**A molecular and morphological re-examination of the generic limits of truffles in the
Tarzetta-Geopyxis lineage – *Densocarpa*, *Hydnocystis*, and *Paurocotylis***

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ABSTRACT

Truffle species within the */tarzetta-geopyxis* lineage share smooth, globose, hyaline spores, but differ in the amount of convolution of hymenia in ascomata. The morphological traits that impart phylogenetic signals are not known, and therefore the relationships among truffle species in this lineage have historically been confused. Phylogenetic analyses of the ITS and 28S nuclear ribosomal DNA from recently collected members of the */tarzetta-geopyxis* lineage from Asia, Austral Asia, North America, and South America prompted a reinvestigation of species and generic limits in the truffle genera *Hydnocystis*, *Paurocotylis*, and *Stephensia*. Our analyses support emendations of *Hydnocystis* and *Paurocotylis*, abandonment of *Stephensia* and the resurrection of the genus *Densocarpa*. Because the type species of *Stephensia*, *S. bombycina*, is

phylogenetically nested within *Hydnocystis*, *Stephensia* is treated as a synonym of *Hydnocystis* and we transfer *S. bombycina* to *Hydnocystis*. *Hydnocystis singeri* is phylogenetically nested within *Paurocotylis* so we transfer *Hydnocystis singeri* to *Paurocotylis*. *Stephensia bynumii* is phylogenetically supported as a species of *Paurocotylis*, so we combine it as *P. bynumii*. This is the first detection of the genus *Paurocotylis* in the Americas. To accommodate other *Stephensia* species, we use the genus *Densocarpa* for a North American species, *D. shanorii*, and a European species, *D. crocea*. We describe three new species, *Hydnocystis transitoria* from North America, *Paurocotylis patagonica* from South America, and *Paurocotylis watlingii* from Australia. We designate a recently collected and sequenced specimen from the holotype collection location as an epitype for *Hydnocystis japonica*. We review anamorphic forms and evaluate the morphological characters used to delimit truffle genera within the /tarzetta-geopyxis lineage in light of new phylogenetic evidence. Our work highlights the unexplored diversity, morphological plasticity, and remaining taxonomic problems among truffles in the /tarzetta-geopyxis lineage.

Key words: Hypogeous fungi, truffle evolution, Pezizales anamorphs

Introduction

The /tarzetta-geopyxis lineage, as defined by Tedersoo *et al.* (2006), includes the truffle genera *Paurocotylis* and *Stephensia*, and the cup fungus genera *Geopyxis* and *Tarzetta* (Tedersoo *et al.* 2006). This lineage was considered part of the *Pyronemataceae* until recently. Phylogenetic analyses based on multiple genetic loci of 93 species across 40 genera did not support a monophyletic *Pyronemataceae* when the earliest diverging lineages were included. One of four lineages recommended for exclusion from the *Pyronemataceae s. str.* was /tarzetta-geopyxis (Hansen *et al.* 2013). Conflicting results from analyses of these four lineages suggest more data are necessary to place them into a new or existing family (Hansen *et al.* 2013). Recent delimitation of the *Geopyxis* species in the /tarzetta-geopyxis lineage left the phylogenetic placement of the truffle genera unresolved (Wang *et al.* 2016). Discoveries of new truffle species in /tarzetta-geopyxis from Asia, Australasia, North America, and South America (Healy *et al.* 2013) prompted us to re-examine the generic limits and ecology of truffles in this lineage. In particular, we re-examined the genera *Hydnocystis*, *Stephensia*, and *Paurocotylis* which

phylogenetic analyses show are included in the /tarzetta-geopyxis lineage (Wang *et al.* 2016). The genus *Densocarpa*, a monotypic genus originally described for *D. shanorii*, is currently considered a synonym of *Stephensia shanorii*. *Densocarpa* is reinstated here to accommodate species in the clade with *D. shanorii*.

Background on truffle genera in the /tarzetta-geopyxis lineage

The genus *Hydnocystis* was described by L. R. and C. Tulasne from ascomata in sandy forests of Europe and Russia (Tulasne & Tulasne 1844). Species of *Hydnocystis* are macroscopically distinguished by an enclosed, finely puberulent to tomentose ascoma that has a single opening leading to a non-convoluted, hollow cavity lined by a white hymenium in a palisade of paraphyses and cylindrical asci with colorless, smooth, uniseriate spores (Tulasne & Tulasne 1844). Burdsall (1968) revised the genus and noted some additional characteristics, the most important of which were eguttulate, globose spores (originally described as ellipsoid by Tulasne & Tulasne 1844) and elongated paraphyses forming an epithecium over the asci.

Over time, 12 species were placed in *Hydnocystis* but most have been transferred to other genera. Gilkey (1916) synonymized *H. compacta* Harkness with *Genea intermedia* Gilkey due to its similarly ornamented spores. This species was subsequently transferred to the monotypic genus *Gilkeya* M.E. Sm. & Trappe as *G. compacta* (Harkn.) M.E. Sm. & Trappe, based on molecular phylogenetic evidence (Smith *et al.* 2006). Burdsall (1968) revised the genus and considered *Hydnocystis* monotypic, including only the type species *H. piligera*. He excluded the other species based on glebal and spore characters. Most excluded species either lacked a true epithecium (*H. convoluta* McAlpine, *H. singeri* Gilkey), had amyloid asci (*H. convoluta*), had ornamented spores (*H. echinospora* Rodway, *H. thwaitesii* Berk. & Br.), or had ellipsoid to subglobose spores (*H. arenaria* Tul. & C. Tul., *H. beccarii* Mattir., *H. californica* Gilkey, *H. clausa* (Tul. & C. Tul.) Ceruti). Burdsall did not suggest an alternate genus for *H. singeri* (Burdsall 1968). After Burdsall's revision, *Hydnocystis japonica* (Kobayasi) Trappe (= *Protogenea japonica* Kobayasi) was added to the genus. Trappe (1975) transferred *P. japonica* to *Hydnocystis* because of its morphological similarities with *H. piligera*. *Hydnocystis japonica* is found among leaf litter in various types of forests and has the main characters of the genus. This species is most frequently under a non-ectomycorrhizal conifer, *Cryptomeria japonica* (Thunb. ex L.f.) D. Don.

At present, *H. piligera* and *H. japonica* are the only undisputed species in *Hydnocystis*. We recently discovered an undescribed North American species of *Hydnocystis*, including its anamorphic state, which stimulated our work on the /tarzetta-geopyxis lineage. From our analyses we infer that *Stephensia bombycina* (Vittad.) Tul. & C. Tul., the type species of *Stephensia*, is nested phylogenetically within the genus *Hydnocystis*. Like *Hydnocystis*, *Stephensia bombycina* also produces an anamorph (Fontana & Giovannetti 1987). Mitospores of *S. bombycina* germinated and grew in culture (Fontana & Giovannetti 1987). Thus, the spores and spore-bearing structures of this state are conidia and conidiophores, respectively.

The genus *Stephensia* (Vittad.) Tul. & C. Tul. was previously shown to be paraphyletic (Læssøe & Hansen 2007, Perry *et al.* 2007, Alvarado *et al.* 2011, Wang *et al.* 2016), though no formal nomenclatural changes have been proposed. With the revelation during our analyses that the type species of *Stephensia* is a *Hydnocystis*, we attempted to place the remaining *Stephensia* species in the most plausible genera for which there is molecular evidence. *Stephensia* currently comprises eight species, including *S. bombycina*, *S. bynumii* Trappe, Bushnell, & Castellano, *S. colomboi* De Vito, *S. crocea* Quél., *S. peyronelii* Mattiolo, *S. sumatrana* Boedijn, *S. shanorii* (Gilkey) Gilkey and *S. varia* Rodway. *Stephensia shanorii* was first described in the monotypic genus *Densocarpa* (Gilkey 1954). After noticing the similarities between *D. shanorii* and *Stephensia crocea*, Gilkey abandoned her new genus and transferred *D. shanorii* to *Stephensia* (Gilkey 1961). However, she noted dissimilarities between *S. shanorii* and the type species, *S. bombycina* (Gilkey 1961). Here we resurrect *Densocarpa* to accommodate both *S. shanorii* and *S. crocea*. The morphological and phylogenetic affiliations of the other species previously treated as *Stephensia* are discussed below.

The genus *Paurocotylis* Berk., with *P. pila* Berk. as the sole member, has been fully and accurately described through the collective efforts of successive mycologists who studied fresh collections at various stages of maturity. Initially the affiliation of this species was uncertain. Berkeley (1855) noted that asci could be present, and that it might be similar to *Stephensia bombycina*. He also mentioned a superficial similarity to *Arachnion* (Lycoperdales). The type specimen was a single, over-mature ascoma in which most of the asci are collapsed in a cavity with a mass of loose spores and glebal hyphae. Berkeley's erroneous interpretation that this taxon was potentially a basidiomycete was followed by Saccardo, thereby causing confusion for later researchers (Dennis 1975). Patouillard (1903) reinterpreted the type, observed asci, and

recognized similarities with *Hydnocystis*, such as the hollow ascoma, excipulum of pseudoparenchyma, and smooth, globose spores. Dennis (1975) studied development in fresh, immature specimens from Great Britain where the species had been introduced. He observed that *P. pila* is initially a solid ascoma with a thin peridium. The asci develop among thin, branched hyphae, and are cylindrical, with 6-8 uniseriate, smooth, globose spores with a single large guttule. The gleba becomes hollow due to evanescent asci that leave powdery spores to partially fill the space in the gleba. Pegler *et al.* (1993) added that the peridium is thick and composed of isodiametric cells that are smaller towards the surface. They observed a poorly developed hymenium with no paraphyses. *Paurocotylis pila* is typically bright red, fruits on the soil surface, and is thought to be saprobic and bird-dispersed (Castellano *et al.* 2004, Læssøe & Hansen 2007, Trappe & Claridge 2015).

