum* (**c**), *Entrophospora infrequens* (**d**), *Cetraspora pellucida* (**e**), *Rhizoglomus microaggregatum* (**f**) inside another, dead AMF spore, resembling *E. infrequens*.

contribute to a better exploration of soil and its consequent geographical expansion to different habitats (Van der Heijden 2001).

Richness of AM fungal molecular species supported by pecan roots was higher in field samples than in pot soils. This was not observed when studying the AM fungal community based on collected spores. This difference between field and pot richness

could be explained by the sporulation of some AM species when growing under greenhouse conditions but only found as mycelium in the field. Frequently, soils under different disturbances (such as reductions in plant biodiversity and/or pot effect) are less rich in AM fungal species than natural soils (Fitter 2005; Lumini et al. 2009). This was expected because previous studies demonstrated that the AM fungal community is as diverse as the host plants community (Fitter 2005; Opik et al. 2009). Other causes could be related with the age and density of roots in plastic containers, changes in soil/substrate chemo-physical properties, differences in plant nutritional needs or inherent differences in mature trees compared to seedlings. However, these variables were not analysed in this work.

The representative nature of the different AM fungal species propagules was more even in T0 samples than in T1 (by both methodologies), suggesting a more equitable distribution of richness and a more biodiverse AM fungal community.

AM richness estimated by the molecular approach was between three and five times higher than that estimated by morphological methods. Differences in numbers of morphological species and MOTUs are expected, due to the potential of metagenomic DNA based techniques to detect AM species from all propagules, in addition to spores. It is also possible that overestimation of molecular richness occurs when using a 97% similarity between sequences to define MOTUs (i.e. different MOTUs corresponded with the same AM fungal species). It has been suggested that, with less than 97% of sequence similarity, it might be possible that the 'query sequence' and the 'reference sequence' represent the same taxonomical unit (i.e. fungal species) (Hibbett et al. 2011). Due to the asexual multinucleated nature of AM fungi, a high intraspecific genetic variability within a single individual has been reported (Fitter 2005). We suggest that, when studying this particular fungal group, a lower percent similarity to design MOTUs could give closer richness estimation to the morphological approach.

Moreover, considering that there are a great number of morphologically defined AM fungal species, which are not yet represented in sequence databases, it is very difficult to match AM environmental sequences with those species (Brock et al. 2009).

Considering that mycorrhizal plants richness was very limited in the sampled *Delta* soils, compared with other agro-ecosystems, the AM fungal richness detected in pecan rhizosphere was higher than expected. The comparison of the AM fungal community composition, associated with pecan rhizosphere, indicated that only three species: *C. lamellosum*, *F. mosseae* and *E. infrequens* were detected using both molecular and morphological approaches. The last two were always present at low frequencies, while *C. lamellosum* was the dominant detected species when using morphological technique, but much less frequent in the metagenomic database. Some AM fungal species, such as *F. coronatum*, *G. margarita*, *C. etunicatum*, *C. pellucida*, *D. eburnea*, *F. rubra* and *S. constrictum*, were detected only as spores in soil samples and trap cultures by the conventional approach, but not by pyrosequencing. Considering that not all AM fungal species, defined by the morphological approach, have been sequenced, it is expected that many of the sequences reported here only reached the generic level, or less, when compared with public databases. Moreover,

dominant sequences in pecan rhizosphere samples, such as *R. irregulare*, along with other less abundant (*D. iranica*, *D. indica*, *S. sinuosa*, *P. laccatum*), were detected only by pyrosequencing. Spores of these species were not observed, evidencing that these AM fungi were only present as mycelium at sampling time.

Our molecular and morphological data indicated the dominance of *Glomerales* amongst other AM orders. Several genera of *Glomerales* have the ability to extensively colonise roots from spores and mycelial fragments as inoculum source. They already have the ability to form mycelial anastomoses after mechanical disruption. These could be the reasons for their dominance compared to other AM groups. The high frequency of these taxa has been previously observed in natural and agronomic ecosystems by several authors (Dumbrell et al. 2011; Lin et al. 2012; Lumini et al. 2009). An absolute predominance of the *Glomeraceae* spores has also been reported for other trees (Husband et al. 2002; Opik et al. 2006; Wubet et al. 2003).

Results of both approaches were not consistent. Both methods should be integrated to provide a more comprehensive estimate of fungal community organisation. Detection of AM fungal species, based on spore morphology in soil and trap culture, could be used in combination with the molecular approach. More pecan orchards should be sampled to determine the regional distribution of AM associated with these forest crops, even though this first study offers a consistent overview of the AM fungal community present in the rhizosphere of transplanted pecan trees. Given the importance of the correct use of mycorrhizal inoculum (natural or external) to maintain or restore forest populations, knowing the type of mycorrhizal association could be helpful to develop strategies for forest management and reforestation practices. Furthermore, since there are water and nutritional needs inherent to each stage of pecan plants development (nursery growth and field transplant), in future, we aim to study how mycorrhization differentially affects nutrients and water access at each stage.

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Taxonomic circumscription of melanconis-like fungi causing canker disease in China

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Abstract

Melanconis-like species comprise latent fungal pathogens with a wide range of woody hosts. Taxonomy of these pathogens is difficult due to their uninformative descriptions and similar asexual morphology. Based on molecular phylogenies, many species of this group were placed in various families of Diaporthales. In this study, eight species of melanconis-like fungi were isolated from *Betula albosinensis*, *B. platyphylla* (Betulaceae), *Cornus controversa* (Cornaceae), *Corylus mandshurica* (Betulaceae) and *Juglans regia* (Juglandaceae) in China. These species were phylogenetically placed in three families of Diaporhthales, i.e. *Juglanconis juglandina*, *J. oblonga* (Juglanconidaceae), *Melanconiella betulicola* **sp. nov.**, *M. corylina* **sp. nov.** (Melanconiellaceae), *Melanconis betulae*, *Ms. itoana*, *Ms. stilbostoma* (Melanconidaceae) and one new genus, *Sheathospora* (Melanconiellaceae). *Sheathospora* is proposed to accommodate *Melanconiella cornuta* with conical and discrete pycnidia with aseptate, hyaline, cylindrical to ellipsoidal conidia with distinct hyaline sheath on branches of *Cornus controversa*. Combined analyses of ITS, LSU, CAL, RPB2 and TEF1-α sequence data were used to construct the molecular phylogeny. Additionally, we provided separate phylogenetic trees for three families (Juglanconidaceae, Melanconidaceae and Melanconiellaceae) to show the species distribution of melanconis-like fungi in China.

Keywords

Diaporthales, phylogeny, taxonomy, wood-inhabiting fungi

Introduction

Melanconium (Diaporthales) was introduced by Link (1809) from dead branches of *Fagus* with *M. atrum* Link as the generic type. Corda (1837) extended this genus to 28 species. Subsequently, the genera *Melanconis* Tul. & C. Tul. and *Melanconiella* Sacc. were described as sexual morphs of *Melanconium* (Wehmeyer 1937, 1941). Sutton (1980) summarised more than 200 binomials that have been described in *Melanconium*, whereas no generic revision is available due to the uninformative descriptions and illustrations, few morphological characteristics, misplacement or poor condition of original specimens and lacking of ex-type cultures. In the Index Fungorum (2018), there are more than 235 species epithets of *Melanconium* with an estimated 50 species epithets by Kirk et al. (2008). Thus *Melanconium* species has serious obstacles for appropriate interpretation and is phylogenetically distributed throughout the Diaporthales, especially in Juglanconidaceae, Melanconidaceae and Melanconiellaceae. Although the genus *Melanconium* may be synonymous with *Melanconis* and would therefore have priority, the true identity of the generic type, *M. atrum*, is obscure and it was recommended to protect *Melanconis* over *Melanconium* (Rossman et al. 2015).

Molecular phylogenetics have had a major impact in taxonomic rearrangements of fungi since the early 1990s (White et al. 1990, Hibbett et al. 2007, Choi and Kim 2017, Fan et al. 2018). Castlebury et al. (2002) re-evaluated Diaporthales based on LSU rDNA sequences, indicating the single genus *Melanconis* with asexual morph *Melanconium* in Melanconidaceae *s. str.* Rossman et al. (2007) followed this differentiation and believed that many additional species of *Melanconis sensu* Wehmeyer (1941) should be separated from Melanconidaceae. One example is *Melanconiella spodiaea* (Tul. & C. Tul.) Sacc., type of the genus *Melanconiella*, which segregated from *Melanconis* (Rossman et al. 2007). Voglmayr et al. (2012) published sequences and molecular phylogenies for species of *Melanconiella* firstly and proposed that *Melanconiella* represented a distinct clade from *Melanconis*. Subsequently, Norphanphoun et al. (2016) introduced Lamproconiaceae to accommodate *Melanconium desmazieri* (Berk. & Broome) Sacc., with its sexual morph *Melanconis desmazieri* Petr. (Grove 1937, Sutton 1980). Voglmayr et al. (2017) proposed Juglanconidaceae to accommodate *Melanconium juglandinum* Kunze. Senanayake et al. (2017) introduced Melanconiellaceae to accommodate the previous unresolved *Melanconiella*.

During trips to collect forest pathogens that cause canker or dieback diseases in China, several melanconis-like taxa associated with various disease symptoms were collected in Beijing, Gansu, Heilongjian, Jilin, Ningxia, Shaanxi and Tibet Provinces. As the higher-level phylogeny of many genera within the melanconis-like taxa remains largely unresolved in China, this project was initiated to address this issue. In this paper, we identified eight melanconis-like species residing in three families of Diaporthales; recognised three genera within Melanoconiellaceae; and described two new species in *Melanconiella* as well as one new genus to accommodate *Melanconiella cornuta*.

Materials and methods

Isolation

Fresh specimens of melanconis-like fungi were collected from infected branches of seven hosts during collection trips in China (Table 1). A total of 47 isolates were established by removing a mucoid spore mass from ascomata or conidiomata, spreading the suspension on the surface of 1.8% potato dextrose agar (PDA) and incubating at 25 °C for up to 24 h. Single germinating conidia/ascospores were removed and plated on to fresh PDA plates. Specimens and isolates were deposited in the Key Laboratory for Silviculture and Conservation of the Ministry of Education in the Beijing Forestry University (BJFU) and the working Collection of X.L. Fan (CF) housed at the BJFU. Axenic cultures are maintained in the China Forestry Culture Collection Centre (CFCC).

Morphological studies

Species identification was based on morphological features of the ascomata or conidiomata produced on infected plant tissues and micromorphology, supplemented by cultural characteristics. Cross-sections were prepared by hand using a double-edge blade under a dissecting microscope. More than 10 conidiomata/ascomata, 10 asci and/or 50 conidia/ascospores were measured to calculate the mean size and standard deviation (SD). Microscopic photographs were captured with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Ri2 high definition colour camera, using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D Package v. 3.00. Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Colony diameters were measured and the colony colours described after 3 weeks according to the colour charts of Rayner (1970).

DNA extraction and sequencing

Genomic DNA was extracted using a modified CTAB method, with fungal mycelium harvested from PDA plates with cellophane (Doyle and Doyle 1990). The DNA was estimated by electrophoresis in 1% agarose gel and the quality was measured by NanoDrop 2000 (Thermo, USA) according to the user's manual (Desjardins et al. 2009). The PCR amplifications were performed in DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA). The ITS region was amplified with the primers ITS1 and ITS4 (White et al. 1990), the LSU region with the primers LR0R and LR5 (Vilgalys and Hester 1990), the CAL gene (for Juglanconidaceae) with primers CAL-228F and CAL-737R (Carbone and Kohn 1999), the RPB2 region with primers

Table 1. Details of the strains included for molecular study used in this study.

Species	Culture/strain/specimen	Location	Host	GenBank accession numbers				
				ITS	LSU	CAL	RPB2	TEF1-α
Apiosporopsis carpinea	CBS 771.79	Switzerland	*Carpinus betulus*	NA	AF277130	NA	NA	NA
Apiosporopsis sp.	11Af2-1	Japan	*Alnus firma*	NA	AB669034	NA	NA	NA
Apoharknessia insueta	**CBS 111377**	Brazil	*Eucalyptus pellita*	JQ706083	AY720814	NA	NA	NA
	CBS 114575	Colombia	*Eucalyptus* sp.	NA	AY720813	NA	NA	NA
Asterosporium asterospermum	MFLU 15-3555	Italy	*Fagus sylvatica*	NA	MF190062	NA	MF377615	NA
	CBS 112404	Italy	*Fagus sylvatica*	NA	AB553745	NA	NA	NA
	KT2138	Japan	*Fagus crenata*	NA	AB553744	NA	NA	NA
Auratiopycnidiella tristaniopsidis	**CBS 132180 = CPC 16371**	Australia	*Tristaniopsis laurina*	JQ685516	JQ685522	NA	NA	NA
Cainiella johansonii	**Kruys 731**	Sweden	*Dryas octopetala*	NA	JF701920	NA	NA	NA
Chapeckia nigrospora	**AR 3809**	USA	*Betula* sp.	JF681957	EU683068	NA	NA	NA
Chiangraiomyces bauhiniae	**MFLUCC 17-1669**	Thailand	*Bauhinia* sp.	MF190118	MF190064	NA	MF377604	NA
	MFLUCC 17-1670	Thailand	*Bauhinia* sp.	MF190119	MF190065	NA	MF377603	NA
Chrysocrypta corymbiae	**CBS 132528**	Australia	*Corymbia* sp.	JX069867	JX069851	NA	NA	NA
Coniella diplodiella	CBS 111858 = CPC 3708	France	*Vitis vinifera*	AY339323	AY339284	NA	KX833423	KX833603
Coniella koreana	CBS 143.97	Korea	NA	KX833584	AF408378	NA	KX833490	KX833684
Coniella musaiensis var. *hibisci*	AR 3534 = CBS 109757	South Africa	*Hibiscus* sp.	KX833589	AF408337	NA	NA	KX833689
Coniella straminea	CBS 149.22 = CPC 3932	USA	*Fragaria* sp.	AY339348	AF362569	NA	KX833506	KX833704
Coniella wangiensis	CBS 132530 = CPC 19397	Australia	*Eucalyptus* sp.	JX069873	JX069857	NA	KX833509	KX833705
Coryneum depressum	AR 3897	Austria	*Quercus cerris*	NA	EU683074	NA	NA	NA
Coryneum modonium	AR 3558	Austria	*Castanea sativa*	NA	EU683073	NA	NA	NA
Coryneum umbonatum	**AR 3541**	Austria	*Quercus cerris*	NA	EU683072	NA	NA	NA
	MFLUCC 15-1110	Italy	*Quercus* sp.	MF190121	MF190067	NA	MF377610	NA
	MFLUCC 13-0658	Italy	*Quercus* sp.	MF190120	MF190066	NA	MF377609	NA
Cryphonectria macrospora	AR 3444 = CBS 109764	Russia	*Quercus mongolica*	EU199182	AF408340	NA	EU220029	NA
Cryphonectria nitschkei	AR 3433 = CBS109776	Russia	*Quercus mongolica*	DQ120761	AF408341	NA	NA	NA
Cryphonectria parasitica	**ATCC 38755**	USA	*Castanea dentata*	AY141856	EU199123	NA	DQ862017	EU222014
Cryptodiaporthe aesculi	**AFTOL-ID 1238 = CBS 109765**	Austria	*Aesculus hippocastanu*m	DQ323530	AF408342	NA	EU199138	GU354004
	AR3640 = CBS 121905	USA	*Aesculus hippocastanum*	EU254994	EU255164	NA	EU219269	DQ313558
	LCM 447.01	Germany	*Aesculus hippocastanum*	GU367076	NA	NA	GU367110	GU354002