Ecologically, the /tarzetta-geopyxis lineage has a mixture of saprobic and biotrophic habits. *Tarzetta* is unequivocally ectomycorrhizal (EcM) (Tedersoo *et al.* 2010) but various trophic strategies have been assigned to taxa in *Densocarpa*, *Geopyxis*, *Hydnocystis*, *Paurocotylis* and *Stephensia*. Wang *et al.* (2016) showed with multigene analyses that *Geopyxis* is paraphyletic, occurring in at least five well-supported clades, and some taxa live as plant or lichen endophytes. Other authors have implicated some *Geopyxis* species as biotrophic (Egger 1986, Egger & Paden 1986b, Vrålstad *et al.* 1998), weakly to moderately pathogenic (Egger 1986, Egger & Paden 1986a), saprobic (Petersen 1970, Egger 1986, Hansen *et al.* 2013), or a combination of trophic strategies (Egger 1986, Vrålstad *et al.* 1998, Hansen *et al.* 2013, Tedersoo *et al.* 2013). *Hydnocystis* species have been considered EcM (Molina *et al.* 1992, Rinaldi *et al.* 2008) or non-ectomycorrhizal (Tedersoo *et al.* 2010). *Stephensia* species have been considered EcM (Molina *et al.* 1992, Rinaldi *et al.* 2008), mycorrhizal with orchids (Bidartondo *et al.* 2004, Wang *et al.* 2016), or non-mycorrhizal (Hutchison 1989, Tedersoo *et al.* 2010). *Paurocotylis* species have been considered EcM (Brundrett *et al.* 1996, Rinaldi *et al.* 2008) or saprobic (Dennis 1975, Castellano *et al.* 2004).

The goals of this work were to: 1) phylogenetically place newly discovered truffle taxa from Austral Asia, North America, and South America in the /tarzetta-geopyxis lineage (*Densocarpa*, *Hydnocystis*, *Paurocotylis*, and *Stephensia*); 2) clarify the taxonomic status of *Stephensia* and assign species of *Stephensia* to appropriate genera based on phylogenetic and morphological evidence; 3) describe three new species (*Hydnocystis transitoria*, *Paurocotylis*

patagonica, *P. watlingii*), designate an epitype for *Hydnocystis japonica*, and transfer *Hydnocystis singeri* to *Paurocotylis*; 4) infer the most likely ecological strategies (endophytic, mycorrhizal, or saprobic) of truffle taxa in the /tarzetta-geopyxis lineage; and 5) identify unifying morphological characters for the genera within the /tarzetta-geopyxis lineage.

Materials and methods

DNA extraction, amplification, and sequencing

We obtained 46 new sequences and downloaded 90 additional nuclear rDNA sequences from NCBI to analyze phylogenetic relationships in the /tarzetta-geopyxis lineage. We used sequences from the internal transcribed spacer regions (ITS1–5.8S–ITS2, referred to as ITS) to aid in species delimitation (Schoch *et al.* 2012), and the 28S rDNA (LSU) to resolve relationships within the /tarzetta-geopyxis lineage. DNA was isolated from unexposed mature glebal tissue of fresh or dried ascomata. Extraction followed a standard chloroform method. The ITS and 5' end of the 28S region of nuclear rDNA were amplified with ITS5 and LR5 (Bertini *et al.* 1999, Vilgalys & Hester 1990). We used NS7/ITS2 (White *et al.* 1990) to obtain sequences of *Stephensia shanorii*. PCR products were visualized on a 1% agarose gel, and excess primer and nucleotides were removed with exonuclease 1 and shrimp alkaline phosphatase (U.S.B. Corporation, Cleveland, OH, USA). Amplicons were sequenced bidirectionally on an ABI PRISM 3700 DNA Analyzer in the Biomedical Genomics Laboratory at the University of Minnesota and the Interdisciplinary Center for Biological Research at the University of Florida using NS7, ITS1f, ITS4, ITS5, LR5, and LR3. Sequences were manually edited and assembled with Sequencher v. 5.1 (Gene Codes Inc., Ann Arbor, MI, USA). New sequences were deposited in GenBank under the numbers JX424575, KT361822–KT361850, KT428713–KT428714, KY321428–KY321441 (Table 1). Alignments and trees are deposited in TreeBase (<http://purl.org/phylo/treebase/phylows/study/TB2:S19360>).

Phylogenetic analyses

For alignment, all sequences were trimmed in SeAl v 2.0a11 (Rambaut 2007) before the “CATTA” motif at the end of the 18S gene and before the “ACCTCNNATCAGGTAGGGAT” motif of the 28S. When numerous hits of highly similar (>97% identity) endophyte ITS sequences were returned from BLAST searches, sequences were chosen to represent host and

geographic diversity. Taxa in the 28S data set included new sequences from this study and sequences representative of major lineages in the Pyronemataceae based on Hansen *et al.* (2013). All sequences were aligned with MAFFT v 7.187 (Katoh & Toh 2010), and manually adjusted in Se-AI. For the ITS alignment, ambiguous regions were excluded in Gblocks under the least stringent settings (Castresana 2000). Phylogenetic inference support included maximum likelihood (ML) bootstrapping and Bayesian posterior probabilities (BPP) from Bayesian inference (BI) analyses. ML was performed with RAxML 8.1.24 (Stamatakis 2014) using a GTR + Gamma model of nucleotide substitution. Rapid bootstrapping was implemented with 1000 replicates. The optimal model of evolution and priors for BI analyses were determined with jModelTest 0.1.1 (Posada 2008) using the Akaike information criterion for each partition. The ITS model was SYM+I+G; the 28S model was TIM3+I+G. Analyses were run for 20,000,000 generations, parameters were sampled every 1000th generation, and the first 25% was discarded as burn-in. Stationarity, mixing, and estimated sample sizes were checked using Tracer v1.4 (Rambaut & Drummond 2007). MAFFT, ML, and BI analyses were implemented in XSEDE on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010). Posterior probabilities were taken out to two decimal points (1/100th). The 28S rDNA alignment had 798 positions for 74 taxa, with *Orbilbia auricolor* serving as the outgroup. After excluding ambiguous regions of the alignment, the ITS rDNA alignment had 464 positions for 77 taxa and was midpoint rooted.

Specimen morphology and microscopy

Macroscopic characteristics were described from field notes of fresh or dried material. Micro-morphological characteristics and measurements were detailed from razor-excised pieces of air-dried ascomata rehydrated in water or 5% KOH. Upon rehydration, excised pieces were squash mounted in water, cotton blue, or Melzer's Reagent and viewed with bright field and differential interference contrast (DIC) microscopy. To determine mean spore diameter spore measurements were taken from 50 randomly selected spores under the highest power of light microscope magnification in water mounts. Color nomenclature follows Kelly & Judd (1955). Fresh material of *H. transitoria* was fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in a gradual ethanol series, exchanged with acetone, and infiltrated with a standard formulation of Spurr's Resin (Spurr 1969), and the resin polymerized for 30 hours at 70°C. Pieces were sectioned 3 µm thick, stained with toluidine blue O, and

mounted with xylol and Permount. For scanning electron microscopy (SEM), small blocks of gleba tissue were fixed as described above, dehydrated in a graded ethanol series to 100% ethanol, critical point dried, attached to aluminum stubs, sputter coated with 1/1 gold/palladium and viewed under 10 KV in a JEOL SEM in the Microscopy and NanoImaging Facility at Iowa State University.

Definitions of morphological terms

The term epithecium refers to paraphyses that are elongated well beyond the asci, that may be branched or unbranched, and may or may not be fused (Kirk *et al.* 2008). If paraphyses are interwoven and fused, the tissue is referred to as pseudoparenchyma, as in species of *Genea*. If the paraphyses are not fused, the tissue is referred to as prosenchyma, as in *Hydnocystis piligera* Tul. & C. Tul. (Kirk *et al.* 2008). In *H. piligera*, the epithecium of prosenchyma forms a distinct layer that is visible with a hand lens.

Results

DNA sequences and phylogenetic analyses

The ML and BI analyses of the 28S alignment confirmed a monophyletic /tarzetta-geopyxis lineage (Fig 1). *Stephensia* was paraphyletic, with *S. bombycina* inferred within *Hydnocystis*, *S. bynumii* affiliated with *Paurocotylis*, and *S. shanorii* and *S. crocea* inferred together in a clade distant from other genera. Since *Densocarpa* is a valid name for *S. shanorii*, we resurrect its use for this species and its closest relative *S. crocea* as well as an undescribed species from Mexico. *Hydnocystis* was also paraphyletic, with *H. singeri* most closely affiliated with *P. pila*. Based on our results three phylogenetically distinct truffle genera are supported: *Densocarpa*, *Hydnocystis*, and *Paurocotylis*. Among the three inferred truffle lineages, *Densocarpa* and *Hydnocystis* had strong bootstrap and posterior probability support, while *Paurocotylis* was weakly supported. *Tarzetta* and *Densocarpa* were inferred as sister to the rest of the /tarzetta-geopyxis lineage. Final ML optimization in RAxML yielded a best tree with log-likelihood of -5787.185458 for the 28S alignment.

Analyses of the ITS region agreed with the placement of truffle species within the /tarzetta-geopyxis lineage as inferred by the LSU analyses. *Stephensia bombycina*, the type species of *Stephensia*, was inferred as a *Hydnocystis*, *S. bynumii* was inferred as a *Paurocotylis*,

249 *S. shanorii*, *S. crocea* were inferred together in a distinct lineage (*Densocarpa*), and *H. singeri*
250 was inferred as a *Paurocotylis*. There was strong support based on ITS for *Densocarpa*,
251 *Hydnocystis*, and *Paurocotylis* (Fig 2). Final ML optimization in RAxML yielded a best tree with
252 log-likelihood of -3321.778401 for the ITS alignment. In Fig 1 and Fig 2 taxon names have been
253 changed to reflect nomenclatural revisions presented here. Table 1 gives a synopsis of the current
254 and previously accepted names for each taxon.

255 Direct comparison of ITS sequences in the 830 bp alignment showed 99% sequence
256 similarity among the nine *Hydnocystis piligera* sequences. ITS sequence similarities between *H.*
257 *piligera* and other species were 96% with *H. japonica*, 95% with an undescribed species from
258 China (FH 00290161), and less than 91% with *Stephensia bombycina* (Serbia and Mexico) and
259 with *H. transitoria* sp. nov. from the USA. There were 26 bp differences (5%) between *H.*
260 *japonica* (507 bp) and the undescribed *Hydnocystis* from China (505 bp) (FH-00290161).
261 RAxML and BI phylogenetic analyses of both ITS and 28S strongly support a monophyletic
262 clade of *H. piligera*, *H. japonica*, *S. bombycina*, *H. transitoria* sp. nov., and *Hydnocystis* sp. nov.
263 from China (FH-00290161) (Fig 1, Fig 2).

264 As surmised by Burdsall (1968), the southern South American species *Hydnocystis*
265 *singeri* was not closely related to the core group of *Hydnocystis*. Instead, our analyses show that
266 it was most closely related to *Paurocotylis pila* from New Zealand. Most taxa in the *Paurocotylis*
267 clade shared a notable insert in the beginning of ITS1. The insert in the *P. pila* sequences (175 bp
268 long of an 873 position alignment) were 93-100% similar (0-11 bp difference and one ambiguity)
269 to each other and were 83-85% similar to the insert of *P. patagonica* sp. nov. (178 bp long),
270 differing in 27-31 nucleotide positions for each *P. pila* sequence (Supplemental Fig 1). The insert
271 in the *H. singeri* sequence (145 bp) was only 33% similar to *P. pila*, and 36% similar to *P.*
272 *patagonica* sp. nov. from Argentina. The insert in the sequence from *P. watlingii* from Australia
273 was relatively short (39 bp), but matched 54% with *H. singeri* across 55 positions. *Stephensia*
274 *bynumii* was missing the insert. The ITS1 insert in *Paurocotylis* species was not similar to the
275 insert discovered in some species of *Geopyxis* (*G. alpine*, *G. deceptive*, *G. majalis*, and *G.*
276 *rehmii*) by Wang et al (2016) (Supplemental Fig S1).

277 *Geopyxis* was inferred as paraphyletic, supporting previous analyses (Perry *et al.* 2007,
278 Tedersoo *et al.* 2013, Wang *et al.* 2016). Endophytic sequences from angiosperms,
279 gymnosperms, lichens, and mosses nested within disparate *Geopyxis* clades (Fig 2). All of the

vascular plant endophytes were isolated from above ground tissues, indicating that *Geopyxis* is not EcM. The only sequences from EcM root tips were resolved within *Tarzetta* (Fig 2).

Taxonomy

Here we describe the morphology of truffles in the genera *Hydnocystis*, *Paurocotylis*, and *Densocarpa*. We describe three new species, make five new combinations, designate one epitype, and amend the descriptions of two species. We provide descriptions of all described truffle species in the /tarzetta-geopyxis lineage in order to effectively compare genera and species.

Hydnocystis Tul. & C. Tul. 1844. *Nuovo Giornale Botanico Italiano* **2**: 59.

Type species. Hydnocystis piligera Tul. and Tul. 1844. *Nuovo Giornale Botanico Italiano* **2**: 59-60.

Etymology. Latin “Hydnon”, Tuber; “cystis”, bladder

Description. Ascoma a ptycothecium, as defined by Weber *et al.* (1997), globose to ovoid, lobed or folded, with either a simple hymenium lining a hollow cavity or infolded with canals and a small cavity, connected to the exterior by a pore or larger opening, whitish to brown.

Excipulum of pseudoparenchyma; if excipular hairs are present, they are granule-encrusted.

Hymenium a palisade of paraphyses and asci.

Paraphyses narrow, septate, often longer than asci, forming epithecium.

Asci inamyloid, cylindrical, indehiscent, spores arranged uniseriately.

Ascospores globose, smooth, hyaline or nearly so.

Comments. We amend the description of the genus *Hydnocystis* with regards to the degree of ascoma infolding and the granules that encrust the excipular hyphae.

Hydnocystis piligera Tul. and Tul. 1844 *Nuovo Giornale Botanico Italiano* **2**: 59-60.

Isotype. **France**, Var, Hyères under terebinth (*Pistacia terebinthus*), Dec. 1844, Tul. and Tul. Antibes (CUP 48960).

Etymology. Latin, “pili”, the hair, possibly referring to the hairy ascomata of this species.

Description. *Ascoma* a ptycothecium subglobose to slightly flattened, sometimes lobed, 0.5-4 cm diam., tomentose, pale brown to yellow brown, usually with one large cavity lined by cottony hyphae, gleba white, odor fruity (Montecchi & Sarasini 2000).

Excipulum 500 µm, hairs 4-6 µm diam., septate, granule-encrusted, brown, outer excipulum composed of pseudoparenchyma with cells 10-30 µm diam., outer cells with brown walls. Inner excipulum with smaller cells, hyaline.

Hymenium a palisade of asci and paraphyses that form a thick epithecium.

Paraphyses narrow, 3-5 µm diam., septate, elongated considerably beyond the asci.

Asci cylindrical, 250-300 µm x 30-50 µm.

Ascospores globose, smooth, hyaline, not guttulate, 28-35 µm diam.

Anamorph unknown.

Distribution and habitat. On soil surface or shallowly hypogeous. In many habitats under Pinaceae or Angiosperms. Widespread in Europe, Russia, and Israel.

Specimens examined. **Austria**, Feldkirch, sn date, (CUP-48863); **France**, Var, Hyères, under terebinth (*Pistacia terebinthus*) Dec. 1844, Tul. and Tul. (Isotype CUP-48960), Antibes, sn date, Torraut (CUP-48975); **Lichtenstein**, Cecinamare, Terremi sabbiosi, under *Pinus*, *Picea*, *Eucalyptus*, sn date, Medici. G. Caro (MA-9982); **Spain**, Mallorca, Banyalbufar, Coll de Sa Bastida, 20 Dec 1990, MA Perez (MA-29812); **Russia**, 6 Aug 1906, F. Bucholtz B311 (CUP-48971, FH-00290167); Bonifa, 18 Jun 1903, R. Maire (CUP-48973); Kemmern, 10 Aug 1901, 8 Jul 1903, F. Bucholtz b311 (CUP-48970, CUP-48972, FH-00290163, FH-00290165).

Comments. There is little to no ITS variability among specimens of *H. piligera*. The sequences in GenBank, most of which are from Alvarado *et al.* (2011), matched the sequences obtained for this study. Morphology is the same as described for the type specimen and the more recently studied specimens treated by Agnello (2011), Alvarado *et al.* (2011), Burdsall (1968), Kaounas *et al.* (2011), and Montecchi & Sarasini (2000).

***Hydnocystis bombycina* (Vittad.) Healy and M. E. Sm., comb. nov.**

MycoBank MB814818

Basionym: *Genea bombycina* Vittad. *Monographia Tuberacearum*. (Milano): 29 (1831).

Synonym: *Stephensia bombycina* (Vittad.) Tul. & C. Tul *Comptes rendus hebdomadaires des séances de l'Académie des Sciences* **21**: 1433 (1845).

341 *Type.* Location unknown. Not examined

342 *Etymology.* Latin, “silky”, referring to the silky ascomata. Vittadini named this species
343 within the genus *Genea*, and most *Genea* have peridia with rough, warted surfaces and obvious
344 peridial hairs. It is possible that Vittadini considered *H. bombycina* to be “silky” and smooth in
345 comparison.

346 *Description.* *Ascoma* a ptycothecium, suglobose, depressed, cracked, up to 2 cm diam.,
347 tomentose, light brown to yellow brown, drying reddish brown (Pegler *et al.* 1993), with a single
348 cavity in the gleba that is connected to the exterior through a pore. Gleba white, marbled with
349 white to cream-colored veins or open canals where the hymenium is folded. Odor of H₂S.

350 *Excipulum* 800 µm, hairs 2-6 µm diam., septate, granule-encrusted, brown with brown
351 walls, outer excipulum composed of somewhat parallel hyphae that radiate to surface, continuing
352 beyond surface as hairs, layer 100 µm thick, outer cell walls brown. Inner excipular layer 700
353 µm thick, of somewhat parallel hyphae (Hawker 1954).

354 *Hymenium* a palisade of paraphyses and asci.

355 *Paraphyses* 2-3 µm diam., uninflated, septate, often elongated beyond ascus tips to grow
356 into cavity between opposing hymenia that result from infolds.

357 *Asci* cylindrical 180-270 x 20-30 µm, tapering to elongated narrow stalk, with up to eight
358 uniseriately (typically) to irregularly biseriately arranged ascospores (Hawker 1954), unreactive
359 in Melzer’s solution.

360 *Ascospores* globose, smooth, hyaline, 19-22 µm diam. (original description), but reported
361 to vary from 16-34 µm diam. (spore measurements of various authors summarized in Gyosheva
362 *et al.* 2012). Contents of mature spores cyanophylous in cotton blue, walls light blue.

363 *Anamorph* produced from ascospores on malt agar. Buff colored. Conidiophores
364 sympodial, septate, repeatedly dichotomously branched, encrusted with granules, with one to
365 three terminal conidia produced, leaving scars at the location of their release. Conidia globose
366 6.5-8 µm diam. to ovoid 7.8 -9.7 x 5.8-6.5 µm, warted (Fontana & Giovannetti 1987).

367 *Distribution and habitat.* On or under the soil surface or in woodland litter in forests
368 dominated by conifers or angiosperms. Europe, Israel, Mexico. Attempts to synthesize
369 mycorrhizae with *Salix* and *Quercus* were unsuccessful and *H. bombycina* was easy to culture
370 (Fontana & Giovannetti 1980). Terrestrial orchid roots have yielded ITS sequences that match *H.*
371 *bombycina* (Fig. 2) (Wang *et al.* 2016).

Specimens examined: **United Kingdom**, England, Somerset, Burrington Coombe, sn date, L. Hawker, Hawker 777 (OSC-34416); **Germany**, Eimersdorf/Saar, 15 August 1968, G. Gross 189 (OSC-131641); **Italy**, Firenze, Mattiolo (OSC-34458); **Serbia**, Grisevac Svrlijig, 21 Oct 1992, Z. Marjanovic, Z. Marjanovic-20, Trappe 23260, (OSC-61969); **Mexico**, Mexico City, Colonia Cuauhtémoc, Shirley Courts Hotel on Sullivan St., under *Pinus*, *Cupressus*, other plants, 14 Jun 1972, J. Trappe, Trappe 3268, (OSC-34395, FH-284692).

Comments. *Hydnocystis bombycina* was described by Vittadini (1831) as *Genea bombycina* from a specimen collected in Italy, and then redescribed as the type species for *Stephensia* by Tulasne & Tulasne (1851). Hawker (1954) added microscopic details in her re-analysis of this species. The specimen from Serbia that was sequenced for our phylogenetic analysis matches the description for *H. bombycina*, which is morphologically similar to other *Hydnocystis* species but varies in a pronounced infolding of the hymenium. Characteristics of *H. bombycina* and other *Hydnocystis* species include the ptycothecial form, ascomatal opening, peridium with encrusted hyphae, cylindrical, stipitate asci in a palisade, slender paraphyses that overgrow the asci, globose spores that are hyaline, inconspicuously guttulate, and uniseriately arranged, and white to cream-colored gleba. Paraphyses of *H. bombycina* are not fused.

The anamorph of *H. bombycina* is similar to the anamorph of *H. transitoria*. Anamorphs of both species are sympodial, have warted, globose to ovoid conidia, septate, branched, warted conidiophores, and conidial scars at the location of conidial release. The anamorph of *H. bombycina* differs from that of *H. transitoria* in producing only terminal conidia. Differences may be due to the growth regimens; the *H. bombycina* anamorph was produced in pure culture whereas the *H. transitoria* anamorph developed in its natural habitat. There were no attempts to culture *H. transitoria*.