Cryptosporella betulae	AR 3524 = CBS 109763	Austria	*Betula pendula*	EU199180	AF408375	NA	EU199139	EU221884
Cryptosporella hypodermia	**AR 3552**	Austria	*Ulmus minor*	EU199181	AF408346	NA	EU199140	NA
Cryptosporella suffusa	AR 3496 = CBS 109750	Austria	*Alnus incana*	EU199207	AF408376	NA	EU199163	EU221945
Cytospora cenisia	AR 3522 = CBS 109752	Austria	*Juniperus communis*	NA	AF408385	NA	NA	NA
Cytospora chrysosperma	**CFCC 89600**	China	*Sophora japonica*	KR045623	KR045623	NA	KU710951	KU710915
Cytospora elaeagni	CFCC 89633	China	*Elaeagnus angustifolia*	KF765677	KF765693	NA	KU710956	KU710919
Cytospora leucostoma	CFCC 50468	China	*Betula platyphylla*	KT732949	KT732968	NA	NA	NA
Cytospora nivea	AR 3512	Austria	*Salix purpurea*	NA	AF408367	NA	NA	NA
Cytospora sacculus	AR 3416 = CBS 109756	Russia	*Quercus mongolica*	NA	AF408386	NA	NA	NA
	AR 3426 = CBS 109777	Austria	*Quercus robur*	NA	AF408387	NA	NA	NA
Dendrostoma mali	**CFCC 52102**	China	*Malus spectabilis*	MG682072	MG682012	NA	MG682032	MG682052
Dendrostoma osmanthi	CFCC 52106	China	*Osmanthus fragrans*	MG682073	MG682013	NA	MG682033	MG682053
	CFCC 52107	China	*Osmanthus fragrans*	MG682074	MG682014	NA	MG682034	MG682054
	CFCC 52108	China	*Osmanthus fragrans*	MG682075	MG682015	NA	MG682035	MG682055
	CFCC 52109	China	*Osmanthus fragrans*	MG682076	MG682016	NA	MG682036	MG682056
Dendrostoma quercinum	CFCC 52103	China	*Quercus acutissima*	MG682077	MG682017	NA	MG682037	MG682057
	CFCC 52104	China	*Quercus acutissima*	MG682078	MG682018	NA	MG682038	MG682058
	CFCC 52105	China	*Quercus acutissima*	MG682079	MG682019	NA	MG682039	MG682059
Diaporthe decedens	AR 3459 = CBS 109772	Austria	*Corylus avellana*	KC343059	AF408348	NA	NA	NA
Diaporthe detrusa	AR 3424 = CBS 109770	Austria	*Berberis vulgaris*	KC343061	AF408349	NA	NA	KC343787
Diaporthe eres	**AR 3538 = CBS 109767**	Austria	*Acer campestre*	KC343075	AF408350	NA	NA	KC343801
Diaporthella corylina	CBS 121124	China	*Corylus* sp.	KC343004	NA	NA	NA	NA
Diaporthella sp.	CN5	Italy	*Corylus avellana*	KP205483	NA	NA	NA	NA
	CN13	Italy	*Corylus avellana*	KP205484	NA	NA	NA	NA
Diaporthosporella cercidicola	**CFCC 51994**	China	*Cercis chinensis*	KY852492	KY852515	NA	NA	NA
	CFCC 51995	China	*Cercis chinensis*	KY852493	KY852516	NA	NA	NA
	CFCC 51996	China	*Cercis chinensis*	KY852494	KY852517	NA	NA	NA
Diaporthostoma machili	**CFCC 52100**	China	*Machilus leptophylla*	MG682080	MG682020	NA	MG682040	MG682060
	CFCC 52101	China	*Machilus leptophylla*	MG682081	MG682021	NA	MG682041	MG682061
Disculoides eucalypti	**CPC 17650**	Australia	*Eucalyptus* sp.	JQ685517	JQ685523	NA	NA	NA
Disculoides eucalyptorum	CBS 132184 = CPC 17648	Australia	*Eucalyptus viminalis*	NR120090	JQ685524	NA	NA	NA
Ditopella ditopa	**AR 3423 = CBS 109748**	Austria	*Alnus glutinosa*	EU199187	EU199126	NA	EU199145	NA
Erythrogloeum hymenaeae	**CPC 18819**	Brazil	*Hymenaea courbaril*	JQ685519	JQ685525	NA	NA	NA
Gnomonia gnomon	**CBS 199.53**	Italy	*Corylus avellana*	AY818956	AF408361	NA	EU219295	EU221885

Harknessia eucalypti	**CBS 342.97**	Australia	*Eucalyptus regnans*	AY720745	AF408363	NA	NA	NA
Harknessia leucospermi	CBS 775.97	South Africa	*Leucospermum* sp.	NR137147	AY720824	NA	NA	NA
Harknessia molokaiensis	AR 3578 = CBS 109779	USA	*Eucalyptus robusta*	NA	AF408390	NA	NA	NA
Harknessia syzygii	CBS 111124 = CPC184	South Africa	*Syzygium cordatum*	AY720738	AY720834	NA	NA	NA
Hercospora tiliae	**AR 3526**	Austria	*Tilia tomentosa*	NA	AF408365	NA	NA	NA
Hyaliappendispora galii	**MFLUCC 16-1208**	Italy	*Galium* sp.	MF190149	MF190095	NA	NA	NA
Involutscutellula rubra	**CBS 192.71**	Japan	*Quercus phillyraeoides*	MG591899	MG591993	NA	MG976476	MG592086
Juglanconis appendiculata	D140	Greece	*Juglans nigra*	KY427138	KY427138	NA	KY427188	KY427207
	D96	Austria	*Juglans nigra*	KY427139	KY427139	NA	KY427189	KY427208
	D96A	Austria	*Juglans nigra*	KY427140	KY427140	NA	KY427190	KY427209
	MC	Greece	*Juglans nigra*	KY427141	KY427141	KY427242	KY427191	KY427210
	MC2	Spain	*Juglans nigra*	KY427142	KY427142	KY427243	KY427192	KY427211
	MC4	Spain	*Juglans nigra*	KY427143	KY427143	KY427244	KY427193	KY427212
	ME17	Austria	*Juglans nigra*	KY427144	KY427144	KY427245	KY427194	KY427213
Juglanconis juglandina	**D142**	Austria	*Juglans nigra*	KY427145	KY427145	NA	KY427195	KY427214
	CFCC 51727*	China	*Juglans nigra*	KY363854	KY363859	MK096394	MK096439	NA
	CFCC 51728*	China	*Juglans nigra*	KY363855	KY363860	MK096395	MK096440	NA
	CFCC 51729*	China	*Juglans nigra*	KY363856	KY363861	MK096396	MK096441	NA
	MC1	Austria	*Juglans nigra*	KY427146	NA	KY427246	KY427196	KY427215
	MC3	Spain	*Juglans nigra*	KY427147	KY427146	KY427247	KY427197	KY427216
	ME16	Austria	*Juglans nigra*	KY427148	KY427147	KY427248	KY427198	KY427217
	ME22	Austria	*Juglans nigra*	KY427149	KY427148	KY427249	KY427199	KY427218
	ME23	Austria	*Juglans nigra*	KY427150	KY427150	KY427250	KY427200	KY427219
Juglanconis oblonga	CFCC 51725*	China	*Juglans nigra*	KY363852	KY363857	MK096392	MK096437	NA
	CFCC 51726*	China	*Juglans nigra*	KY363853	KY363858	MK096393	MK096438	NA
	ME14	USA	*Juglans cinerea*	KY427151	KY427151	KY427251	KY427201	KY427220
	ME15	USA	*Juglans cinerea*	KY427152	KY427152	KY427252	KY427202	KY427221
	ME18	Japan	*Juglans ailanthifolia*	KY427153	KY427153	KY427253	KY427203	KY427222
	ME19	Japan	*Juglans ailanthifolia*	KY427154	KY427154	KY427254	KY427204	KY427223
Juglanconis pterocaryae	ME20	Japan	*Pterocarya rhoifolia*	KY427155	KY427155	KY427255	KY427205	KY427224
Lamproconium desmazieri	**MFLUCC 14-1047**	Russia	*Tilia cordata*	KX430132	KX430133	NA	NA	MF377592
	MFLUCC 15-0870	Russia	*Tilia tomentosa*	KX430134	KX430135	NA	MF377605	MF377591
Lasmenia sp.	CBS 124123	Puerto Rico	*Nephelium lappaceum*	GU797406	JF838338	NA	NA	NA
	CBS 124124	Puerto Rico	*Nephelium lappaceum*	JF838336	JF838341	NA	NA	NA

Luteocirrhus shearii	**CBS 130776**	Australia	*Banksia baxteri*	NR120254	NG042770	NA	NA	NA
Macrohilum eucalypti	**CPC 10945**	New Zealand	*Eucalyptus* sp.	DQ195781	DQ195793	NA	NA	NA
	CPC 19421	Australia	*Eucalyptus piperita*	KR873244	KR873275	NA	NA	NA
Melanconiella betulicola	CFCC 52482*	China	*Betula albosinensis*	MK096312	MK096352	NA	MK096397	MK096272
	CFCC 52483*	China	*Betula albosinensis*	MK096313	MK096353	NA	MK096398	MK096273
Melanconiella carpinicola	MNM	Austria	*Carpinus betulus*	JQ926232	JQ926232	NA	JQ926304	JQ926370
	MNUK	UK	*Carpinus betulus*	JQ926234	JQ926234	NA	JQ926306	JQ926372
	MSMI	Austria	*Carpinus betulus*	JQ926235	JQ926235	NA	JQ926307	JQ926373
Melanconiella chrysodiscosporina	MCH	Austria	*Carpinus betulus*	JQ926238	JQ926238	NA	JQ926310	JQ926376
	MEE	Austria	*Carpinus betulus*	JQ926240	JQ926240	NA	JQ926312	JQ926378
	MGG	Austria	*Carpinus betulus*	JQ926242	JQ926242	NA	JQ926314	JQ926380
Melanconiella chrysomelanconium	MCM	Austria	*Carpinus betulus*	JQ926247	JQ926247	NA	JQ926319	JQ926385
	MEUK	UK	*Carpinus betulus*	JQ926249	JQ926249	NA	JQ926321	JQ926387
	MGUK	UK	*Carpinus betulus*	JQ926255	JQ926255	NA	JQ926327	JQ926393
Melanconiella chrysorientalis	MGB	Croatia	*Carpinus orientalis*	JQ926256	JQ926256	NA	JQ926328	JQ926394
	MGP	Croatia	*Carpinus orientalis*	JQ926257	JQ926257	NA	JQ926329	JQ926395
	MVH	Croatia	*Carpinus orientalis*	JQ926259	JQ926259	NA	JQ926331	JQ926397
Melanconiella corylina	CFCC 52484*	China	*Corylus mandshurica*	MK096314	MK096354	NA	MK096399	MK096274
	CFCC 52485*	China	*Corylus mandshurica*	MK096315	MK096355	NA	MK096400	MK096275
Melanconiella decorahensis	CBS 159.26	USA	*Betula* sp.	JQ926260	JQ926260	NA	JQ926332	JQ926398
	MD	France	*Betula pendula*	JQ926261	JQ926261	NA	JQ926333	JQ926399
	MED	France	*Betula pendula*	JQ926262	JQ926262	NA	JQ926334	JQ926400
Melanconiella echinata	DAOM 121196	USA	*Carpinus caroliniana*	JQ926263	JQ926263	NA	N/A	N/A
Melanconiella elegans	AR 3830	USA	*Carpinus caroliniana*	JQ926264	JQ926264	NA	JQ926335	JQ926401
	BPI 843574	USA	*Carpinus caroliniana*	JQ926266	JQ926266	NA	JQ926337	JQ926403
	BPI 872067	USA	*Carpinus caroliniana*	JQ926267	JQ926267	NA	JQ926338	JQ926404
Melanconiella ellisii	BPI 843491	USA	*Carpinus caroliniana*	JQ926268	JQ926268	NA	N/A	JQ926405
	BPI 878343	USA	*Carpinus caroliniana*	JQ926271	JQ926271	NA	JQ926339	JQ926406
	BPI 883227	USA	*Carpinus caroliniana*	JQ926269	JQ926269	NA	N/A	N/A
Melanconiella flavovirens	MFV1	Austria	*Corylus avellana*	JQ926274	JQ926274	NA	JQ926342	JQ926409
	MFV2	Austria	*Corylus avellana*	JQ926275	JQ926275	NA	JQ926343	JQ926410
	MFV3	Italy	*Corylus avellana*	JQ926276	JQ926276	NA	JQ926344	JQ926411
Melanconiella hyperopta	MCHBV	Austria	*Carpinus betulus*	JQ926280	JQ926280	NA	JQ926346	JQ926413
	MCR	Austria	*Carpinus betulus*	JQ926283	JQ926283	NA	JQ926349	JQ926416
	MHG	Switzerland	*Carpinus betulus*	JQ926285	JQ926285	NA	JQ926351	JQ926418

Melanconiella hyperopta var. *orientalis*	MHP	Croatia	*Carpinus orientalis*	JQ926288	JQ926288	NA	JQ926352	JQ926420
	MHVA	Croatia	*Carpinus orientalis*	JQ926287	JQ926287	NA	JQ926353	JQ926419
	MSK	Croatia	*Carpinus orientalis*	JQ926286	JQ926286	NA	JQ926354	JQ926421
Melanconiella meridionalis	MOA	Austria	*Ostrya carpinifolia*	JQ926289	JQ926289	NA	JQ926355	JQ926422
	MOK	Croatia	*Ostrya carpinifolia*	JQ926290	JQ926290	NA	JQ926356	JQ926423
	MOM	Austria	*Ostrya carpinifolia*	JQ926291	JQ926291	NA	JQ926357	JQ926424
Melanconiella ostryae	CBS 208.38	USA	*Ostrya virginiana*	JQ926297	JQ926297	NA	JQ926363	JQ926430
Melanconiella spodiaea	**MVS**	Croatia	*Carpinus orientalis*	JQ926299	JQ926299	NA	JQ926365	JQ926432
	MSH	Austria	*Carpinus betulus*	JQ926298	JQ926298	NA	JQ926364	JQ926431
	SPOD	Croatia	*Carpinus betulus*	JQ926300	JQ926300	NA	JQ926366	JQ926433
Melanconis alni	AR 3529	Russia	*Duschekia maximowiczii*	NA	AF362566	NA	NA	NA
	AR 3748	Austria	*Alnus viridis*	EU199195	EU199130	NA	EU199153	NA
	AR 4016 = CBS 121480	Austria	*Alnus alnobetula*	EU254863	NA	NA	EU219298	EU221894
	CBS 109773	Austria	*Alnus viridis*	DQ323523	AF408371	NA	EU219300	EU221896
Melanconis betulae	CFCC 50471*	China	*Betula albosinensis*	KT732952	KT732971	NA	KT732984	KT733001
	CFCC 50472*	China	*Betula albosinensis*	KT732953	KT732972	NA	KT732985	KT733002
	CFCC 50473*	China	*Betula albosinensis*	KT732954	KT732973	NA	KT732986	KT733003
Melanconis italica	MFLUCC 16-1199	Italy	*Alnus cordata*	MF190151	MF190096	NA	NA	NA
	MFLUCC 17-1659	Italy	*Alnus cordata*	MF190151	MF190097	NA	MF377602	NA
Melanconis itoana	CFCC 50474*	China	*Betula albosinensis*	KT732955	KT732974	NA	KT732987	KT733004
	CFCC 52876*	China	*Betula albosinensis*	MK096324	MK096364	NA	MK096409	MK096284
	CFCC 52877*	China	*Betula albosinensis*	MK096326	MK096366	NA	MK096411	MK096286
	CFCC 52878*	China	*Betula albosinensis*	MK096327	MK096367	NA	MK096412	MK096287
	MAFF 410080	Japan	*Betula ermanii*	JX522738	NA	NA	NA	NA
Melanconis marginalis	AR 3442 = CBS 109744	Canada	*Alnus rubra*	EU199197	AF408373	NA	EU219301	EU221991
	MAFF 410218	Japan	*Alnus maximowiczii*	JX522742	NA	NA	NA	NA
Melanconis stilbostoma	**CBS 109778 = AR 3501**	Austria	*Betula pendula*	DQ323524	AF408374	NA	EU219299	EU221886
	CBS 121894 = MS	NA	*Betula pendula*	JQ926229	JQ926229	NA	JQ926302	JQ926368
	CFCC 50475*	China	*Betula platyphylla*	KT732956	KT732975	NA	KT732988	KT733005
	CFCC 50476*	China	*Betula platyphylla*	KT732957	KT732976	NA	KT732989	KT733006
	CFCC 50477*	China	*Betula platyphylla*	KT732958	KT732977	NA	KT732990	KT733007
	CFCC 50478*	China	*Betula platyphylla*	KT732959	KT732978	NA	KT732991	KT733008
	CFCC 50479*	China	*Betula platyphylla*	KT732960	KT732979	NA	KT732992	KT733009
	CFCC 50480*	China	*Betula platyphylla*	KT732961	KT732980	NA	KT732993	KT733010