Similarity among the 28S sequences and the ITS sequences of the Serbian and Mexican collections were within 99%. Both are well supported within the *Hydnocystis* clade.

Since the type species of *Stephensia* is a *Hydnocystis* species, the genus *Stephensia* is a later synonym. *Hydnocystis* (described in 1844) has priority over the name *Stephensia* (described in 1845). *Hydnocystis bombycina* was previously recognized as having a close relationship with *H. piligera* (Alvarado *et al.* 2011) and the paraphyly of *Stephensia* was also recognized previously (Læssøe & Hansen 2007, Perry *et al.* 2007, Alvarado *et al.* 2011, Wang *et al.* 2016).

Hydnocystis transitoria L. Kumar, Healy & M.E. Sm., **sp. nov.**

MycoBank: MB814213

Fig 3

Diagnosis. *Hydnocystis transitoria* is a North American species distinguished from other *Hydnocystis* species by its distinct ITS sequence, nearly smooth ascoma, its thick, well-developed epithecium of parallel and interwoven paraphyses tips, and its envelopment of asci within paraphyses that are interwoven and sometimes fused. Among *Hydnocystis* species with a simple cavity, it is distinguished by much smaller spores.

Holotype designated here. **United States of America**, Minnesota, Fillmore Co., Forestville State Park, hypogeous in mesic *Quercus*-dominated woodland. N43°38' 28.3" W92°13'17.3", 21 Jul 2009, Leticia Kumar, RH950 (MIN-928483).

Etymology. Latin, “transitoria”, intermediate, referring to our interpretation of this species having paraphyses intermediate between the unfused elongated paraphyses in the prosenchymatic epithecia of *H. piligera* and the pseudoparenchymatic epithecia of more highly modified *Hydnocystis* such as *H. bombycina* comb. nov.

Description. Ascoma a ptycothecium, hypogeous, laterally flattened and somewhat rounded in outline, 4–8 mm diam. x 1.5–3 mm high with an opening 1–4 mm diam. (Fig 3 A), leading into a simple cavity lined by a regular, unfolded hymenium that is cottony in appearance due to a well-developed epithecium (Fig 3 B, arrows). Peridium light brown (ISCC-NBS 74) when fresh, turning darker (ISCC-NBS 78) when dried, covered with sparse well-spaced short granule-encrusted hairs (Fig 3 C). The scent of fresh fruiting bodies a mixture of a sour odor (reminiscent of soy sauce) and a sweet odor (reminiscent of maple syrup). When dried, the ascoma had a strong sweet scent of maple syrup or pearly everlasting flowers (*Anaphalis margaritacea*).

Excipulum 500 µm, of pseudoparenchyma, short microscopic excipular hairs, 4–8 µm wide, arising sporadically from the outermost cells (Fig 3 D), outer excipulum 100–170 µm, light golden brown (ISCC-NBS 74) with cells up to 30 µm thick, walls 2–3 µm thick. The inner excipulum, 240–300 µm thick, walls 1 µm thick. The excipular layers are of textura angularis (Fig 3 E).

Hymenium a palisade of asci and paraphyses.

433 *Paraphyses* 2–10 μm diam., parallel to interwoven and sometimes fused to form a
434 hyaline textura intricata (Fig 3 F–I) that embeds the asci and extends beyond them to form a
435 well-defined epithecium, 63–197.5 μm thick, composed of parallel to interwoven and sometimes
436 fused hyphae (Fig 3 F, I). Subhymenium hyaline with cells up to 20 μm diam., of textura
437 intricata. Epithecial cells 2–16 μm wide, with average diam. of 4 μm . Paraphyses tips separate
438 and distinct at the epithecium leading edge.

439 *Asci* 185–217 μm long, 16–23.5 μm diam. tapering to their bases (Fig 3 J), with obtuse
440 apices, 8-spored, uniserate, J- in Melzer's. Croziers not observed.

441 *Ascospores* globose, smooth, containing inconspicuous oil droplets (Fig 3 J–K) 18–24.5
442 μm , with an average size of 21 μm in diam.

443 *Anamorph.* (Fig 3 L–P). Mycelial spore mat on woody debris, colonies small (~ 1 cm in
444 diam.), pinkish-brown (Fig 3, L). Hyphae, including conidiophores, encrusted with granules (Fig
445 3 M–N), 4–6 μm diam. at the septum, even or swollen up to 6–8 μm between septa.
446 Conidiophores sympodial, terminal, or at the base of septa along the main axis (Fig 3 N
447 arrowhead), with one to four branches observed per node, one to two concurrent conidia per
448 apex, and up to five conidiogenous scars per apex (Fig 3 O arrows). Branches 2 μm long with
449 conidium almost sessile (Fig 3 N arrow) to 32 μm long, tapering at apex (Fig 3 N). Mature
450 conidia globose to ovoid (laterally or longitudinally flattened) with closely spaced truncated
451 spines (Fig 3 O–P). Size excluding ornamentation: (6.5–) 7–9 (–11) with a median of 8 μm for
452 globose spores or 7–8.5 x 7–9 μm with a median of 8 x 8.5 μm for ovoid spores. Spines 1–1.5
453 μm long. There was no attempt to culture the anamorph.

454 *Distribution and habitat.* Two ascomata were collected in July and the anamorph was
455 collected two years later in October from the same site in Minnesota. This site is on the
456 Paleozoic Plateau, characterized by limestone/dolostone/sandstone and shale bedrock, covered
457 by silty glacial deposits in the uplands and sandy gravel in the river valleys. The area is known
458 for its karst topography (Williams 2009). The truffles were shallowly hypogeous under leaf litter
459 in a hardwood forest of *Quercus rubra*, *Q. alba*, *Carya ovata*, *Tilia americana*, *Ostrya*
460 *virginiana*, *Acer* sp. and *Celtis occidentalis*. The anamorph was collected on a fallen twig in the
461 same woods. These are the only known collections of *H. transitoria*. This species is presumed to
462 be biotrophic, saprobic, or of a non-ectomycorrhizal habit such as orchid mycorrhizae, since
463 there is no EcM evidence of any *Hydnocystis* from root tips.

Anamorph examined. **United States of America**, Minnesota, Fillmore Co., Forestville State Park, hypogeous in mesic *Quercus*-dominated woodland. 2 Oct 2011, R. Healy, AM488 (MIN-929321).

Comments. There are key microscopic morphological differences between *H. transitoria* and the two previously described and accepted *Hydnocystis* species, *H. japonica* and *H. piligera*. *Hydnocystis transitoria* has parallel to interwoven paraphyses that encase the asci in textura intricata, the epithecium is composed of parallel to interwoven hyphae, and sparse excipular hairs are present on the ascoma. In contrast, *H. piligera* has separate (non-fused) paraphyses (Tulasne & Tulasne 1851), an epithecium formed by unfused paraphyses, and ascomata that are densely tomentose (Montecchi & Sarasini 2000). Similar to *H. japonica*, the paraphyses of *H. piligera* overgrow the asci to form a prosenchymatic epithecium. The peridium of *H. piligera* is more densely tomentose than the peridium of either *H. japonica* or *H. transitoria*. *Hydnocystis bombycina* comb. nov. differs in its densely tomentose ascoma and highly invaginated hymenium. The spores of *H. transitoria* are smaller than those reported for *H. piligera* and *H. japonica*, but are within the size range reported for *H. bombycina* (de Vries 1985, Gyosheva et al. 2012).

Hydnocystis japonica (Kobayasi) Trappe, Mycotaxon 2: 115. 1975.

Mycobank (epitype): MBT374117

Fig 4, A-E

Basionym: *Protogenea japonica* Kobayasi, 1964 [1963]. *Transactions of the Mycological Society of Japan* 4:119-120.

Types. **Japan**, Kagoshima Prefecture, Utizume, collected by H. Indoh, K. Aoshima, Y. Kobayasi on 14 Nov 1962 (Isotype CUP-JA-003467) annotated as *Hydnocystis japonica* by J.M. Trappe 12 Nov 1974. Epitype designated here: **Japan**, Kagoshima Pref., Mt. Koba, Minamiosumi-cho, collected by T. Orihara on 12 May 2015 (KPM-NC-0024873).

Etymology. “japonica”, Japan, the country from where this species was described.

Description. Ascoma a ptycothecium, laterally flattened, more or less rounded, 8–12 mm diam. x 5–7 mm high with an opening up to 2 mm in diam. (Fig 4 A) into a large central cavity lined with a regular unfolded to once folded white hymenium that is cottony on the surface due

to a well-developed epithecium. The peridium light brown (ISCC-NBS 74), smooth except for a basal tuft of white mycelium.

Excipulum 500–550 µm, projecting hyphal tips (“hairs”) with cell walls 1µm thick, light golden brown (ISCC-NBS 74), with short granule-encrusted, outer excipulum composed of textura angularis with more or less isodiametric cells, outermost cell walls 1–1.5 µm thick (Fig 4 B). The subtending layer is of textura intricata, 100–150 µm thick, hyaline.

Hymenium a palisade of paraphyses and asci.

Paraphyses 3–4 µm diam., septate, parallel (Fig 4 C), extending beyond the asci, where they appear to adhere at the tips (although in the type description, the tips are free). Disentangled tips of paraphyses are irregularly rough (Fig 4 D).

Asci cylindrical, 270–400 x 35–40 µm, tapering to a narrow elongated base (Fig 4 E), with three to eight uniseriately arranged spores of varying sizes.

Ascospores globose, smooth, hyaline, not guttulate, 25–32 µm diam., average of 30 µm.

Anamorph unknown.

Distribution and habitat. On soil or shallowly hypogeous, Japan.

Specimens examined. **Japan**, Kagoshima Prefecture, Utizume, 14 Nov 1962, H. Indoh, K. Aoshima, Y. Kobayasi (Isotype CUP-JA-003467 as *Protogenea japonica* annotated as *Hydnocystis japonica* by JM Trappe 12 Nov 1974); Minamiosumi-cho, Mt. Koba, on ground in deciduous broadleaf forest, 12 May 2015 T. Orihara (Epitype KPM-NC-0024873); Nishinoomote-shi, Anjoh, along Waseda River on ground under *Cryptomeria japonica* 12 August 2015 T. Orihara (KPM-NC0024892); Kanagawa Prefecture, Yugawara-machi, Mt. Tensho, 14 Nov 2011 T. Orihara (KPM-NC 18030, FLAS F-59178); Hayama-machi, Nangoh-uenoyama Park, 23 Oct 2011, S. Inoue (KPM-NC 18029, FLAS F-59177); Isehara-shi, Hinata, 17 Nov 1999 Y. Degawa (KPM-NC 8613); Odawara-shi, Iryuda, K. Maruyama, 25 Oct 2003, J. Ohta (KPM-NC 11669, FLAS F-59176).

Other specimens examined:

China, Mixed forest of *Picea* and *Abies* with occasional *Cupressus*, *Juniperus* and *Betula* along stream. 31°41'46" N, 100°42'19" E, 3620 m, May 1998, D. Hibbett and Z. Wang wz2209 (FH-00290161, FH-00290162). (*Hydnocystis* sp., originally identified as *H. piligera*).

Comments. Kobayasi (1963) described the ascomata as having an opening but he indicated that some ascomata were closed. This was one of the reasons, along with the nearly

smooth peridium and occurrence on the soil surface, that he erected a new genus (*Protogenea*) for this species. Trappe (1975) recognized the key similarities of *P. japonica* with *H. piligera*, along with the variability of the diagnostic criteria, and correctly placed it in *Hydnocystis*. The opening in *H. japonica* is typically larger than the opening in *H. piligera*. Since the type specimen is too old for effective sequencing, we have designated as epitype a recently sequenced Japanese collection (KPM-NC0024873) collected ca. 6 km west of the holotype locality that agrees in every respect with the type description. A specimen collected in China (*Hydnocystis* sp. FH 290161) was originally identified as *H. piligera* but later identified as *H. japonica* by Wang & Pei (2001) based on morphological similarity. With phylogenetic analysis, however, the Chinese specimen apparently represents an undescribed species. This specimen should be studied in greater detail to determine if there are consistent morphological differences.

Paurocotylis Berkeley in Hooker, 1855. *Botany of Antarctic Voyage of H.M. Discovery Ships Erebus and Terror, in the years 1839-1843: Flora Novae-Zelandiae, II*. London, pp. 188. *Type species. Paurocotylis pila* Berkeley in Hooker, 1855. *Botany of Antarctic Voyage of H.M. Discovery Ships Erebus and Terror, in the years 1839-1843: Flora Novae-Zelandiae, II*. London, pp. 188.

Etymology: Greek, “pauro”, few, “cotylis”, a cavity, presumably referring to the cavity that characterizes the type species of this genus.

Description. Ascoma a ptycothecium with solid, white gleba that may develop a cavity.

Excipulum. Outer excipulum of interwoven hyphae. Projecting hyphae granule-encrusted.

Hymenium. Paraphyses and asci in a palisade.

Paraphyses thin, branched.

Asci stalked, inamyloid, indehiscent, cylindrical, early deliquescing in some species.

Ascospores globose, smooth, hyaline, not conspicuously guttulate.

Anamorphs unknown.

Paurocotylis pila Berkeley in Hooker, 1855. *Botany of Antarctic Voyage of H.M. Discovery Ships Erebus and Terror, in the years 1839-1843: Flora Novae-Zelandiae, II*. London, pp. 188. Fig 4 F–J.

Type. **New Zealand**, North Island, Tehawera collected by Colenso on the ground, (K-171066). Not examined.

Etymology. Latin, “pila”, sphere, presumably referring to the globose shape of the ascomata.

Amended description (from Dennis 1975). *Ascoma* a globose ptycothecium bright crimson (v.R 11) drying to a dull rufous (Berkeley 1855), soft, up to three cm, collapsing and becoming wrinkled. Gleba solid, white, and veined in young ascomata, becoming cream-colored with powdery spores surrounding large cavity in mature specimens. Although the peridium of *Paurocotylis pila* appears smooth (Fig 4 F–G), there are microscopic granule-encrusted hyphae that project from the excipulum (Fig 4 H–I). Odor of ascomata not distinctive.

Excipulum. Outer excipulum 200–300 μm thick, of compactly interwoven hyphae 3–12 μm broad partially inflated to form pseudoparenchyma (Fig 4 G); surface and projecting hyphae in outermost layer granule-encrusted (Fig 4 H–I); walls 1 μm thick. Inner excipulum colorless, pseudoparenchymatous, composed of subglobose cells 12–40 μm in diam.

Hymenium of paraphyses and asci, but asci are early evanescent.

Paraphyses slender, branched, septate, hyaline, 3 μm diam.

Asci cylindrical with long stalk, 200–300 x 20–25 μm , inamyloid, with 4–6 spores

Ascospores globose, smooth, non-guttulate, hyaline to yellowish, 17–23 μm in diam. with walls ~ 1 μm thick (Fig 4 J).

Distribution and habitat. Native to New Zealand, Australia, and Tasmania but apparently naturalized in the United Kingdom (Dennis 1975). It is typically found under *Podocarpus* in New Zealand (Trappe & Claridge 2015), and in a gravel pit under *Crataegus*, *Sambucus*, *Urtica*, *Chamaenerioun*, and *Brachythecium* in Nottingham, England.

Specimens examined. **New Zealand**, det. Lloyd, sent to Roland Thaxter 1921, (FH-374294); North Island, Tongariro National Park, Ketetahi Road, under *Podocarpus* sp., 19 May 2013, T. Orihara TO-13-NZ-19-1 KPM-NC 23051); Bay of Plenty, Whinray Scenic Reserve, 14 May, 2013, Petra White TO-13-NZ-11-5 (KPM-NC 23032); Kaitoke Regional Park, Swingbridge Trail, on humus in broadleaf and podocarp forest, 7 May 1997, D.J. and E.G. McLaughlin DJM1050 (MIN-863002). **Australia**, Tasmania, Mt. Wellington, Hobart, in wet sclerophyll litter, elevation 300m; 42° 54'S 147° 15'E, 30 May 2016, G. Gates EB263 (FLAS-F-59803)

Comments. The specimen from Tasmania differs from the New Zealand specimens because the Tasmanian specimen is light brown with encrusted hairs in the excipulum whereas

the specimens from New Zealand are red and mostly smooth or with minute hairs. The hairs of the Tasmanian specimen were generally short, in scattered clusters, and extended away from the exciple. However, some were long, repent, forming a loosely interwoven outer layer in some places along the excipulum. They originated from cells that were in the outer layer of the exciple. At the molecular level, the ITS sequence from the Tasmanian specimen was ca. 99% similar to other *P. pila* collections from New Zealand and Australia. Further investigation using other loci may help to clarify whether the Tasmanian collection represents a new species or a divergent morphology within *P. pila*. It has been suggested that the New Zealand taxon adapted to bird dispersal, as evidenced by its attractive bright red coloration and smooth peridium. The color and shape resemble the podocarp fruits of the bushes that it is often found fruiting under (Trappe & Claridge 2015).

***Paurocotylis singeri* (Gilkey) Nouhra, Healy & ME Sm., comb. nov.**

MycoBank: MB814821

Basionym: *Hydnocystis singeri* Gilkey, 1962 [1961]. *Mycologia* **53**: 216.

(Fig 4 K–N)

Holotype. **Argentina**, Tucumán, 10 March 1955 R. Singer Gilkey850 (Holotype OSC-25345).

Etymology. “singeri”, In honor of the mycologist Rolf Singer who collected the type specimen.

Description (from Gilkey 1961). *Ascoma* a ptycothecium, subglobose, 1 cm, even to furrowed, white with a simple to partially partitioned cavity, minutely puberulent. Surface hairs originate within the excipulum rather than from surface cells (Burdson 1968). Gleba white, with one or two cavities.

Excipulum composed of pseudoparenchyma

Hymenium of asci and paraphyses in palisade.

Paraphyses simple to branched, shorter than the asci.

Asci cylindrical, 190–228 µm long, 8-spored.

Ascospores globose, smooth, hyaline, 21µm diam.

Description of Paurocotylis singeri (CORD-C00004224 Robledo #1858). *Ascoma* a ptycothecium, white, 15 x 11 x 8 mm when dry, with chambers in the gleba, tomentose with light

brown encrusted projecting hyphae (Fig 4 K) which are the ends of interwoven, unfused, branching hyphae that can be traced to various levels within the outer excipulum, hyphae 6–8 μm diam., occasionally swollen to 9–10 μm in projecting hyphae.

Excipulum. Outer excipulum of loosely interwoven hyphae, (Fig 4 L) constricted at the septum and swollen up to 16 μm between septa. Inner excipulum similar but tightly packed, and fused to form pseudoparenchyma.

Hymenium of cylindrical asci with narrow paraphyses that are about 20 μm shorter than the asci (Fig 4 M–N). The hymenium is invaginated and lines a convoluted chamber.

Subhymenium of interwoven to parallel hyphae, 3–8 μm diam.

Paraphyses 3 μm in diam., septate, even, not inflated at the tips.

Asci 210–268 x 29–36 μm with 2–8 spores per ascus. In asci with fewer than 8 spores, degenerated spore remnants are often visible.

Ascospores globose, smooth, inconspicuously guttulate, 21–24 μm diam. with 2 μm thick walls (Fig 4 N).

Distribution and habitat. Known only from the Yungas forest region of Argentina.

Specimens examined. **Argentina**, Tucumán, 10 March 1955, R. Singer Gilkey850 (Holotype OSC-25345, Isotype CUP-048961); Jujuy Province, Departamento Ledesma, Parque Nacional Calilegua, Sendero Tataupa, Latitude 23° 44' 33.3" south, Longitude 64° 51' 8" west, epigeous on soil in montane subtropical Yunga forests, 2 April 2008, Robledo and Rajchenberg Robledo1858 (CORD-C00004224, FLAS F-59171).

Comments. The Robledo 1858 specimen described above was collected near the type locality and fits the type description in most regards. It differs in that the asci are often less than 8-spored and the spores are 21–24 μm with an average of 22 μm whereas spores for *H. singeri* were described as being 21 μm in diam. or less. Although the spore size and number of spores per ascus differ from the type description, the overall similarities and proximity of collection to the type locality support our identification of this taxon as *P. singeri*. Further collections will be needed to help resolve the morphological concept of this species. Our new collection is clearly similar to the type and both morphological and molecular data indicate that this species is a *Paurocotylis*, not a *Hydnocystis*. This specimen was collected in a type of forest with only one EcM host, the infrequently occurring subcanopy tree *Pisonia zapallo* Griseb. *Paurocotylis* has

not been detected on the roots of *Pisonia zapallo*, but few studies have included this host (Geml *et al.* 2014).

Paurocotylis patagonica Nouhra, Healy and M.E. Sm. **sp. nov.**

MycoBank: MB814820

Fig 4 O–W.