Melanconis stilbostoma	**CFCC 50481***	China	*Betula platyphylla*	KT732962	KT732981	NA	KT732994	KT733011
	CFCC 50482*	China	*Betula platyphylla*	KT732963	KT732982	NA	KT732995	KT733012
	CFCC 52843*	China	*Betula platyphylla*	MK096338	MK096378	NA	MK096423	MK096298
	CFCC 52844*	China	*Betula platyphylla*	MK096341	MK096381	NA	MK096426	MK096301
	CFCC 52845*	China	*Betula platyphylla*	MK096343	MK096383	NA	MK096428	MK096303
	CFCC 52846*	China	*Betula platyphylla*	MK096347	MK096387	NA	MK096432	MK096307
	CFCC 52847*	China	*Betula platyphylla*	MK096348	MK096388	NA	MK096433	MK096308
	CFCC 52848*	China	*Betula platyphylla*	MK096349	MK096389	NA	MK096434	MK096309
	CFCC 52849*	China	*Betula platyphylla*	MK096328	MK096368	NA	MK096413	MK096288
	CFCC 52850*	China	*Betula platyphylla*	MK096329	MK096369	NA	MK096414	MK096289
	CFCC 52851*	China	*Betula platyphylla*	MK096330	MK096370	NA	MK096415	MK096290
	CFCC 52852*	China	*Betula platyphylla*	MK096331	MK096371	NA	MK096416	MK096291
	CFCC 52853*	China	*Betula platyphylla*	MK096332	MK096372	NA	MK096417	MK096292
	CFCC 52854*	China	*Betula platyphylla*	MK096333	MK096373	NA	MK096418	MK096293
	CFCC 52855*	China	*Betula platyphylla*	MK096334	MK096374	NA	MK096419	MK096294
	CFCC 52856*	China	*Betula platyphylla*	MK096335	MK096375	NA	MK096420	MK096295
	CFCC 52857*	China	*Betula platyphylla*	MK096336	MK096376	NA	MK096421	MK096296
	CFCC 52858*	China	*Betula platyphylla*	MK096337	MK096377	NA	MK096422	MK096297
	CFCC 52859*	China	*Betula platyphylla*	MK096339	MK096379	NA	MK096424	MK096299
	CFCC 52860*	China	*Betula platyphylla*	MK096340	MK096380	NA	MK096425	MK096300
	CFCC 52861*	China	*Betula platyphylla*	MK096342	MK096382	NA	MK096427	MK096302
	CFCC 52862*	China	*Betula platyphylla*	MK096344	MK096384	NA	MK096429	MK096304
	CFCC 52863*	China	*Betula platyphylla*	MK096345	MK096385	NA	MK096430	MK096305
	CFCC 52864*	China	*Betula platyphylla*	MK096346	MK096386	NA	MK096431	MK096306
	CFCC 52865*	China	*Betula platyphylla*	MK096316	MK096356	NA	MK096401	MK096276
	CFCC 52866*	China	*Betula platyphylla*	MK096317	MK096357	NA	MK096402	MK096277
	CFCC 52867*	China	*Betula platyphylla*	MK096318	MK096358	NA	MK096403	MK096278
	CFCC 52868*	China	*Betula platyphylla*	MK096319	MK096359	NA	MK096404	MK096279
	CFCC 52869*	China	*Betula platyphylla*	MK096320	MK096360	NA	MK096405	MK096280
	CFCC 52870*	China	*Betula platyphylla*	MK096321	MK096361	NA	MK096406	MK096281
	CFCC 52871*	China	*Betula platyphylla*	MK096322	MK096362	NA	MK096407	MK096282
	CFCC 52872*	China	*Betula platyphylla*	MK096323	MK096363	NA	MK096408	MK096283
	CFCC 52873*	China	*Betula platyphylla*	MK096350	MK096390	NA	MK096435	MK096310
	CFCC 52874*	China	*Betula platyphylla*	MK096351	MK096391	NA	MK096436	MK096311
	CFCC 52875*	China	*Betula platyphylla*	MK096325	MK096365	NA	MK096410	MK096285

Microascospora fragariae	CBS 118.16	USA	*Fragaria* sp.	NR156500	NA	NA	NA	NA
	CBS 128350	USA	*Rubus* sp.	JF514854	NA	NA	NA	NA
	1-1	China	*Fragaria ananassa*	HM854850	NA	NA	NA	NA
	1-2	China	*Fragaria ananassa*	HM854849	NA	NA	NA	NA
	1-3	China	*Fragaria ananassa*	HM854852	NA	NA	NA	NA
Microascospora rubi	**MFLU 15-1112**	Italy	*Rubus ulmifolia*	MF190154	MF190098	NA	MF377581	MF377611
	MFLU 17-0883	Italy	*Rubus ulmifolia*	MF190153	MF190099	NA	MF377582	MF377612
Nakataea oryzae	**CBS 243.76**	NA	NA	KM484861	DQ341498	NA	NA	NA
Oblongisporothyrium castanopsidis	**ATCC 22470**	Japan	*Castanopsis cuspidata*	MG591850	MG591943	NA	MG592038	MG976454
Ophiodiaporthe cyatheae	**YMJ1364**	China	*Cyathea lepifera*	JX570889	JX570891	NA	JX570893	NA
Pachytrype princeps	**Rogers S**	USA	NA	NA	FJ532382	NA	NA	NA
Pachytrype rimosa	FF1066	Costa Rica	NA	NA	FJ532381	NA	NA	NA
Paradiaporthe artemisiae	**MFLUCC 14-0850**	Italy	*Artemisia* sp.	MF190155	MF190100	NA	NA	NA
	MFLUCC 17-1663	Italy	*Artemisia* sp.	MF190156	MF190101	NA	NA	NA
Phaeoappendispora thailandensis	**MFLUCC 13-0161**	Thailand	*Quercus* sp.	MF190157	MF190102	NA	MF377613	NA
Phaeodiaporthe appendiculata	**CBS 123821 = D77**	Austria	*Acer campestre*	KF570156	KF570156	NA	NA	NA
	CBS 123809 = D76	Austria	*Acer campestre*	KF570155	KF570155	NA	NA	NA
Phragmoporthe conformis	**AR 3632 = CBS 109783**	Canada	*Alnus rubra*	DQ323527	AF408377	NA	NA	NA
Plagiostoma euphorbiae	**CBS 340.78**	Netherlands	*Euphorbia palustris*	EU199198	AF408382	NA	DQ368643	NA
Plagiostoma salicellum	AR 3455 = CBS 109775	Austria	*Salix* sp.	DQ323529	AF408345	NA	EU199141	EU221916
Prosopidicola mexicana	**CBS 113530**	USA	*Prosopis glandulosa*	AY720710	NA	NA	NA	NA
	CBS 113529	USA	*Prosopis glandulosa*	AY720709	KX228354	NA	NA	NA
Pseudomelanconis caryae	**CFCC 52110**	China	*Carya cathayensis*	MG682082	MG682022	NA	MG682042	MG682062
	CFCC 52111	China	*Carya cathayensis*	MG682083	MG682023	NA	MG682043	MG682063
	CFCC 52112	China	*Carya cathayensis*	MG682084	MG682024	NA	MG682044	MG682064
	CFCC 52113	China	*Carya cathayensis*	MG682085	MG682025	NA	MG682045	MG682065
Pseudoplagiostoma eucalypti	**CBS 124807**	Venezuela	*Eucalyptus urophylla*	GU973512	GU973606	NA	NA	NA
	CBS 116382	Thailand	*Eucalyptus camaldulensis*	GU973514	GU973608	NA	NA	NA
Pseudoplagiostoma oldii	CBS 115722	Australia	*Eucalyptus camaldulensis*	GU973535	GU973610	NA	NA	NA
Pseudoplagiostoma variabile	CBS 113067	Uruguay	*Eucalyptus globulus*	GU973536	GU973611	NA	NA	NA
Pyricularia grisea	**Ina168**	NA	NA	AB026819	AB026819	NA	NA	NA
Racheliella saprophytica	NTCL052-1	Thailand	*Syzygium cumini*	KJ021933	KJ021935	NA	NA	NA
Racheliella wingfieldiana	**CBS 143669**	South Africa	*Syzigium guineense*	MG591911	MG592006	NA	MG592100	MG976487
Rossmania ukurunduensis	**AR 3484**	Russia	*Acer ukurunduense*	NA	EU683075	NA	NA	NA

Saprothyrium thailandense	**MFLU 13-0260**	Thailand	Decaying leaf	MF190163	MF190110	NA	NA	NA
Sheathospora cornuta	**CFCC 51990***	China	*Cornus controversa*	MF360006	MF360008	NA	MF360002	MF360004
	CFCC 51991*	China	*Juglans regia*	MF360007	MF360009	NA	MF360003	MF360005
Sillia ferruginea	**AR 3440 = CBS 126567**	Austria	*Corylus avellana*	JF681959	EU683076	NA	NA	NA
Sphaerosporithyrium mexicanum	**CFNL 2945**	Mexico	*Quercus eduardi*	MG591896	MG591990	NA	MG592083	MG976473
Stegonsporium protopyriforme	CBS 117041	Austria	*Acer pseudoplatanus*	NR126119	EU039992	NA	NA	NA
Stegonsporium pyriforme	**CBS 124487**	UK	*Acer heldreichii*	KF570160	KF570160	NA	KF570190	NA
Stilbospora macrosperma	**CBS 121883**	Austria	*Carpinus betulus*	JX517290	JX517299	NA	KF570196	NA
	CBS 121695	Netherlands	*Carpinus betulus*	JX517288	JX517297	NA	NA	NA
Sydowiella depressula	CBS 813.79	Switzerland	*Rubus* sp.	NA	EU683077	NA	NA	NA
Sydowiella fenestrans	**AR 3777 = CBS 125530**	Russia	*Chamerion angustifolium*	JF681956	EU683078	NA	NA	NA
Synnemasporella aculeans	AR 3878 = CBS 126566	USA	*Rhus glabra*	NA	EU255134	NA	NA	NA
	CFCC 52094	China	*Rhus chinensis*	MG682086	MG682026	NA	MG682046	MG682066
	CFCC 52095	China	*Rhus chinensis*	MG682087	MG682027	NA	MG682047	MG682067
	CFCC 52096	China	*Rhus chinensis*	MG682088	MG682028	NA	MG682048	MG682068
Synnemasporella toxicodendri	**CFCC 52097**	China	*Toxicodendron sylvestre*	MG682089	MG682029	NA	MG682049	MG682069
	CFCC 52098	China	*Toxicodendron sylvestre*	MG682090	MG682030	NA	MG682050	MG682070
	CFCC 52099	China	*Toxicodendron sylvestre*	MG682091	MG682031	NA	MG682051	MG682071
Tubakia japonica	**ATCC 22472**	Japan	*Castanea crenata*	MG591886	MG591978	NA	MG592071	MG976465
	CBS 191.71	Japan	*Castanea crenata*	MG591885	MG591977	NA	MG592070	MG976464
	MUCC 2297	Japan	*Castanea crenata*	NA	MG591979	NA	MG592072	MG976466
	MUCC 2298	Japan	*Castanea crenata*	NA	MG591980	NA	MG592073	MG976467
	MUCC 2300	Japan	*Castanea crenata*	NA	MG591981	NA	MG592074	MG976468
	MUCC 2301	Japan	*Castanea crenata*	NA	MG591982	NA	MG592075	MG976469
Tubakia seoraksanensis	CBS 127490	South Korea	*Quercus mongolica*	MG591907	KP260499	NA	MG592094	NA
Tubakia sutoniana	ICMP 14042	New Zealand	*Quercus* sp.	KC145909	NA	NA	NA	KC145954
	ICMP 14043	New Zealand	*Quercus ilex*	KC145858	NA	NA	NA	KC145955

Note: ATCC: American Type Culture Collecton, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Centre, Beijing, China; CFNL: Herbarium and culture collection at the Faculty of Forestry Sciences, University of Nuevo León, México; CPC: Culture collection of Pedro Crous, The Netherlands; ICMP: International Collection of Microorganisms from Plants, New Zealand; MFLU: Mae Fah Luang University herbarium, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; NA: not applicable. All the new isolates used in this study are marked by an asterisk (*) and the strains from generic type species are in bold.

fRPB2-5F and fRPB2-7cR (Liu et al. 1999), the TEF1-α gene with the primers EF1-728F and EF1-LLErev for Melanconiellaceae (Carbone and Kohn 1999, Jaklitsch et al. 2005) and the primers EF1-983F and EF1-1567R for Melanconidaceae (Carbone and Kohn 1999, Rehner and Buckley 2005). The PCR mixture for all the regions consisted of 1 μl genomic DNA, 3 mM $MgCl_2$, 20 μM of each dNTP, 0.2 μM of each primer and 0.25 U BIOTAQ DNA polymerase (Bioline). Conditions for PCR of ITS and LSU regions constituted an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s at 51 °C and 1 min at 72 °C and a final extension step of 8 min at 72 °C, while the TEF1-α gene was performed using an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s at 56 °C and 1 min at 72 °C and a final extension step of 8 min at 72 °C. For the RPB2 amplification, conditions consisted of five cycles of 45 s at 95 °C, 45 s at 56 °C and 2 min at 72 °C, then five cycles with a 53 °C annealing temperature and 30 cycles with a 50 °C annealing temperature. The DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyzer with BigDye Terminater Kit v. 3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

DNA sequences generated by each primer combination were used to obtain consensus sequences using SeqMan v. 7.1.0 in the DNASTAR Lasergene Core Suite software package (DNASTAR Inc., Madison, WI, USA). Reference sequences were selected based on ex-type or ex-epitype sequences available from relevant published literature (Voglmayr et al. 2012, 2017, Fan et al. 2016, 2018, Du et al. 2017, Senanayake et al. 2017) (Table 1). All sequences were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html) and edited manually using MEGA v. 6 (Tamura et al. 2013). Phylogenetic analyses were performed using PAUP v. 4.0b10 for maximum parsimony (MP) analysis (Swofford 2003), MrBayes v. 3.1.2 for Bayesian Inference (BI) analysis (Ronquist and Huelsenbeck 2003) and PhyML v. 7.2.8 for Maximum Likelihood (ML) analysis (Guindon et al. 2010). The first analyses were performed on the combined multi-gene dataset (ITS, LSU, RPB2, TEF1-α) to compare isolates of Diaporthales species to ex-type sequence data from recent studies (Table 1).