Diagnosis. *Paurocotylis patagonica* is a South American species that can be distinguished from other *Paurocotylis* by its genetic ITS profile, its white ascoma, lack of a distinct simple cavity, its outer excipular and projecting hyphae of granule-encrusted moniliform cells, and its indeterminate paraphyses.

Holotype designated here. **Argentina**, Neuquen Province, Lanin National Park, Ruta a Hua Hum, 12 May 2009, Eduardo Nouhra EN59 (CORD-C00004222).

Etymology. “patagonica”, in reference to Patagonia, the region of South America where this species was discovered.

Description. *Ascoma* a ptycothecium, small, 7 x 4 mm, white, with one hymenium-lined central region stuffed with sterile hyphae that are slightly yellowish in mass (Fig 4 O); exterior minutely felty.

Excipulum. The outer excipulum is 60–120 µm thick, slightly brownish. Emanating hyphae are septate, hyaline, finely encrusted hyphae that originate from various levels within the outer excipulum. The free hyphal tips are 4 µm diam. at septum, many swollen 10–12 µm between septa (Fig 4 P–Q). The excipulum is 280–400 µm thick, hyaline, and composed of interwoven hyphae that fuse to form pseudoparenchyma (Fig 4 R). The outer excipulum can be teased apart to reveal many unfused interwoven hyphae (Fig 4 S). Hyphae constricted to 5 µm at the septum, and swollen to 8–36 µm between septa, appearing beaded or moniliform (Fig 4 S), with walls that are about 1 µm thick. Subhymenium is of interwoven hyphae, 5 µm diam. at septum, sometimes swollen 16–40 µm between septa (Fig 4 T).

Hymenium a palisade of asci and modified paraphyses.

Paraphyses very slender, not fused, 2–4 µm wide, some straight, others tortuously bent between and beyond asci. The paraphyses are greatly elongated, and stuff the region above the asci where they are parallel to interwoven, hyaline, and are often swollen between the septa. This tissue may be thought of as a modified epithecium.

679 *Asci* cylindrical to slightly clavate (Fig 4 U), tapered to the forked base (Fig 4 V), 24–25
680 (–28) μm x 100–270 μm , walls 1 μm thick, usually about 170 μm in length and of even width
681 tapering to base, with 4– typically 7–8 uniseriate spores at maturity.

682 *Ascospores* globose, smooth, with inconspicuous guttules (Fig 4 W), pinkish, (16–)19–26
683 μm , with an average of 22.6 μm diam., varying in size, with walls two layered 1.6 μm thick (Fig
684 4 W).

685 *Distribution and habitat.* Known from mixed forests in the Patagonian region of the
686 Andes Mountains in Argentina and Chile.

687 *Specimens examined.* **Argentina**, Neuquen Province, Lanin National Park, Ruta a Hua
688 Hum, Sitio 3 (b1), in organic soil with roble pellin (*Nothofagus obliqua*), Latitude 40° 08' 56.6"
689 south, Longitude 71° 36' 29.8" west, 12 May 2009, Eduardo Nouhra EN59 (Holotype CORD-
690 C00004222). **Chile**, Puyehue National Park, Anticurra, Sendero La Princesa, in mixed forest, 5
691 May 2016, Pablo Sandoval Leiva, MES-1666 (SGO, FLAS-F-59804).

692 *Comments.* This species shares a long insert near the beginning of the ITS with *P. pila*
693 that has over 80% similarity in DNA sequence (Supplemental Fig S1). Phylogenetic analyses of
694 the ITS and 28S place these two species closer to each other than to the other species in the
695 clade. *Paurocotylis patagonica* differs from *P. singeri* and *P. bynumii* in having paraphyses that
696 are much longer than the asci, with the ends incorporated into a modified epithecium.

697 *Paurocotylis patagonica* differs from *P. pila* in having ascoma that are white rather than red,
698 having asci that are not evanescent, and having a chamber above the asci that is stuffed by a
699 modified epithecium rather than containing a hollow cavity. *Paurocotylis patagonica* is most
700 similar in morphology to *Paurocotylis watlingii* from Australia (FLAS F-59172), but in *P.*
701 *patagonica*, the tomentum is less dense and the spores are smaller on average.

702
703 ***Paurocotylis watlingii* Healy and M.E. Sm. sp. nov.**

704 MycoBank: MB 819377

705 Fig 5 A–E.

706 *Diagnosis.* *Paurocotylis watlingii* is an Australian species distinguished from other
707 *Paurocotylis* species by its distinct ITS sequence and combination of brown tomentose ascoma,
708 modified epithecium in which paraphyses are indeterminate, lack of glebal cavity, and relatively
709 large spores.

Holotype designated here. Australia, Queensland, Lamington National Park, Binna Burra, 27 Mar 1974, coll. R. Watling, Trappe 11725 (FLAS F-59172)

Etymology. In honor of the collector, the eminent mycologist Roy Watling.

Description. *Ascoma* a ptycothecium, clay-colored with white, unstratified gleba with slightly sinuous streaks, 8 x 8 mm, slightly flattened, slightly subtomentose with granule-encrusted hyphae (Fig 5 A).

Excipulum. Outer excipulum composed of brown-walled *textura intricata*, mostly unfused and can be teased apart (Fig 5 B). Outer excipular hyphae emanate from the ascoma to form the tomentum (Fig 5 B–C); hyphal ends 6 μm , swollen to 10 μm , or of even diam. Outer excipular hyphae constricted at the septum (5 μm) and inflated between septa to 26 μm (Fig 5 B). Inner excipulum similar but hyphae fused and more densely packed together (Fig 5 D).

Hymenium a palisade of asci and elongated paraphyses, 3 μm diam., that form a modified epithecium.

Paraphyses are indeterminate in a modified epithecium. Hyphae are tortuously bent. Subhymenium of uninflated, interwoven hyphae.

Asci long, narrow, with 8 or fewer uniseriate spores/ascus.

Ascospores smooth, globose, pink-walled, inconspicuously guttulate, 22–28 μm diam., with an average size of 25 μm (Fig 5 E).

Distribution and habitat. Only known from a subtropical rainforest in Australia, from a single collection found on the soil surface by a mammal burrow, evidently tossed up to the soil surface.

Comments. The paraphyses and modified epithecium of this species are similar to those of *Paurocotylis patagonica*. However, the ascoma of *Paurocotylis watlingii* (FLAS F-59172) is more densely tomentose, with outer excipular hyphae that are darker brown than in *P. patagonica* and the spores are larger on average. This species is described from a single collection.

Paurocotylis bynumii (Trappe, Bushnell & Castellano) Healy & M.E. Sm., **comb. nov.**

MycoBank: MB814822

Basionym: *Stephensia bynumii* Trappe, Bushnell & Castellano, *Mycotaxon* **64**: 432 (1997)

(basionym)

Holotype. United States of America, Oregon, Turner, under *Corylus* and *Rhus*, 20 June 1980 O. Bynum Trappe 5832 (OSC-58834).

Etymology. “bynumii”, in honor of O. Bynum, the collector of the type specimen.

Description. *Ascoma* a ptycothecium, subglobose, lobed, folded, 1–2 x 1.5–2 cm, yellowish brown with brown tomentum, gleba white to cream-color with pink-brown to orange brown veins. Odor not distinctive.

Excipulum. Outer excipulum 50–150 μm thick, composed of loosely interwoven hyphae that form moniliform cells, 4–9 μm diam., cells inflated to 10–40 μm . Tomentum granule-encrusted, granules hyaline to light yellow. Inner excipulum hyaline, tightly interwoven.

Hymenium a palisade of paraphyses and asci.

Paraphyses narrow 2–3.5 μm diam., septate, same length as asci or slightly longer.

Asci cylindrical, 230–250 x 18–25 μm tapering to long stem and forked base, 6–8 spores arranged uniseriately

Ascospores globose, smooth, hyaline, 16–21 μm diam.

Distribution and habitat. Only known from Oregon, USA. Hypogeous under *Pseudotsuga menziesii*, *Corylus cornuta* var. *californica*.

Specimens examined: United States of America, Oregon, Marion Co., Turner, Marion Hill Rd, WVNMM, under *Corylus* and *Rhus*, 600' elevation, 20 June 1980, O. Bynum Trappe 5832 (Holotype OSC-58834); 28 June 1980, Trappe 5855 (OSC-58835); under *Tsuga heterophylla*, 10 July 1980, Trappe 5868 (OSC-58836); 26 July 1980, Trappe 5884 (OSC-58837); 2 August 1980, Trappe 5942 (OSC-58838); Junction of County Rds.-Howell Prairie No. 51 and Waconda Rd. No. 6, under maple, 200' elevation, 9 April 1986, W. Bushnell Trappe 8881 (OSC-58839); Clackamas Co., Beaver Cr., WVCL, under *Pseudotsuga menziesii*, *Alnus rubra* & *Corylus californica*, elevation 500 ft., 15 August 1986, D. Wheeler Trappe 9004 (OSC-58840); Paul Bishop's Tree Farm, 26 July 1987, Trappe 9574 (OSC-58841).

Comments. *Paurocotylis bynumii* is the only member of the genus *Paurocotylis* to have a native distribution in the Northern Hemisphere. It is also the most disjunct, as it occurs in the Northwestern USA, while the others have a Gondwanan distribution. It is sister to the other species in *Paurocotylis*. We place it in *Paurocotylis* as the best current hypothesis, acknowledging that further data may reveal it to be a distinct genus. Morphological features that help to unite this species with other *Paurocotylis* include the brown tomentum of granule-

encrusted hyphae, outer excipulum composed of loosely interwoven hyphae that form chains of moniliform cells, and asci that taper to the forked base. The paraphyses are as long as or slightly longer than the asci, and there is no epithecium. In this regard, the morphology is more like that of *P. singeri* than the other *Paurocotylis* species. *Paurocotylis bynumii* is reported as a probable mycorrhizal associate with *Pseudotsuga menziesii* and *Corylus cornuta*, but molecular evidence from root tips is lacking.

Densocarpa Gilkey *N. Amer. Fl.*, Ser. 2 (New York) **1**: 16 1954.

Type species. ***Densocarpa shanorii*** Gilkey [as '*shanori*'], 1954. *N. Amer. Fl.*, Ser. 2 (New York) **1**: 16, 1954[1955]. *Mycologia* **46**: 786.

Fig 5 F-H

Types. United States of America, Illinois, Champaign-Urbana, 14 June 1953 L. Shanor Gilkey 764a (Holotype ILL-10894, Isotype OSC-38610).