A partition homogeneity test (PHT) with heuristic search and 1 000 search replicates was performed using PAUP to test for incongruence amongst the ITS, LSU, RPB2 and TEF1-α sequence datasets in reconstructing phylogenetic trees. Maximum parsimony (MP) analysis was run using 1 000 heuristic search replicates with random-additions of sequences with a tree bisection and reconnection (TBR) algorithm. Max-trees were set to 5 000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). Maximum likelihood (ML) analysis was performed with a GTR site substitution model, including a gamma-distributed rate heterogeneity and a proportion of invariant sites

(Guindon et al. 2010). The branch support was evaluated with a bootstrapping (BS) method of 1 000 replicates.

MrModeltest v. 2.3 was used to estimate the best nucleotide substitution model settings for each gene (Posada and Crandall 1998). Bayesian inference (BI) was performed based on the DNA dataset from the results of the MrModeltest, using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). Two MCMC chains were run from random trees for 1 000 M generations and stopped when the average standard deviation of split frequencies fell below 0.01. Trees were saved each 1 000 generations. The first 25% of trees were discarded as the burn-in phase of each analysis and the posterior probabilities (BPP) were calculated from the remaining trees (Rannala and Yang 1996).

In addition to the above analyses, we provided separate phylogenetic trees for Juglanconidaceae, Melanconidaceae and Melanconiellaceae, based on various gene regions (see below) and the same analyses parameters as given above. Phylograms were edited using FigTree v. 1.3.1 (Rambaut and Drummond 2010). Novel sequences generated in the current study were deposited in GenBank (Table 1). The aligned matrices used for phylogenetic analyses and the resulting trees can be found in TreeBASE (www.treebase.org; accession number: S23477).

Results

Phylogenetic analyses

The combined matrix of ITS, LSU, RPB2 and TEF1-α of Diaporthales included 209 ingroup and two outgroup taxa, comprising 3 269 characters including gaps (776 characters for ITS, 517 for LSU, 1107 for RPB2 and 869 for TEF1-α) in the aligned matrix. Of these, 1 417 characters were constant, 192 variable characters were parsimony-uninformative and 1 660 characters were parsimony informative. The MP analysis resulted in 100 most parsimonious trees (TL = 10 370, CI = 0.341, RI = 0.806, RC = 0.275) and the first tree is shown as Fig. 1. The MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Branches with significant Bayesian posterior probability (≥ 0.95) in Bayesian analyses were thickened in the phylogenetic tree. The phylogram based on four genes resolved 28 known lineages, representing 26 known families and two *incertae sedis* genera *Diaporthella* and *Phaeoappendispora* due to lack of sequence data on their types. The current 47 melanconis-like isolates are herein placed within Juglanconidaceae, Melanconidaceae and Melanconiellaceae in Diaporthales (Fig. 1). A phylogenetic tree of each family or genus was constructed separately based on different DNA datasets. Tree topologies of all genera computed from the MP, ML and Bayesian analyses were similar for the individual gene region and in the combined dataset.

For the single genus *Juglanconis* (Juglanconidaceae), a combined ITS, LSU, CAL and RPB2 matrix of 23 ingroup accessions (five from this study and 18 retrieved from

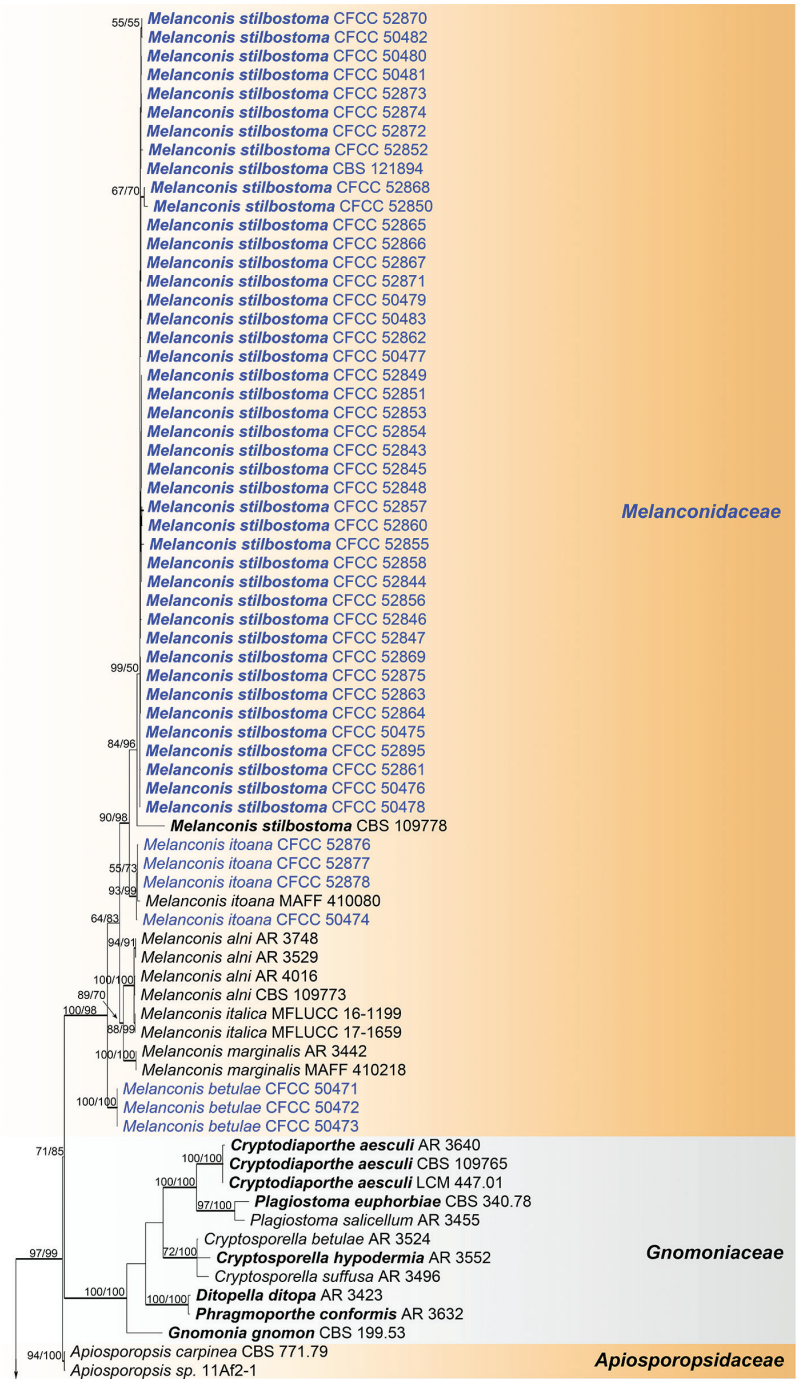

Figure 1. Phylogram of Diaporthales obtained from an MP analysis of a combined matrix of ITS, LSU, RPB2 and TEF1-α. MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 200 changes. Type species are in bold. Strains obtained in the current study are in blue.

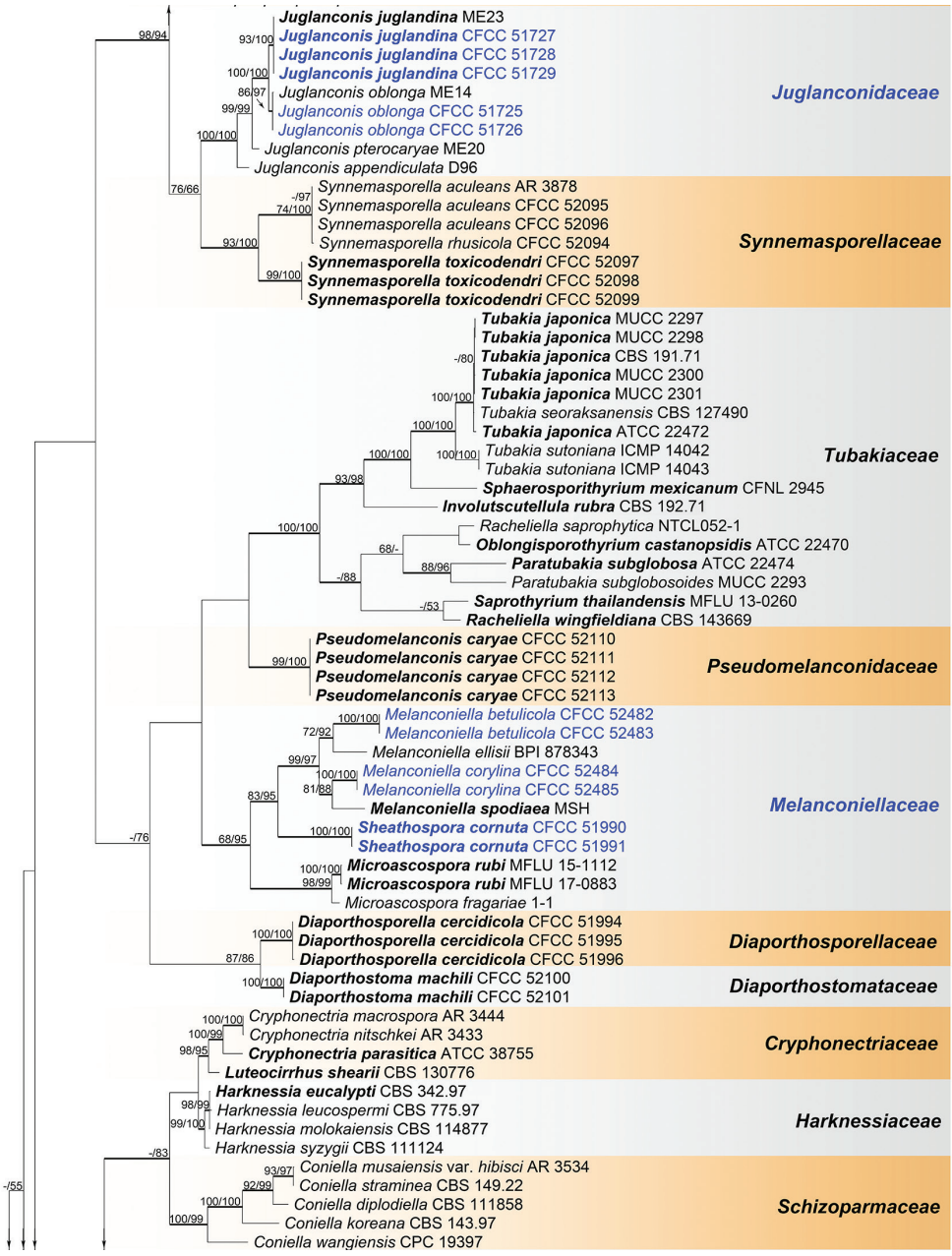

Figure 1. Continued.

GenBank) was produced, which comprised 2 736 characters including gaps (2 427 constant, 216 variable and parsimony-uninformative, 93 parsimony-informative). A heuristic MP search generated nine equally most parsimonious trees (TL = 332, CI = 0.976, RI = 0.985, RC = 0.961), one of which is shown in Fig. 2. Isolates of *Juglanconis* clustered in four clades, corresponding to the four known species in this genus.

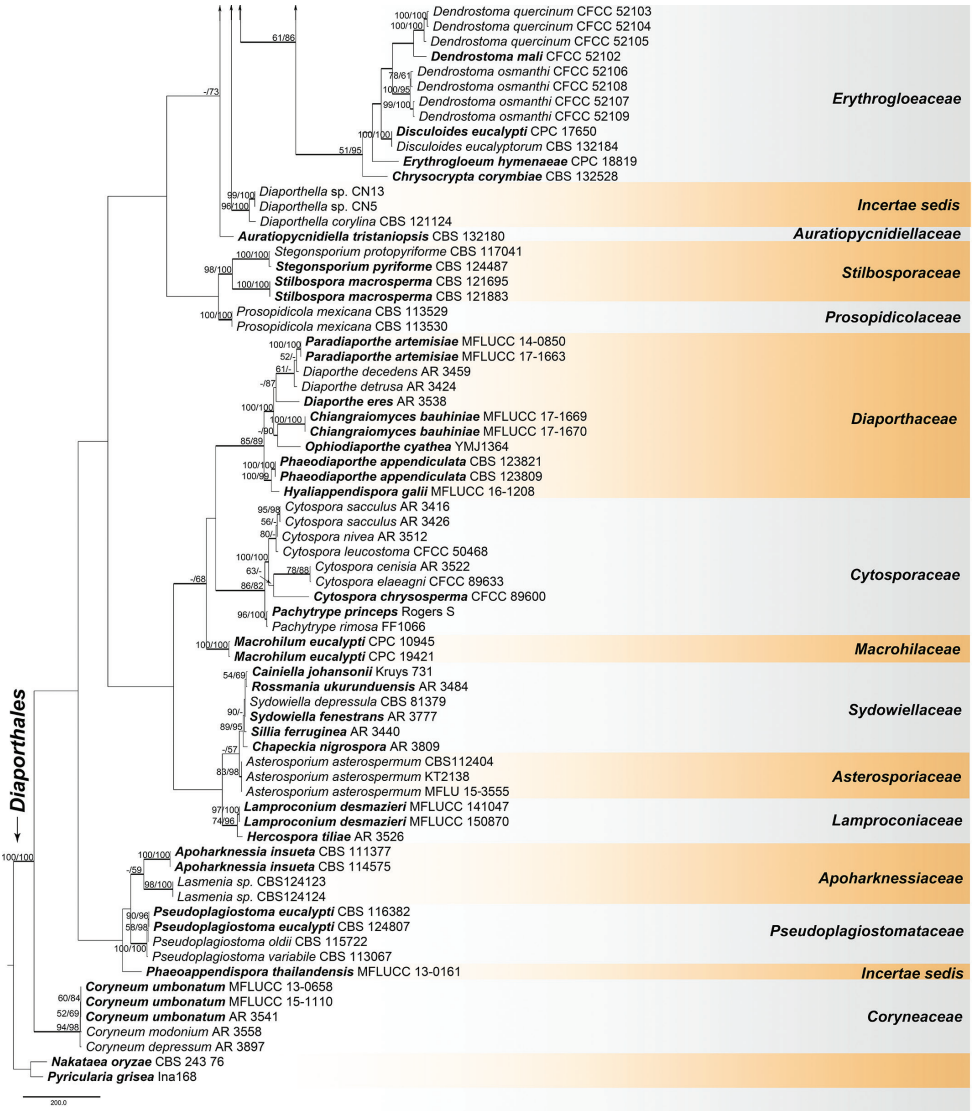

Figure 1. Continued.

The five Chinese strains sequenced in this study were revealed to belong to *Juglanconis juglandina* (3) and *J. oblonga* (2).

For Melanconiellaceae, a combined ITS, LSU, RPB2 and TEF1-α matrix was produced from 53 ingroup accessions (six from this study and 47 retrieved from GenBank), which comprised 4 122 characters including gaps (2 829 constant, 87 variable and parsimony-uninformative, 1 206 parsimony-informative). A heuristic MP search generated 24 most parsimonious trees (TL = 2 716, CI = 0.652, RI = 0.880, RC = 0.573), one of which is shown in Fig. 5. Isolates of Melanconiellaceae clustered in three clades, corresponding to the type genus *Melanconiella*, *Microascospora* and a line-

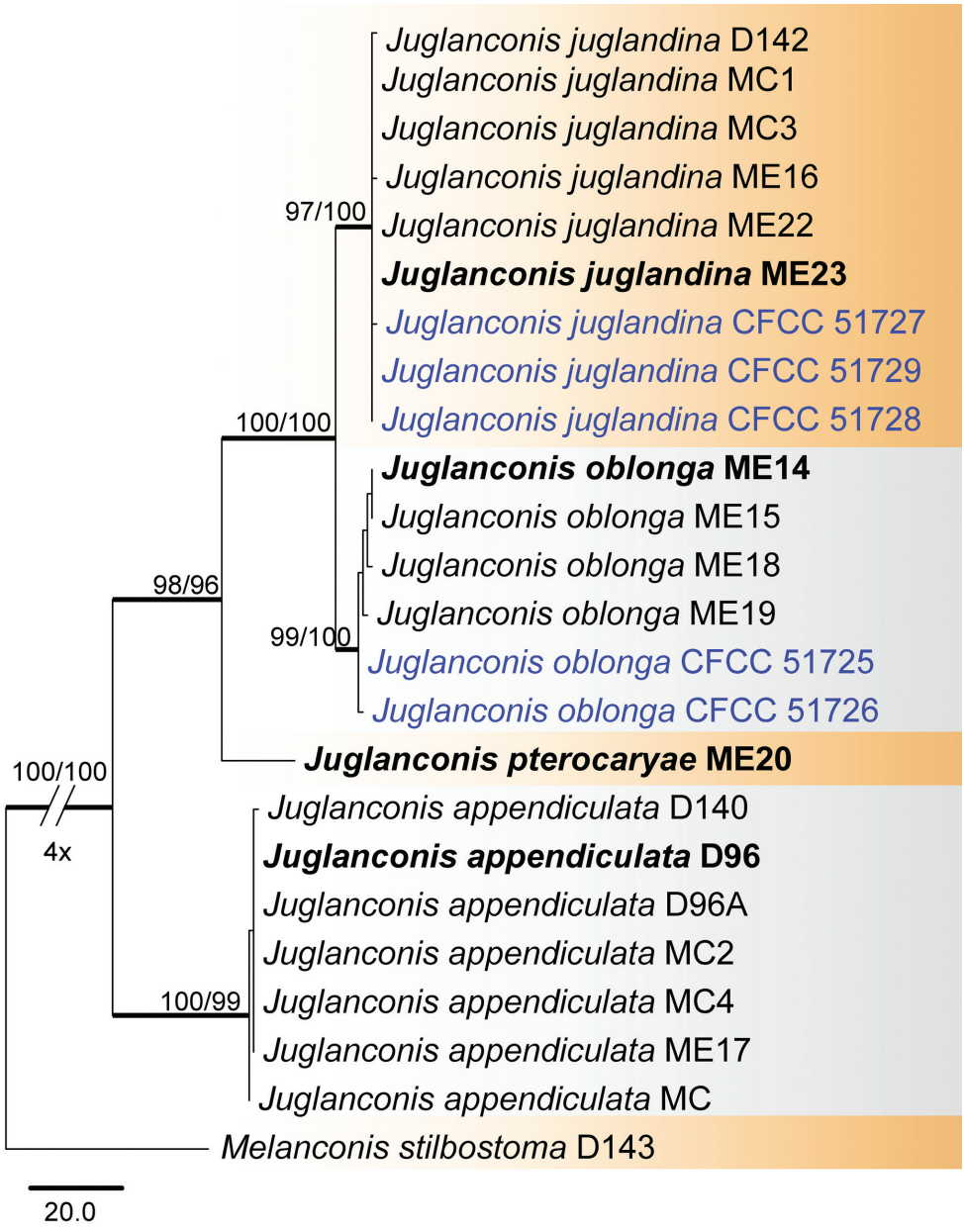

Figure 2. Phylogram of *Juglanconis* (Juglanconidaceae) obtained from an MP analysis of a combined matrix of ITS, LSU, CAL and RPB2. MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 20 changes. Type species are in bold. Strains obtained in the current study are in blue.

age described as the new genus *Sheathospora* below. *Melanconiella betulicola* and *M. corylina* formed two distinct strongly supported clades (MP/ML/BI = 100/100/1), which differ from the other species of the *Melanconiella* clade.

For the single genus *Melanconis* (Melanconidaceae), a combined ITS, LSU, RPB2 and TEF1-α matrix was produced for 57 ingroup accessions (49 from this study and eight retrieved from GenBank), which comprised 2 597 characters including gaps (2 238 constant, 219 variable and parsimony-uninformative, 140 parsimony-informative). A heuristic MP search generated 144 most parsimonious trees (TL = 459, CI = 0.861, RI = 0.919, RC = 0.791), one of which is shown in Fig. 6. Isolates of *Melanconis* clustered in six clades, corresponding to six known species in this genus. *Melanconis betulae*, *Ms. stilbostoma* and *Ms. itoana* were confirmed from China in this study.

Taxonomy

Juglanconidaceae Voglmayr & Jaklitsch, Persoonia 38: 142 (2017)

Type genus. *Juglanconis* Voglmayr & Jaklitsch, Persoonia 38: 142 (2017)

Notes. Juglanconidaceae, with the single genus *Juglanconis*, was newly introduced by Voglmayr et al. (2017) for *Melanconium juglandinum*, *M. oblongum* and *M. pterocaryae*. In this paper, we provide an updated tree including accessions of two *Juglanconis* species from China (Fig. 2).

Juglanconis Voglmayr & Jaklitsch, Persoonia 38: 142 (2017)

Type species. *Juglanconis juglandina* (Kunze) Voglmayr & Jaklitsch, Persoonia 38: 144 (2017).

Notes. *Juglanconis* was newly introduced by Voglmayr et al. (2017). The genus is characterised by having perithecial ascomata, octosporous asci with an apical ring, hyaline, bicellular ascospores with or without gelatinous appendages and acervular conidiomata with brown conidia with gelatinous sheaths and with verruculous inner surface of the conidal wall (Voglmayr et al. 2017). *Juglanconis* includes four species (*J. appendiculata, J. juglandina, J. oblonga* and *J. pterocariae*), which were restricted to host in Juglandaceae (Voglmayr et al. 2017).

Juglanconis juglandina (Kunze) Voglmayr & Jaklitsch, Persoonia 38: 144 (2017)
Fig. 3

≡*Melanconium juglandinum* Kunze, Fl. Dresd., 2. Aufl.: 260. 1823.

Descriptions. Conidiomata acervular, immersed in host bark, erumpent from surface of host branches, scattered or occasionally confluent, 1.5–2.5 mm, covered by black discharged conidial masses at maturity, usually conspicuous. Ectostromatic disc straw to honey, surrounded by bark or not. Central column beneath the disc more

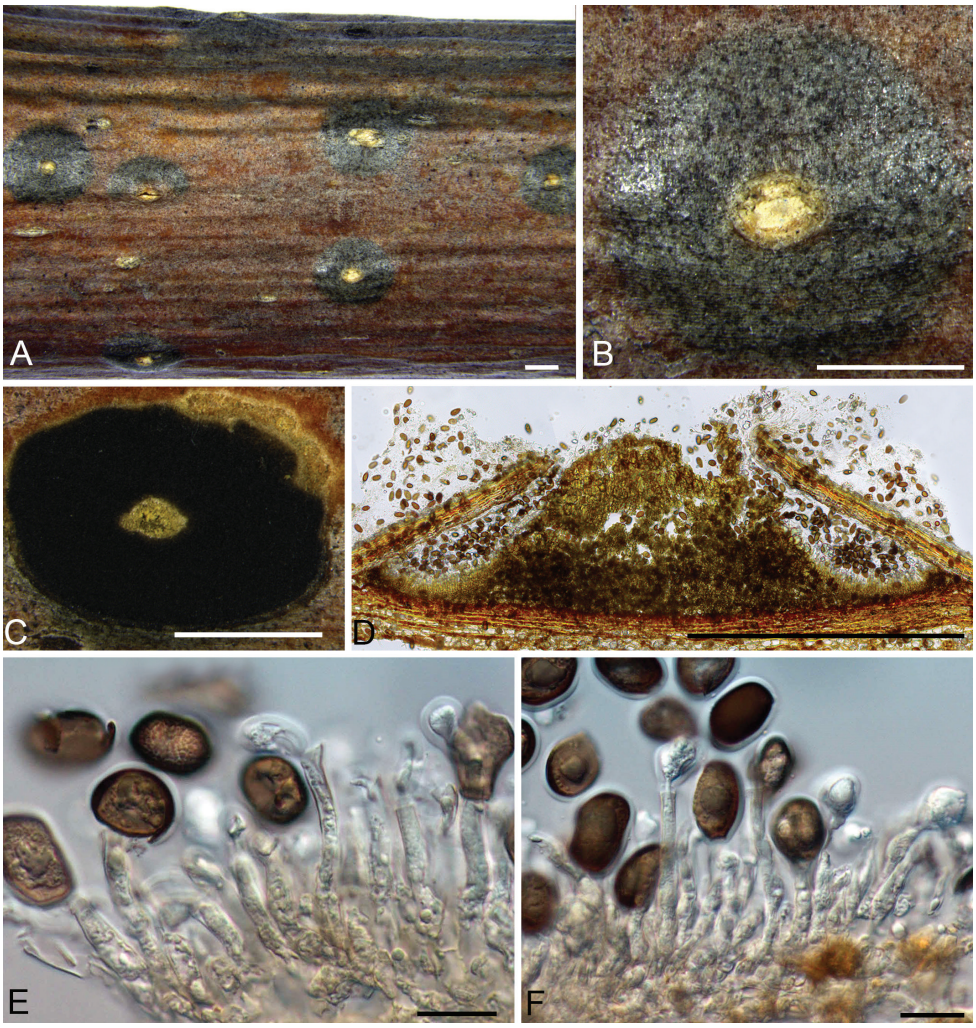

Figure 3. Morphology of *Juglanconis juglandina* from *Juglans regia*. **A–B** habit of acervuli on branches **C** transverse section through acervulus **D** longitudinal section through acervulus **E–F** conidiophores, conidiogenous cells and conidia. Scale bars: 1 mm (**A–D**), 20 µm (**E–F**).

or less conical, straw to buff. Conidiophores cylindrical to lageniform, simple, rarely branched at the base, smooth, subhyaline to pale brown. Conidiogenous cells annellidic with distinct annellations, integrated. Conidia unicellular, initially hyaline, becoming brown to blackish when mature, broadly ellipsoid to broadly pip-shaped, truncate with distinct scar at the base, densely multiguttulate, thick-walled, (17–)19–22(–24.5) × (9–)11–14(–16.5) µm (av. = 20 × 13 µm, n = 50), with 0.8–1 µm wide gelatinous sheath. Sexual morph was not observed.

Culture characteristics. On PDA, cultures are initially white, becoming straw after 3–5 d and grey olivaceous after 7–10 d. The colonies are felty with an irregular edge; sterile.

Materials examined. (all on twigs and branches of *Juglans regia*). CHINA, Gansu Province, Qingyang City, Shishe village, 35°38'17.08"N, 107°47'48.68"E, 14 July 2013, X.L. Fan (BJFC-S908; living culture, CFCC 51727); Gansu Province, Qingyang City, Zhongwan Forest Farm, 35°26'26.33"N, 108°34'09.38"E, 11 July 2013, X.L. Fan (BJFC-S947; living culture, CFCC 51728); Gansu Province, Qingyang City, Zhongwan Forest Farm, 35°26'25.52"N, 108°34'09.03"E, 11 July 2013, X.L. Fan (BJFC-S955; living culture, CFCC 51729).

Notes. *Juglanconis juglandina* is the type species of *Juglanconis* and is thus far only known to occur on *Juglans regia* distributed in Asia and Europe (Voglmayr et al. 2017). *Juglanconis juglandina* is described based on *Melanconium juglandinum* (= *Melanconis carthusiana*) (Voglmayr et al. 2017), which was regarded as the main causal agent of canker and dieback disease of *Juglans regia* in China (China Microbiology and Virology Databases, http://www.micro.csdb.cn/).

***Juglanconis oblonga* (Berk.) Voglmayr & Jaklitsch, Persoonia 38: 147 (2017)**
Fig. 4

≡ *Melanconium oblongum* Berk., Grevillea 2 (no. 22): 153. 1874.
= *Diaporthe juglandis* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 45: 448. 1893.
≡ *Melanconis juglandis* (Ellis & Everh.) A.H. Graves, Phytopathology 13: 311. 1923.

Descriptions. Pseudostromata immersed in host bark, distinctly erumpent from surface of host branches, 1.5–3 mm diam. Ectostromatic disc indistinct, usually circular, greyish to brownish. Perithecia often appearing as rounded bumps beneath the bark surface surrounding the ectostromatic disc, prolonged black neck from the top, (450–)525–700(–780) µm diam. (av. = 580 µm, n = 30). Asci hyaline, clavate to fusoid, (120–)122–135 × (12.5–)13–16.5 (–17) µm (av. = 126.5 × 15 µm, n = 20). Ascospores hyaline, ellipsoid, broadly ellipsoid or broadly fusoid, symmetric to slightly asymmetric, straight, rarely slightly curved, constricted at the septum, (17–)17.5–22(–23.5) × (7.5–)8–10.5(–11) µm (av. = 19.5 × 9.5 µm, n = 50). Conidiomata acervular, immersed in host bark, erumpent from surface of host branches, scattered or occasionally confluent, 1–2 mm, covered by black discharged conidial masses at maturity, usually conspicuous. Ectostromatic disc buff to honey, surrounded by bark or not. Central column beneath the disc more or less conical, isabelline to olivaceous grey. Conidiophores cylindrical to lageniform, simple, rarely branched at the base, smooth, subhyaline to pale brown. Conidiogenous cells annellidic with distinct annellations, integrated. Conidia unicellular, initially hyaline, becoming brown to blackish when mature, broadly ellipsoid to broadly pip-shaped, truncate with distinct scar at the base, densely multiguttulate, thick-walled, (14–)19–23.5(–28) × (6.5–)9–13(–15) µm (av. = 22 × 12.5 µm, n = 50), with 0.8–1 µm wide gelatinous sheath.