Synonym: *Stephensia shanorii* (Gilkey) Gilkey (1962) [1961] *Mycologia* **53**(3): 219.

Etymology. “*shanorii*”, in honor of L. Shanor, the collector of the type specimen.

Description. *Ascoma* a ptycothecium, knobby, folded, sandy in appearance, smooth to slightly verrucose, often cracked, often grub-infested, sometimes with ants; 0.5–2 cm, light ochraceous buff (Gilkey 1954), light yellowish-pink (ISCC-NBS 28) to light orange brown (ISCC-NBS 57) (Fig 5 F), with basal tuft of granule-encrusted hyphae. Gleba pale ochraceous buff (Gilkey 1954), white to cream white with meandering canals (Fig 5 F). Odor unpleasant, raffinaceous.

Excipulum 80–180 μm thick. Outer excipulum pale yellowish-brown, of interwoven hyphae 5–10 μm at septum with cells inflated to 15 μm composed of textura intricata that is fused to form small-celled pseudoparenchyma. Excipular hairs sparse, of smooth to encrusted hyphae (granules up to 0.6 μm diam., orange in mounting medium with ethanol), hyaline to yellowish, 5 μm diam. Inner excipular hyphae hyaline, cells less swollen, but otherwise similar to outer excipulum, hyphae 2.5 μm at septum swollen to 5 μm .

Hymenium a palisade of paraphyses and asci, infolded with canals between opposing hymenia.

Paraphyses hyaline, slightly swollen at the tips, 2.5–5 μm diam., some about the same length as asci and others greatly elongated to form a web of hyphae in the canal between

opposing hymenia. Elongated paraphyses fuse, and connect opposing hymenia or partially stuff the canals (Fig 5 G).

Asci inamyloid, cylindrical, 150–188 μm x 17.5–22.5 μm , walls 1 μm , (3–) 4–8 uniseriate spores, spores in a single ascus often of different sizes, thin-walled smaller spores apparently disintegrating. Ascus tapers, narrowing towards base.

Ascospores smooth, globose, with one large guttule that is irregular in appearance (Fig 5 H), (14–) 15.6–17.5 (–20) μm diam. with thick pinkish wall, 1 μm diam.

Anamorph produced in culture from ascospores germinated in 1% malt extract broth and showed best growth on oatmeal agar (Uecker 1967). Colonized agar becomes brown to gray. Conidiophores verrucose, producing conidia blastically, sympodially, terminally or laterally on long or short branches, singly or in groups of two or more. Conidia warted, brown, globose, 6–8 μm diam., to ovoid, 6–10 x 5–7 μm sometimes with a short stalk (e.g. remnant of the conidiophore). The conidiophores encrusted with dark brown warts. The older hyphae in the colonies becoming brown and granule-encrusted, similar to those of the conidiophores and hyphae at the base of mature ascomata (Uecker 1967).

Distributions and habitat. *Densocarpa shanorii* is known from the Northeast U.S.A., collected in lawns, garden soil, and on wood or woody debris in mixed deciduous and coniferous woods (<http://mycoportal.org/portal/index.php>, accessed May 2016).

Specimens examined. **United States of America**, Illinois, Champaign Co., Urbana, Pennsylvania St. at Busey, 14 June 1953 L. Shanor Gilkey 764a (Isotype OSC-150061); Illinois St., 1 June 1953 H. Ahles Gilkey 782 (OSC-146659); Brownfield Woods, 1 July 1960 D.D. McLain (OSC-80635); Iowa, Decatur County, near Leon, in bark litter in oak savanna, 12 Apr 1999, Sibylla Brown RH328 (ISC); Story Co., Ames, Ames High School Woods, on ground in bark litter of elm tree, 29 Jun 1998, R. Healy 136 (FLAS-F-59175); Story Co., near Ames, McFarland Park, erumpent in soil under *Quercus macrocarpa* and *Tilia americana*, 24 Jun 2005, R. Healy (FLAS-F-59174); Missouri, St. Louis Co., St. Louis, erumpent in soil in cultivated garden adjacent to natural area, 10 May 2016, Ken Gilberg (FLAS-F-59805). Michigan, Washtenaw Co., Ann Arbor, University of Michigan campus, 17 July 1945 A.H. Smith 34280 (OSC-150059); **Mexico**, Chiapas, ca 5 km S. of Ixtacomitan, Carmino Teapa-Tuxtla, 13 July 1972 Trappe 3319 (OSC-150060).

Comments. The asci in specimens studied here often had more than four mature spores per ascus while the type description states that four or fewer spores per ascus typically reach maturity (Gilkey 1961). However, Uecker (1967) also reported up to eight spores maturing per ascus. The guttules in our *Densocarpa* specimens are not smooth and uniform as in some other Pezizales known to have “guttulate” spores, and are perhaps of a different composition. Ours are similar to those depicted in Uecker (1967).

Gilkey originally described *Densocarpa* in 1954. Later, however, she observed strong similarities between *D. shanorii* and *Stephensia sumatrana* and decided to transfer *D. shanorii* to the genus *Stephensia* (1961). When she transferred the species Gilkey noted that *D. shanorii* and *S. sumatrana* had more similarities to each other than to *H. bombycina* (referred to in that publication as *Stephensia bombycina*, the type of the genus *Stephensia* – Gilkey, 1961). In particular, Gilkey noted the lack of a basal cavity in *D. shanorii* and *S. sumatrana*. Nevertheless, she amended the generic description of *Stephensia* to accommodate *D. shanorii*, adding the character of a notable and foul H₂S odor to her description of *D. shanorii*. Uecker (1967) added more details to Gilkey’s description during his cytological study of *D. shanorii*. One of Gilkey’s questions regarding differences between *D. shanorii* and *H. bombycina* was answered when Uecker documented that, unlike *H. bombycina*, there is no universal tomentum during any stage of development in *D. shanorii*. However, he noted that the ascomata are completely enclosed throughout all developmental stages and he noted the absence of a basal cavity. He also observed that the asci disintegrate at maturity to release spores into the gleba and he described the spores as having one large or several smaller oil drops.

Densocarpa crocea (Quél.) Healy and M.E. Sm., **comb. nov.**

MycoBank: MB814819

Basionym: *Stephensia crocea* (Quél) Quél. (1887) *Comptes Rendus de l’Association Française pour l’Avancement des Sciences* 15: 489.

Synonym: *Stephensia bombycina* var. *crocea* Quél. (1886) *Enchir. fung.* (Paris): 258.

Type. **Netherlands**, Utrecht, Bunnik, Oud Amelisweerd, 20 Oct 1984 G. van Immerzeel & G. de Vries (CBS H-18547). Not examined.

Etymology. Latin, “crocea”, saffron-yellow, referring to the orangish yellow color of the peridium.

Description (from De Vries 1985). *Ascoma* a ptycothecium, up to 2 cm diam., yellow, oblong, covered with tomentum, pale orange, darker brown in crevices, gleba whitish to yellow with canals stuffed by elongated paraphyses. Odor raphinoid.

Excipulum 400–700 µm, outer excipulum pale yellow, 40–45 µm thick, composed of pseudoparenchyma, cells 10–20 µm diam., tomentum of granule-encrusted, hyaline to brown, short hyphae. Inner excipulum of hyaline textura intricata.

Hymenium a palisade of paraphyses and asci.

Paraphyses hyaline to brown, septate, branched, 2.5–5 µm diam., smooth except for the granule-encrusted region elongated beyond the ascus tips.

Asci cylindrical to oblong, tapering to base, 120–200 x 18–23 µm, 4–8 spores.

Ascospores hyaline, globose, smooth, 10–17 (–22) µm diam., wall 1 µm thick.

Anamorph not observed, but cultures were “easily” obtained on malt extract agar and Sabourad’s agar (de Vries 1985).

Specimens examined. **Netherlands**, Noord-Brabant, Drimmelen, in garden near *Betula* and ornamental shrubs, 15 October 1982, Mrs. W. Sommer-Kenniphaas G.A. deVries 1030 & J. Trappe (OSC-41582, as *S. densocarpa*); **Spain**, Asturias, Covadonga, 17 Aug 2001, E. Rubio (MA-56988).

Comments. If a type specimen exists, its location is unknown. De Vries (1985) described specimens of *S. crocea* as so similar to *S. shanorii* as to be conspecific, as did Fontana & Giovannetti (1987) though no formal transfer was made. Our molecular data supports the placement of *S. crocea* in *Densocarpa*, but suggest *D. crocea* and *D. shanorii* are distinct species. The two species are also geographically separated with *Densocarpa crocea* documented only from Europe and *D. shanorii* known only from North America. A collection tentatively labeled *S. shanorii* from Mexico is molecularly divergent in both ITS and LSU from *S. shanorii* from the USA, suggesting it is a novel species.

Taxa to consider for future analysis of the /tarzetta-geopyxis lineage

No sequence data are currently available for *Berggrenia cyclospora* (Cooke) Sacc. (1889). *Syll. fung.* (Abellini) 8: 152 but this species has some morphological features in common with the other truffles in the /tarzetta-geopyxis lineage. This pinkish, lobed, terrestrial fungus has globose hyaline spores, no paraphyses, and is known only from New Zealand. Originally described as *B.*

895 *aurantiaca* var. *cyclospora* by Cooke (1886), Saccardo (1898) recognized the difference in spore
896 shape (ellipsoid in *B. aurantiaca* and globose in *B. cyclospora*) as worthy of species status and
897 elevated it to *Berggrenia cyclospora*. These are the only two recognized species in the genus
898 *Berggrenia*. Lack of paraphyses is a characteristic of *Berggrenia*, but it is not clear from
899 descriptions whether the paraphyses are missing altogether or whether they are modified as
900 indeterminate or interwoven hyphae among the asci. The genus *Berggrenia* is currently
901 considered *incertae sedis* within the Pezizomycotina (Lumbsch & Huhndorf 2007) but the
902 Austral distribution and the globose spores may indicate an affinity with the /tarzetta-geopyxis
903 lineage. Fresh collections and DNA sequences are needed to resolve the phylogenetic affinity of
904 both species in this genus.