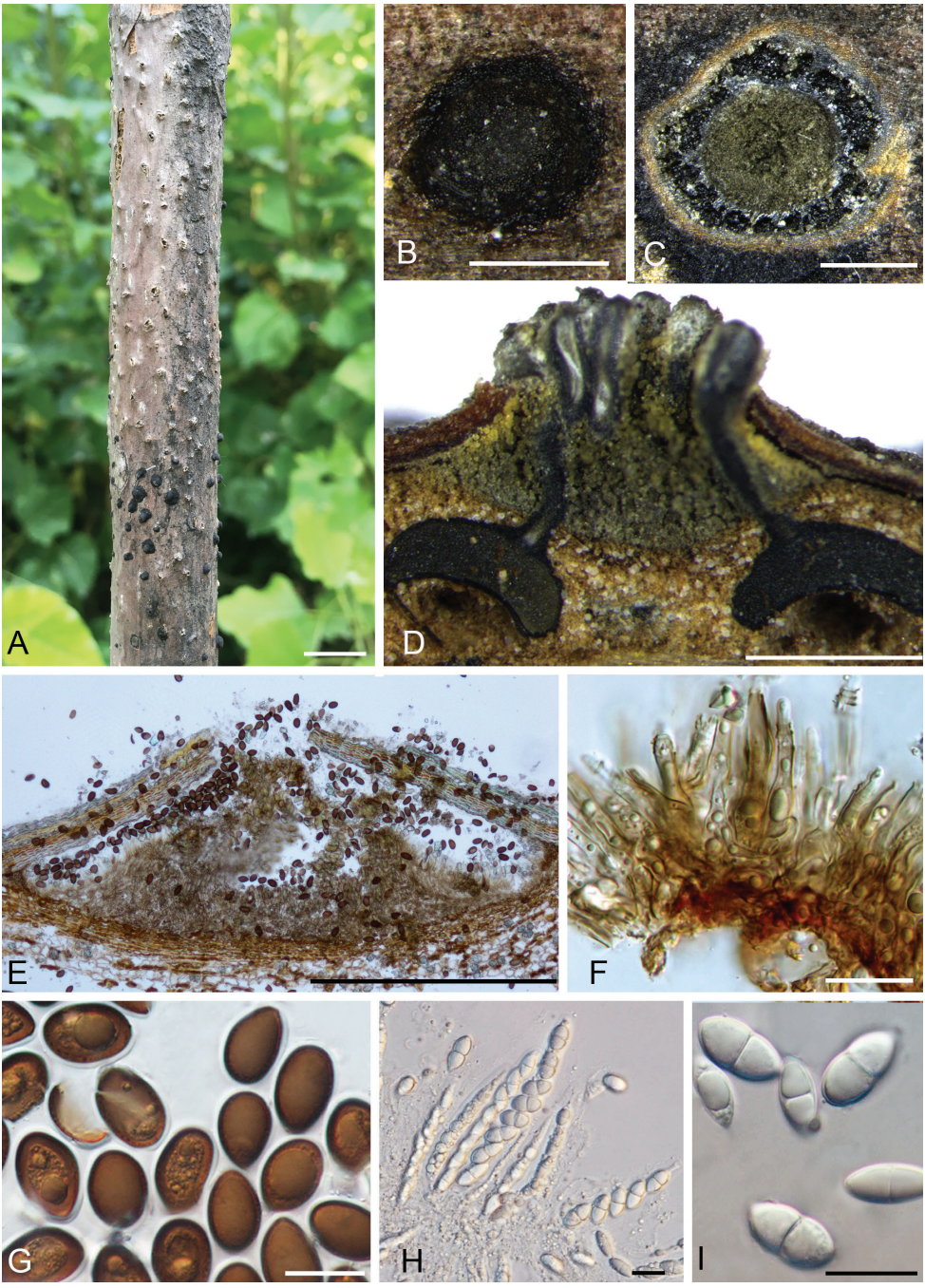

Figure 4. Morphology of *Juglanconis oblonga* from *Juglans regia*. **A–B** habit of acervuli on branches **C** transverse section through acervulus **D** longitudinal section through perithecia **E** longitudinal section through acervulus **F** conidiophores, conidiogenous cells **G** conidia **H** asci and ascospores **I** ascospores. Scale bars: 10 mm (**A**), 500 µm (**B–E**), 20 µm (**F–I**).

Culture characteristics. On PDA, cultures are initially white, becoming pale olivaceous grey after 10 d. The colonies are felty with an irregular edge; texture uniform; sterile.

Materials examined. (all on twigs and branches of *Juglans regia*). CHINA, Heilongjiang Province, Harbin City, Linan, Heilongjiang Botanical Garden, 45°42'21.10"N, 126°38'42.87"E, 2 August 2016, Q. Yang & Z. Du (BJFC-S1374; living culture, CFCC 51725; *ibid.* CFCC 51726).

Notes. *Juglanconis oblonga* is based on *Melanconium oblongum* (= *Melanconis juglandis*) (Voglmayr et al. 2017). This species can be distinguished from *J. juglandina* by on average longer length of conidia (22 × 12.5 *vs.* 20 × 13 µm). However, there is a substantial size overlap between both species and sequence data are sometimes necessary for reliable species identification. It was also recorded to cause canker and dieback disease of *Juglans regia* in China (China Microbiology and Virology Databases, http://www.micro.csdb.cn/).

Melanconidaceae G. Winter, Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 764 (1886)

Type genus. *Melanconis* Tul. & C. Tul., Select. fung. carpol. (Paris) 2: 115 (1863)

Notes. Melanconidaceae was introduced by Winter (1886) and subsequently involved many genera with perithecia immersed in a well-developed stroma with ostioles (beaks) that emerge through an ectostromatic disc (Barr 1978). Castlebury et al. (2002) and Rossman et al. (2007) reduced this family to the type genus *Melanconis* based on LSU rDNA sequences. In this paper, we provide an updated tree with additional isolates of *Melanconis* (Melanconidaceae) from China (Fig. 5). All species have been described and illustrated by Fan et al. (2016).

Melanconis Tul. & C. Tul., Select. fung. carpol. (Paris) 2: 115 (1863)

Type species. *Melanconis stilbostoma* (Fr.) Tul. & C. Tul., Select. fung. carpol. (Paris) 2: 115 (1863)

Notes. The type genus *Melanconis* was established by Tulasne and Tulasne (1863) based on *Sphaeria stilbostoma* Fr. This genus is characterised by circularly arranged perithecia immersed in well developed to reduced entostromata with a concolourous central column and ostioles erumpent through a light-coloured ectostromatic disc with hyaline, one-septate ascospores; acervuli with light-coloured central column producing brown to olive-brown, fusiform to pyriform alpha conidia and hyaline, cylindrical or allantoid beta conidia (Barr 1978; Castlebury et al. 2002; Voglmayr et al. 2012; Fan et al. 2016). *Melanconis* has approximately 105 species epithets recorded in Index Fungorum (2018), whereas Rossman et al. (2007) suggested that many of the species previously residing in *Melanconis* may belong somewhere else. Fan et al. (2016) provided an account on this genus including five species (*Melanconis alni*, *Ms. betulae*, *Ms. marginalis*, *Ms. itoana* and the type species *Ms. stilbostoma*), which were restricted to hosts in Betulaceae.

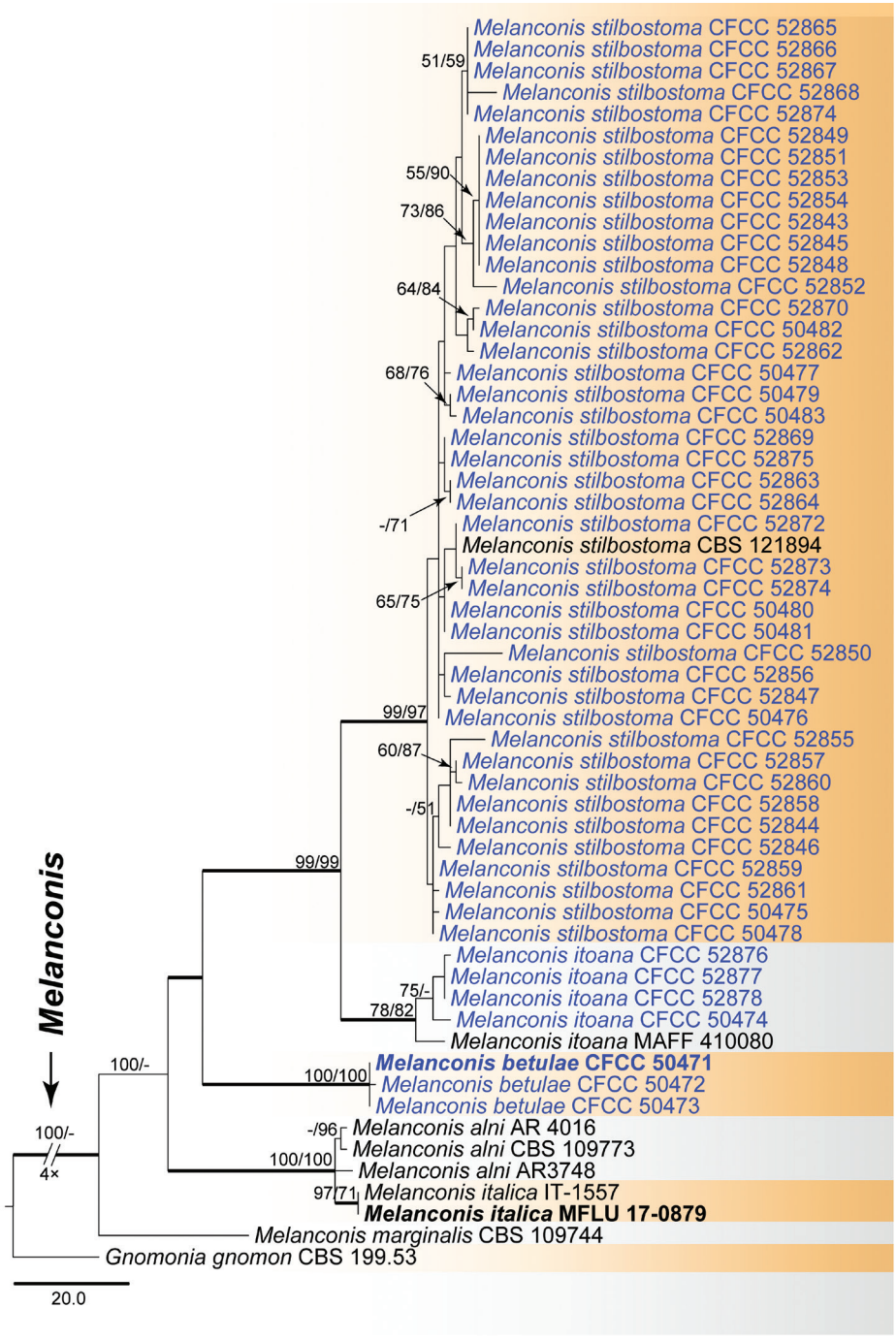

Figure 5. Phylogram of *Melanconis* (Melanconidaceae) obtained from an MP analysis of a combined matrix of ITS, LSU, RPB2 and TEF1-α. MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 20 changes. Type species are in bold. Strains obtained in the current study are in blue.

***Melanconis betulae* C.M. Tian & X.L. Fan, Mycol. Progr. 15(4/40): 4 (2016)**

Materials examined. (all on twigs and branches of *Betula albosinensis*). CHINA, Gansu Province, Gannan Tibetan Autonomous Prefecture, Zhouqu County, Qiban Forestry Centre, 33°56'35.36"N, 104°07'13.03"E, 20 August 2014, Y.M. Liang (BJFC-S1319, holotype; living ex-type culture, CFCC 50471); Gansu Province, Gannan Tibetan Autonomous Prefecture, Zhouqu County, Qiban Forestry Centre, 33°56'37.05"N, 104°07'13. 78"E, 20 August 2014, Y.M. Liang (BJFC-S13200; living culture, CFCC 50472); Gansu Province, Gannan Tibetan Autonomous Prefecture, Zhouqu County, Qiban Forestry Centre, 33°56'34.44"N, 104°07'15.59"E, 20 August 2014, Y.M. Liang (BJFC-S1321; living culture, CFCC 50473).

Notes. *Melanconis betulae* was described from *Betula albosinensis* (Fan et al. 2016). Morphologically, *M. betulae* is characterised by ovoid, olive-brown, aseptate alpha conidia, which are different from other *Melanconis* species but similar to the type species *Ms. stilbostoma*. However, it can be distinguished by the smaller length of its alpha conidia (10 *vs.* 12 μm) and sequence data.

***Melanconis itoana* Tak. Kobay., Bull. Govt Forest Exp. Stn Meguro 226: 19 (1970)**

Materials examined. (all on twigs and branches of *Betula albosinensis*). CHINA, Gansu Province, Gannan Tibetan Autonomous Prefecture, Zhouqu County, Qiban Forestry Centre, 33°56'34.49"N, 104°07'15.21"E, 20 August 2014, X.L. Fan (BJFC-S1322; living culture, CFCC 50474); Shaanxi Province, Ankang City, Ningshan County, Huoditang Forest Farm, 33°26'24.80"N, 108°26'45.10"E, 3 August 2015, Q. Yang (BJFC-S1349; living culture, CFCC 52877; ibid, CFCC 52878); Jilin Province, Jiaohe City, Haiqing Forest Farm, 43°79'88.71"N, 127°15'83.04"E, 26 June 2017, X.W. Wang (CF 20170668; living culture, CFCC 52876).

Notes. *Melanconis itoana* was described from *Betula ermanii* in Japan (Kobayashi 1970). Fan et al. (2016) isolated it from *Betula albosinensis* as a new record in China. *Melanconis itoana* is characterised by fusoid, green-brown alpha conidia with acute ends (13 × 4 μm) and hyaline, cylindrical or crescent beta conidia (9.5 × 1.5 μm).

***Melanconis stilbostoma* (Fr.) Tul. & C. Tul., Select. fung. carpol. (Paris) 2: 115 (1863)**

Materials examined. (all on twigs and branches of *Betula platyphylla*). CHINA, Tibet Autonomous Region, Linzhi City, Juemu Valley, 29°39'50.13"N, 94°18'50.70"E, 22 July 2016, X.L. Fan (CF 20160703; living culture, CFCC 528433); Heilongjiang Province, Yichun City, Dailing District, Liangshui Natural Reserve, 47°11'05.26"N, 128°57'26.15"E, 29 July 2016, Q. Yang & Z. Du (CF 20161703; living culture, CFCC 52867); Heilongjiang Province, Harbin City, Heilongjiang Botanical Garden, 45°42'27.58"N, 126°38'36.72"E, 2 August 2016, Q. Yang & Z. Du (CF 20161709; living culture, CFCC 52868); Qinghai Province, Menyuan City, Xianmi Forest Farm,

37°16'35.27"N, 101°46'53.78"E, 3 September 2016, J.H. Zuo (CF 20160911; living culture, CFCC 52865); Ningxia Autonomous Region, Yinchuan City, Helan County, Taihedizhonghai, 38°31'50.40"N, 106°17'46.10"E, 5 August 2015, X.L. Fan & Z. Du (CF 20150802; living culture, CFCC 52873); Ningxia Autonomous Region, Jingyuan City, Jingguan Road, 35°29'50.32"N, 106°18'27.10"E, 13 August 2014, X.L. Fan & Z. Du (BJFC-S1324; living culture, CFCC 50476); Beijing City, Tongzhou District, Song Village, 35°59'49.50"N, 116°39'32.35"E, 20 May 2015, X.L. Fan (BJFC-S1325; living culture, CFCC 50477); other materials with similar locations and hosts are listed in Table 1.

Notes. *Melanconis stilbostoma* is the type species of *Melanconis* and is thus far only known to occur on *Betula* spp. with a worldwide distribution (Fan et al. 2016). *Betula pendula*, *B. rotundifolia* and *B. tianschanica* are recorded as hosts in China (Zhuang 2005). The current investigation suggested that this species is restricted to and widespread on *Betula platyphylla* in China.

Melanconiellaceae Senan., Maharachch. & K.D. Hyde, Stud. Mycol. 86: 275 (2017)

Type genus. *Melanconiella* Sacc., Syll. fung. (Abellini) 1: 740 (1882)

Notes. Melanconiellaceae was validated by Senanayake et al. (2017) for the invalid Melanconiellaceae of Locquin (1984). Senanayake et al. (2017) emended this family to accommodate *Dicarpella*, *Greeneria*, *Melanconiella*, *Microascospora* and *Tubakia*. Braun et al. (2018) recommended an exclusion of *Dicarpella*, *Greeneria* and *Tubakia*. In this paper, we introduce the new genus *Sheathospora* and two new species of *Melanconiella* in Melanconiellaceae (Fig. 6).

***Melanconiella* Sacc., Syll. fung. (Abellini) 1: 740 (1882)**

Type species. *Melanconiella spodiaea* (Tul. & C. Tul.) Sacc., Syll. fung. (Abellini) 1: 740 (1882)

Notes. The genus *Melanconiella* was established by Saccardo (1882) for two species, *Melanconis spodiaea* Tul. & C. Tul. and *M. chrysostroma* (Fr.) Tul. & C. Tul. The genus subsequently entered a long period of confusion with a broad concept of the melanconidaceous genera *Melanconium* and *Melanconis* Tul. & C. Tul. (Wehmeyer 1937, 1941; Barr 1987). *Melanconiella* has 37 species epithets recorded in Index Fungorum (2018). Voglmayr et al. (2012) revised the generic circumscriptions of *Melanconiella* with 13 accepted species, excluded numerous species and confirmed that it is genetically distinct from the genus *Melanconis* based on morphology and multi-gene phylogeny (ITS, LSU, RPB2 and TEF1-α). *Melanconiella* is characterised by forming circularly arranged perithecia immersed in the substrate with oblique or lateral ostioles convergent and erumpent through an ectostromatic disc with dark coloured or hyaline ascospores; acervuli with light-coloured central column, producing dark brown melanconium-like or hyaline discosporina-like conidia (not in the same species) (Barr 1978;

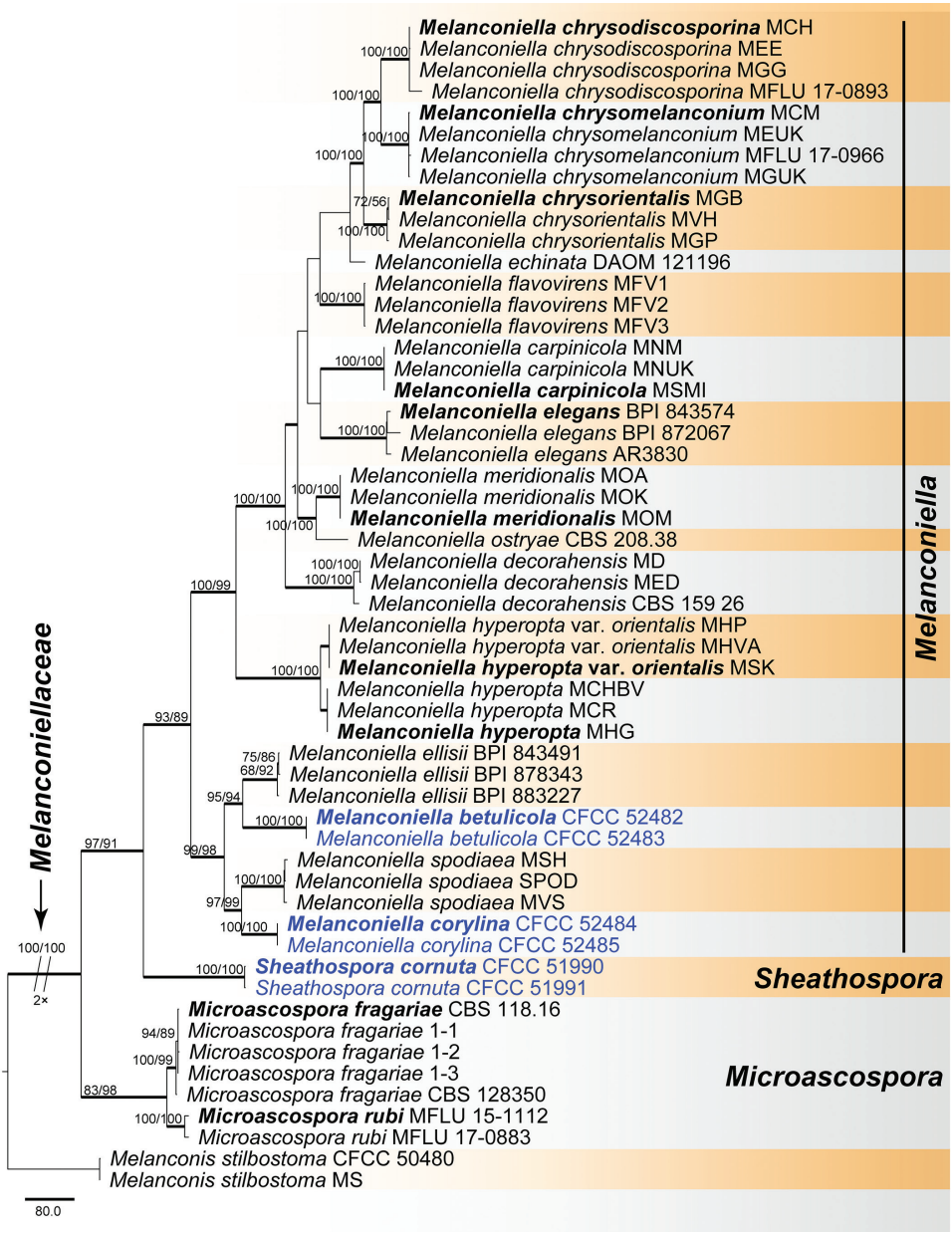

Figure 6. Phylogram of Melanconiellaceae obtained from an MP analysis from a combined matrix of ITS, LSU, RPB2 and TEF1-α. MP and ML bootstrap support values above 50% are shown at the first and second position, respectively. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 80 changes. Type species are in bold. Strains obtained in the current study are in blue.

Voglmayr et al. 2012). *Melanconiella* species were observed to be highly host-specific, as they were found to be confined to a single genus or sometimes even species within the host family Betulaceae from Europe and North America (Voglmayr et al. 2012).

***Melanconiella betulicola* Fan, sp. nov.**
MycoBank MB828427
Fig. 7

Etymology. *betulicola* (Lat.): referring to the host genus on which it was collected, *Betula*.

Diagnosis. This species is distinguished by hyaline ascospores, (16.5–)18–22(–24) × (3–)4–6 μm, with slightly constricted at the septum and with hyaline broad cap-like appendages at both ends.

Holotype. CHINA. Shaanxi Province: Ningshan County, Huoditang Forest Farm, Huodi Valley, 33°26'36.32"N, 108°26'46.48"E, 3 August 2015, on twigs and branches of *Betula albosinensis*, Q. Yang (BJFC-S1347 holotype; living culture, CFCC 52482).

Descriptions. Pseudostromata inconspicuous, immersed in host bark, slightly erumpent from surface of host branches, 1.5–3 mm diam. Ectostromatic disc indistinct, usually circular, buff to hazel. Central column circular, mouse grey to iron grey. Ostioles numerous, violaceous black to black, scarcely projecting, 70–150 μm diam. Perithecia flask-shaped to spherical, arranged circularly or irregularly, 7–12 per disc, often appearing as rounded bumps beneath the bark surface surrounding the ectostromatic disc, (320–)350–550(–610) μm diam. (av. = 480 μm, n = 30). Asci hyaline, clavate to fusoid, (50–)55–65(–70) × (7–)8.5–14(–16) μm (av. = 60 × 11 μm, n = 20). Ascospores hyaline, ellipsoid, broadly ellipsoid or broadly fusoid, 2–4 guttulate, symmetric to slightly asymmetric, straight, rarely slightly curved, slightly constricted at the septum, (16.5–)18–22(–24) × (3–)4–6 μm (av. = 20 × 4.5 μm, n = 50), with hyaline broad cap-like appendages at both ends. Conidiomata acervular, immersed in host bark, erumpent from surface of host branches, scattered or occasionally confluent, 1.3–2.5 mm, covered by fawn to dark brick discharged conidial masses at maturity, usually conspicuous. Ectostromatic disc inconspicuous. Central column beneath the disc more or less conical, olivaceous grey to iron grey. Conidiophores hyaline, smooth, cylindrical to lageniform, simple, rarely branched at the base. Conidiogenous cells hyaline, phialidic. Conidia unicellular, hyaline, narrowly ellipsoid, elongate to slightly allantoid, (9.5–)10–13.5(–15) × (2–)3–4.5(–5.5) μm (av. = 13 × 3.5 μm, n = 50), with 0.5 μm wide gelatinous sheath.

Culture characteristics. On PDA, cultures are initially white, becoming greyish-sepia after 3 d and distensible radially after 10 d. The colonies are felty with an irregular edge; texture uniform; sterile.

Additional material examined. CHINA. Shaanxi Province: Ningshan County, Huoditang Forest Farm, Huodi Valley, 33°26'37.53"N, 108°26'44.14"E, 3 August 2015, on twigs and branches of *Betula albosinensis*, Q. Yang (CF 20150847; living culture, CFCC 52483);

Notes. *Melanconiella betulicola* is associated with canker disease of *Betula albosinensis* in China. It is similar to *M. ellisii* but differs by larger ascospores (18–22 × 4–6 *vs.* 12.5–16 × 4.0–5.5 μm) with hyaline, broad cap-like appendages at both ends (Voglmayr et al. 2012), distribution (China *vs.* eastern North America) and a different host,

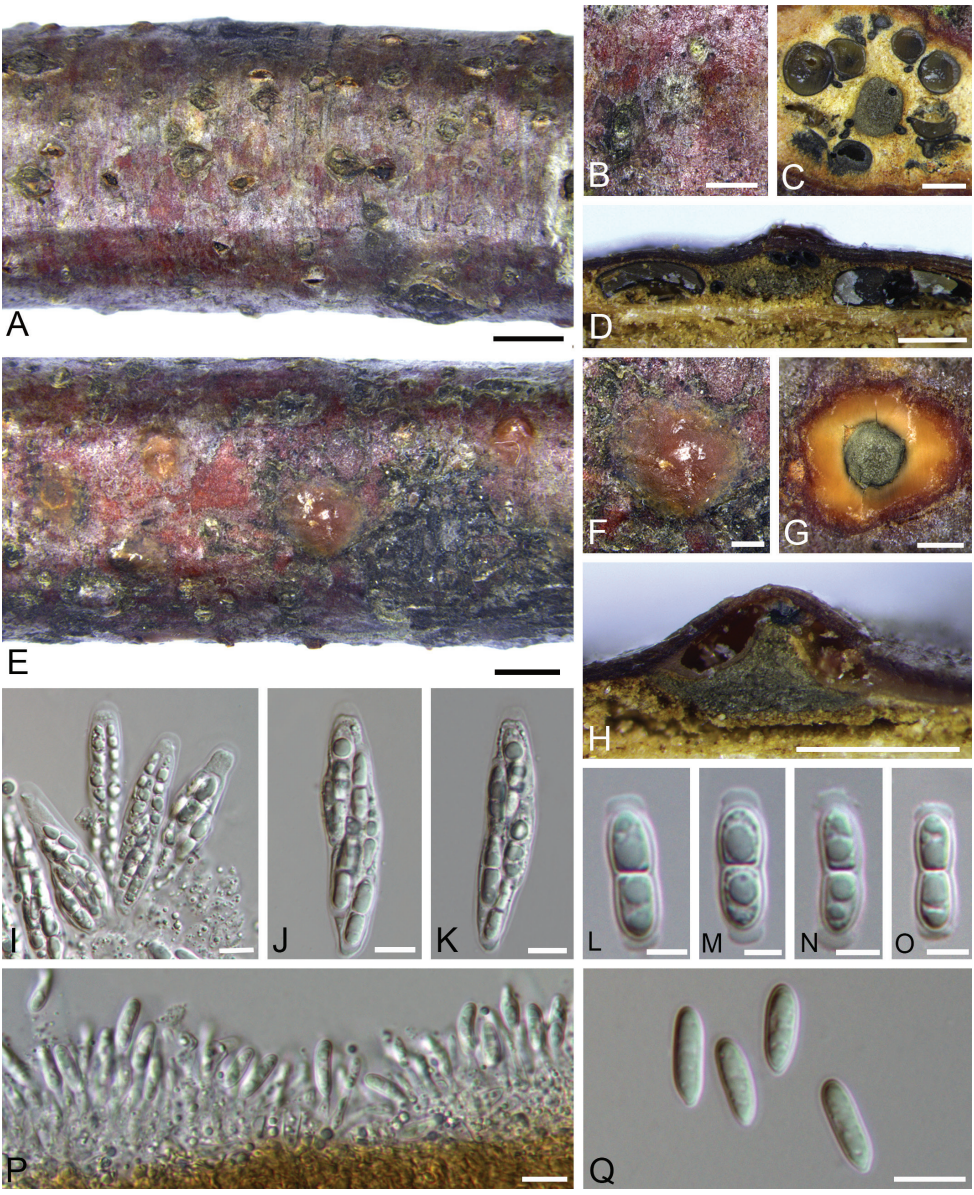

Figure 7. Morphology of *Melanconiella betulicola* from *Betula albosinensis*. **A–B** habit of pseudostromata on branches **C** transverse section through perithecia **D** longitudinal section through perithecia **E–F** habit of acervuli on branches **G** transverse section through acervulus **H** longitudinal section through acervulus **I** asci and ascospores **J–K** ascus and ascospores **L–O** ascospores **P** conidiophores, conidiogenous cells and conidia **Q** conidia. Scale bars: 2 mm (**A, E**), 500 μm (**B–D, F–H**), 10 μm (**J–K, P–Q**), 5 μm (**L–O**).

Betula albosinensis vs. Carpinus caroliniana. *Melanconiella decorahensis* also occurs on *Betula* (in Europe and North America) and it can be distinguished from *M. betulicola* based on dark brown ascospores without appendages and dark brown conidia (Vogl-

mayr et al. 2012). The clear phylogenetic position confirmed a distinction from all other available strains included in this study and we therefore result in our decision to describe this species as new, based on DNA sequence data and morphology.

Melanconiella corylina **Fan, sp. nov.**
MycoBank MB828428
Fig. 8

Etymology. *corylina* (Lat.): referring to the host genus on which it was collected, *Corylus*.

Diagnosis. This species is distinguished by acervuli erumpent through circularly cracked host bark and covered by olivaceous buff to honey discharged conidial masses at maturity; conidia unicellular, hyaline, with various shapes and 1–3 guttulate, (7–)8–13.5(–14.5) × (2–)2.5–4(–5) μm.

Holotype. CHINA. Shaanxi Province: Baoji County, Taibai Mountain, 34°15'43.32"N, 107°88'42.16"E, 13 July 2017, on twigs and branches of *Corylus mandshurica*, N. Jiang (BJFC-FB56 holotype; living culture, CFCC 52484).

Descriptions. Conidiomata acervular, immersed in host bark, erumpent from surface of host branches, scattered or occasionally confluent, 1–1.5 mm, erumpent through circularly cracked host bark and covered by olivaceous buff to honey discharged conidial masses at maturity, usually conspicuous. Ectostromatic disc inconspicuous and cracked circularly at maturity. Central column beneath the disc more or less oblate, iron grey to dark grey. Conidiophores hyaline, smooth, cylindrical, simple, rarely branched at the base. Conidiogenous cells hyaline, phialidic. Conidia unicellular, hyaline, narrowly ellipsoid to fusoid, elongate to slightly allantoid, 1–3 guttulate, (7–)8–13.5(–14.5) × (2–)2.5–4(–5) μm (av. = 10 × 3.5 μm, n = 50) μm (av. = 13 × 3.5 μm, n = 50). Sexual morph was not observed.

Culture characteristics. On PDA, cultures are initially white, becoming fuscous black in the centre and edge after 5 d. The colonies are felty with an irregular edge; texture uniform; sterile.

Additional material examined. CHINA. Shaanxi Province: Baoji County, Taibai Mountain, 34°15'40.05"N, 107°88'43.33"E, 13 July 2017, on twigs and branches of *Corylus mandshurica*, N. Jiang (CF 20170756 holotype; living culture, CFCC 52485).

Notes. *Melanconiella corylina* is associated with canker disease of *Corylus mandshurica* in China. It can be distinguished from its closest relative, the generic type *M. spodiaea* growing in *Carpinus* spp., by its hyaline, discosporina-like conidia, and the smaller size of conidia (8–13.5 × 2.5–4 *vs.* 13.3–15.2 × 7.5–8.5 μm) as well as the hosts (Voglmayr et al. 2012). *Melanconiella flavovirens* also occurs on *Corylus* (in Europe and North America), and it can be distinguished from *M. corylina* based on larger conidia (12–15 × 5.0–5.5 *vs.* 8–13.5 ×2.5–4 μm) (Voglmayr et al. 2012). The phylogenetic inferences indicated *M. corylina* as an individual well-supported clade (MP/ML/BI=100/99/1) within *Melanconiella* and we therefore describe it as new, based on sequence data and morphology.

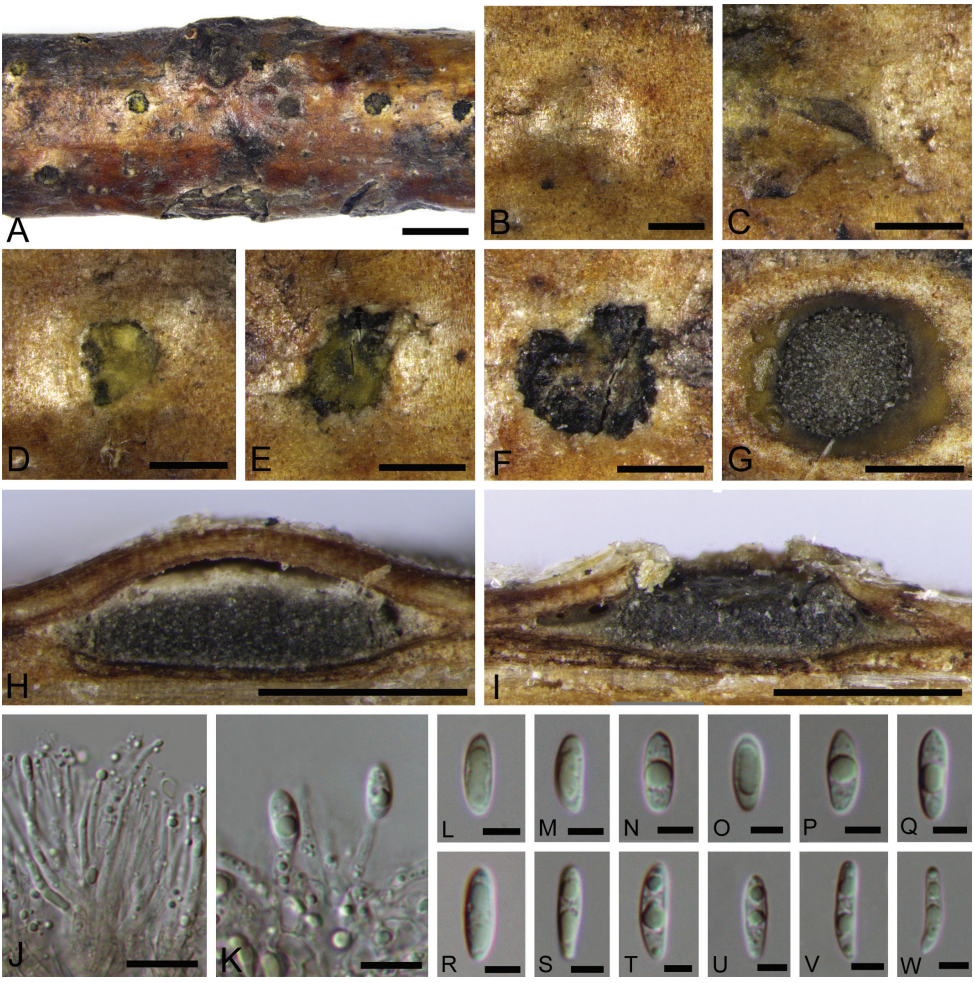

Figure 8. Morphology of *Melanconiella corylina* from *Corylus mandshurica*. **A** habit of acervuli on branches **B–F** process of development of acervulus **G** transverse section through acervulus **H–I** longitudinal section through acervulus **J** conidiophores **K** conidiogenous cells and conidia **L–W** conidia. Scale bars: 2 mm (**A**), 500 μm (**B–I**), 10 μm (**J–K**), 5 μm (**L–W**).

***Sheathospora* Fan, gen. nov.**
MycoBank MB828429

Etymology. *Sheathospora* (Lat.): referring to the conidia with distinct hyaline sheath.

Diagnosis. This genus differs from other genera in Melanconiellaceae by conical and discrete pycnidia with aseptate, cylindrical to ellipsoidal conidia with distinct hyaline sheath.

Type species. *Sheathospora cornuta* (C.M. Tian & Z. Du) Fan.

Descriptions. Conidiomata pycnidial, immersed in host bark, erumpent through the surface of host branches. Ectostromatic disc inconspicuous and ex-

tended to form a beak at maturity. Central column absent. Conidiophores hyaline, smooth, cylindrical, simple, rarely branched at the base. Conidiogenous cells hyaline, phialidic. Conidia hyaline, aseptate, with distinct hyaline sheath. Sexual morph was not observed.

Notes. *Sheathospora* is established for *Melanconiella cornuta*, which was previously included in the *Melanconiella* clade (Voglmayr et al. 2012; Du et al. 2017). Morphologically, it differs from other genera in Melanconiellaceae by pycnidial conidiomata and conidia with distinct hyaline sheath. In our phylogenetic analyses, *Melanconiella cornuta* formed a distinct clade basal to *Melanconiella* within Melanconiellaceae. Based on morphology and different hosts (*Cornus* and *Juglans vs.* Betulaceae), it is here excluded from *Melanconiella* and transferred to the new genus *Sheathospora.* In our revised circumscription, Melanconiellaceae include three genera named *Melanconiella*, *Microascospora* and *Sheathospora*.

***Sheathospora cornuta* (C.M. Tian & Z. Du) Fan, comb. nov.**
MycoBank MB828430
Fig. 9

Basionym. *Melanconiella cornuta* C.M. Tian & Z. Du, Phytotaxa 327(3): 257 (2017)

Diagnosis. This species is distinguished by conical and discrete pycnidia without central column and aseptate, cylindrical to ellipsoidal, (19–)19.5–22.5(–23) × (8–)8.5–10.5(–11) μm conidia, with a distinct hyaline sheath 1–1.5 μm wide.

Holotype. CHINA. Shaanxi Province: Ankang City, Ningshan County, Huoditang Forest Farm, 33°26'04.46"N, 108°26'59.91"E, 3 July 2016, on twigs and branches of *Cornus controversa*, X.L. Fan (BJFC-S1375 holotype; living ex-type culture CFCC 51990).

Descriptions. Conidiomata pycnidial, immersed in host bark, conical, with single necks erumpent through the surface of host branches, scattered, (250–)270–330(–410) μm (av. = 300 μm, n = 20) diam. Ectostromatic disc inconspicuous and extended to form a beak at maturity, pale luteous to amber. Central column absent. Conidiophores hyaline, smooth, cylindrical, simple, rarely branched at the base, 17–24(–25) × 2.5–4(–4.5) μm (av. = 21.5 × 3.5 μm, n = 50). Conidiogenous cells hyaline, phialidic. Conidia hyaline, aseptate, cylindrical to ellipsoidal, (19–)19.5–22.5(–23) × (8–)8.5–10.5(–11) μm (av. = 21 × 10 μm, n = 50), with distinct hyaline sheath, 1–1.5 μm wide at maturity. Sexual morph was not observed.

Culture characteristics. Colony growth on PDA originally white, becoming pale yellowish after 7–10 days. Colony flat, felty-like, with a uniform texture and yellowish to dark brown conidiomata irregularly scattered on the colony surface.

Additional specimens examined (paratypes). CHINA. Shaanxi Province: Ankang City, Ningshan County, Huoditang Forest Farm, 36°26'13.30"N, 108°26'48.32"E, 3 August 2015, on twigs and branches of *Juglans regia*, Q. Yang (BJFC-S1345 paratype; living ex-paratype culture CFCC 51991).

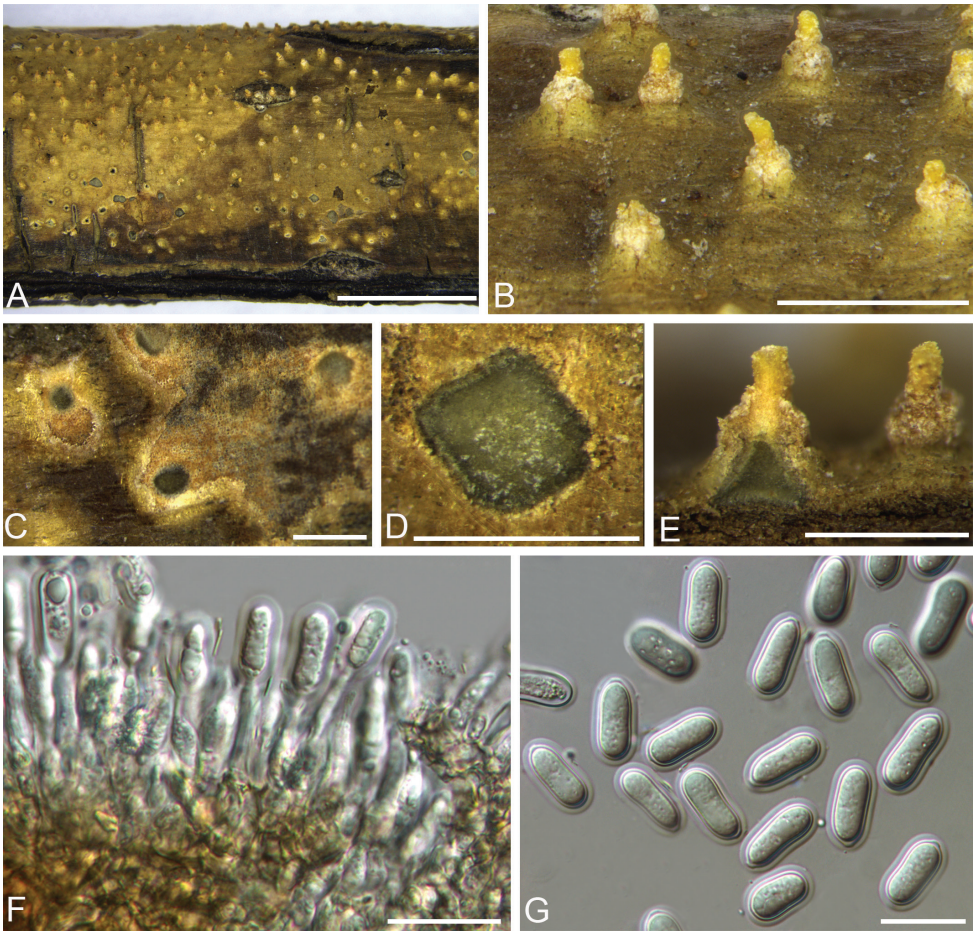

Figure 9. Morphology of *Sheathospora cornuta* from *Cornus controversa*. **A–B** Habit of pycnidia on branches **C–D** transverse section through pycnidium **E** longitudinal section through pycnidium **F** conidiophores, conidiogenous cells **G** conidia. Scale bars: 5 mm (**A**), 1 mm (**B**), 500 µm (**C–E**), 20 µm (**F–G**).

Notes. *Sheathospora cornuta* is proposed here as a new combination for *Melanconiella cornuta*. It is the type and currently only species of *Sheathospora* and so far known from *Cornus controversa* and *Juglans regia* in China. The sexual morph of this species is unknown and further collections are required to elucidate its life cycle.

Discussion

During the investigation of melanconis-like fungi in China, we identified eight species residing in three families (Juglanconidaceae, Melanconidaceae and Melanconiellaceae) of Diaporthales. It includes *Juglanconis juglandina*, *J. oblonga*, *Melanconis betulae*, *Ms. itoana*, *Ms. stilbostoma*, the two new species *Melanconiella betulicola* and *M. corylina* and the new combination *Sheathospora cornuta* in the new genus *Sheathospora*.

All specimens in the current study were collected from symptomatic branches and twigs associated with canker or dieback diseases, of which *Juglanconis* (Juglanconidaceae) species were isolated from *Juglans regia* (Juglandaceae), *Melanconiella* (Melanconiellaceae) species from *Betula albosinensis* and *Corylus mandshurica* (Betulaceae) and *Melanconis* (Melanconidaceae) species from *Betula albosinensis* and *Betula platyphylla* (Betulaceae). It may indicate that many melanconis-like species have obvious host specificity. The type species of the new genus *Sheathospora* (Melanconiellaceae) was isolated from Cornaceae (*Cornus controversa*) and *Juglans regia* (Juglandaceae), suggesting a low host specificity and that additional undiscovered hosts species of this taxon may exist in China.

As the morphological features in previous melanconis-like fungi are highly overlapping, phylogenetic studies using DNA sequences have been useful to elucidate the diversity and systematics in this group. The current results indicated that *Juglanconis* and *Melanconis* are still unique, the only genera in Juglanconidaceae and Melanconidaceae, respectively, due to the lacking of extensive fresh collections. The family Melanconiellaceae was recently proposed by Senanayake et al. (2017) to accommodate *Dicarpella*, *Greeneria*, *Melanconiella*, *Microascospora* and *Tubakia* based on morphological features and phylogenetic analyses. In this study, the phylogenetic affinity of *Dicarpella*, *Greeneria* and *Tubakia* was evaluated in Diaporthales (Fig. 1), which conformed to the recently described family Tubakiaceae (Diaporthales) (Braun et al. 2018). We here establish a new genus within Melanconiellaceae, *Sheathospora*, which is characterised by typical diaporthalean-like pycnidia and aseptate, cylindrical to ellipsoidal conidia with distinct hyaline sheath. Thus Melanconiellaceae is here restricted to the three genera *Melanconiella*, *Microascospora* and *Sheathospora* (Fig. 6).

As shown in this paper, future studies addressing the fungal diversity associated with canker or dieback diseases should routinely include sequence data for protein-coding genes to achieve stable, supported topologies in phylogenetic trees. It is hoped that the classification proposed here will also provide an updated phylogenetic framework that will facilitate further revision of the families with melanconis-like asexual morphs. Although the current study provides additional new data on melanconis-like genera, typification, species concept and taxonomic affiliation of many described *Melanconium* species are yet unclear, including the type species *M. atrum*, which currently represents a doubtful taxon (Rossman et al. 2015). In addition, sequence data are missing for most described *Melanconium* species. Thus, a thorough revision of the genus *Melanconium* based on robust sampling, reliable identification, cultures and DNA data is urgently needed. The fact that new records and species from three related families of Diaporthales were recorded in China further suggests that Asia may harbour many more species awaiting collections and descriptions.

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