905 *Stephensia sumatrana* Boedijn, (1939) was described from Sumatra by Boedijn, and later
906 reported by Gilkey (1961) to be more similar to *D. shanorii* than to *H. bombycina*. Illustrations
907 and description by Boedijn (1939) appear similar to *Densocarpa* in the invaginated hymenia
908 lining the canals, and the excipulum composed of pseudoparenchyma. This species is so far
909 known only from Sumatra. Ascomata of this species have a gleba with meandering veins, one
910 small ostiole, a thick excipulum of pseudoparenchyma, an irregular palisade of asci and
911 paraphyses that grow beyond the asci to merge with the hyphae that stuff the canals between
912 opposing hymenia. The asci are cylindrical, with eight or fewer globose, smooth ascospores.
913 The presence of an ostiole in *S. sumatrana* is notably different than other described species in
914 *Densocarpa* but may suggest an affinity with *Paurocotylis* or *Hydnocystis*. Fresh collections and
915 DNA sequences are needed to resolve the phylogenetic affinity of this fungus.

916 *Stephensia colomboi* is said to be similar to *H. bombycina* (Læssøe & Hansen 2007).
917 DNA sequences and a morphological comparison with other taxa in the /tarzetta-geopyxis
918 lineage will help to place this species.

919 *Stephensia peyronelii* has spores that are not globose, but rather ellipsoid, and may be
920 twice as long as wide (Trappe *et al.* 1997). This species has only known from the type specimen
921 and description. The isotype examined (OSC-131642) was too scanty and old to sample for
922 DNA. A fresh sample would help to place this species. If it belongs in any of the three truffle
923 genera treated here, it will be the only one with ellipsoid rather than globose spores.

Wenyingia sichuanensis Zheng Wang & Pfister (2001), a monotypic species, is described as morphologically similar to *Tarzetta*. Sequence data are needed to determine whether this taxon may also belong to the /tarzetta-geopyxis lineage.

Doubtful and excluded taxa

Additional species placed in *Hydnocystis* include *H. arenaria* Tul., *H. beccari* Mattir., and *H. echinosperma* Rodway. The first two species were placed in *Geopora* by Burdsall (1968) and it was Burdsall's opinion that *H. echinosperma* was a *nomen superfluum* for *Sphaerosoma tasmanica*. Accordingly, we consider these species to belong outside of the /tarzetta-geopyxis lineage.

About half of the legitimately named species originally placed in *Paurocotylis* were later determined to be Glomeromycota. These include *Redeckera fulvum* (Berk. & Broome) C. Walker & A. Schüßler (synonym: *Paurocotylis fulva* Berk. & Broome 1873), *Glomus fragile* (Berk. & Broome) Trappe & Gerd. (synonym: *Paurocotylis fragilis* Berk. & Broome) and *Glomus macrocarpum* Tul. & Tul. (synonym: *Paurocotylis fulva* var. *zealandica* Cooke).

Placement of the remaining species in the /tarzetta-geopyxis lineage is doubtful because none of these taxa have smooth spores, a defining feature of all known taxa in the /tarzetta-geopyxis lineage. Descriptions of *Paurocotylis echinosperma* Cooke, *P. niveus* Rodway and *P. prima* Rick clearly include spiny spores. In addition, *P. niveus* is described with globose asci and this feature has not been found in any other taxa in the /tarzetta-geopyxis lineage. No sequences are available for any of these taxa.

Discussion

Phylogenetic analyses

Phylogenetic analyses of the ITS strongly supported three monophyletic truffle genera in the /tarzetta-geopyxis lineage: *Hydnocystis*, *Paurocotylis*, and *Densocarpa*, while inferring a paraphyletic *Geopyxis* as shown by previous work (Hansen *et al.* 2013). *Hydnocystis* and *Densocarpa* were strongly supported by phylogenetic analyses of the 28S region, while *Paurocotylis* was moderately supported by ML only. Unifying morphological characters for the three truffle genera include ascoma with a ptycothecium form, projecting excipular hyphae encrusted with granules, a hymenium of cylindrical asci in a palisade, and smooth, hyaline, globose spores. Anamorphs are known for *Hydnocystis*, *Densocarpa*, and *Geopyxis*, including

Hydnocystis bombycina (Fontana & Giovannetti 1987) and *H. transitoria*, (this study), *D. shanorii* (Uecker 1967), *G. majalis* and *G. carbonaria* (Paden 1972, Vrålstad *et al.* 1998). The anamorphs in the /tarzetta-geopyxis lineage are unified by globose to ovoid warted conidia produced at the tips of branched or simple-septate conidiophores. Species of *Geopyxis*, *H. bombycina*, and *D. shanorii* have all been readily grown in axenic culture and produced sympodial, blastosporic conidia on conidiophores (Paden 1972, Fontana & Giovannetti 1987, Uecker 1967).

Anamorphs may have been described for species in the /tarzetta-geopyxis lineage under other names. Chamuris & Wang (1990, 1998) made a nomenclatural recombination of the anamorph *Rhinotrichum alutaceum* Peck (1881) to their new genus *Stenocephalopsis subalutacea* (Peck) Chamuris & Wang (1998). Peck's species was collected on decayed wood in New York. Chamuris & Wang gained access to fresh collections of the same fungus from plant debris in New York State. Their descriptions and images are strikingly similar to the anamorphs of *Hydnocystis transitoria* and *H. bombycina*. The similarities include spore mat color, granule-encrusted conidia and conidiophores, denticles remaining on smooth apices after spore release, and branching and conidiation patterns. Chamuris & Wang (1990) also noted that their anamorph was similar to that of *Geopyxis majalis* (Paden 1972). Within the taxon *S. subalutacea*, Chamuris & Wang (1990) included spore mats from North America, New Zealand and the United Kingdom. In addition to these localities, Holubová-Jechová (1982) putatively reported the same species (as *Basifimbria subalutacea*) from the Czech Republic, Russia, and Germany. The variability in spore size and ornamentation noted by Holubová-Jechová (1982) may signal that taxa across this wide area are different species. Based on our phylogenetic analyses, it is doubtful that species of truffles in the /tarzetta-geopyxis lineage are found on multiple continents, except in cases where they have been introduced due to anthropogenic activities. It would be interesting to recollect similar spore mats from these localities and to sequence DNA from them to determine if they fall within the /tarzetta-geopyxis lineage. If this is the case, it may help to further elucidate the ecology, and geographic distributions of this group of fungi.

Morphological features that characterize the /tarzetta-geopyxis lineage include ascomata and anamorphs with granule-encrusted hyphae, cylindrical asci, and smooth spores. *Geopyxis* differs from the truffle genera in the clade by the lack of excipular hairs and in the ellipsoid rather than globose spores. Morphological similarities among the epigeous and hypogeous

genera include smooth spores, asci that taper at their bases, and similar anamorph characters. In the truffle lineages, further unifying characteristics include globose spores, and the presence of asci and paraphyses in a more or less organized hymenium. Features that help to differentiate among genera in the /tarzetta-geopyxis lineage include excipular structure and spore shape, presence of a well-defined epithecium, and presence of a glebal cavity. The outer excipulum of most *Paurocotylis* is composed of partially to fully unfused interwoven, branched hyphae. *Hydnocystis* can be differentiated by the presence of a cavity, often with an opening connecting the cavity to the exterior, and the presence of a well-defined epithecium in most species. Species of *Densocarpa* have ascomata with open to stuffed canals between folded opposing hymenia but they lack a glebal cavity.

Ecology of the /tarzetta-geopyxis lineage

It is often assumed that truffles and false truffles are EcM and some authors have erroneously assigned an EcM lifestyle to truffles that are not EcM (e.g. Rinaldi *et al.* 2008, Molina *et al.* 1992). *Geopyxis* was previously considered EcM by some authors (Vrålstad *et al.* 1998, Rinaldi *et al.* 2008). However, evidence suggests that *Geopyxis* and the related truffle genera discussed here do not form EcM symbioses. Lines of evidence include the rapid growth in axenic culture (Vrålstad *et al.* 1998), conidial germination on media (Fontana & Giovannetti 1987), the development of anamorphs in culture, and the development of ascomata among woody litter on the soil surface. Furthermore, except for *Tarzetta*, many of the species in the /tarzetta-geopyxis lineage consistently fruit away from EcM host plants in nature (Pegler *et al.* 1993, Dennis 1975, Trappe & Claridge 2015) and except for *Tarzetta*, there are no credible ITS rDNA sequences from this lineage reported on EcM root tips, even though there have been many molecular studies of roots in areas where *Geopyxis* and allies are regularly collected (Tedersoo *et al.* 2010). In contrast, the anamorphs and ascomata of EcM Pezizales are typically found on bare soil and are regularly recovered from ITS rDNA sequencing of EcM roots (Petersen 1985, Tedersoo *et al.* 2010, Healy *et al.* 2013)

Except for *Tarzetta* species, taxa in the /tarzetta-geopyxis lineage that have been found on roots, have also survived for an extensive period of time after the death of the plant, suggesting they are not obligately mycorrhizal (Vrålstad *et al.* 1998). In addition, some studies have found a positive correlation between burned trees and *Geopyxis* fruiting, a pattern which is atypical for

EcM fungi Greene *et al.* 2010). Other than *Tarzetta*, members of the /tarzetta-geopyxis clade may have biotrophic, saprobic, or mixed trophic modes. Our phylogenetic analyses included many endophyte sequences of angiosperms, gymnosperms, lichens and mosses nested within the /tarzetta-geopyxis lineage. An endophytic lifestyle was previously suggested for *Geopyxis* by Hansen *et al.* (2013) and Tedersoo *et al.* (2013).

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- Figure legends**
- Fig 1 – Phylogenetic tree based on maximum likelihood (ML) analysis of 28S rDNA, with node support from 1000 bootstrap iterations and Bayesian posterior probabilities (BPP). BPP ≥ 0.95 / Bootstrap values $\geq 70\%$ are indicated at branch nodes. Thickened branches indicate support from both methods of phylogenetic analyses. Bolded taxa are newly generated sequences. Non-bolded sequences are from GenBank, “EcM” stands for “ectomycorrhizal”. Taxon names presented here reflect nomenclatural revisions introduced in the taxonomy section of this paper.
- Fig 2 – Phylogenetic tree based on maximum likelihood (ML) analysis of ITS rDNA, with node support from 1000 bootstrap iterations and Bayesian posterior probabilities (BPP). BPP ≥ 0.95 / Bootstrap values $\geq 70\%$ are indicated at branch nodes. Thickened branches indicate support from both methods of phylogenetic analyses. Bolded taxa are newly generated sequences. Non-bolded sequences are from GenBank, “EcM” stands for “ectomycorrhizal”. Taxon names presented here reflect nomenclatural revisions introduced in the taxonomy section of this paper.
- Fig 3 – *Hydnocystis transitoria* (A–P). (Type specimen RH950) (A). Ascoma with opening into cavity; (B). Ascoma cut in half to expose cavity lined with regular palisade of hymenium overtopped by cottony epithecium (arrows); (C–K). Brightfield microscopy; (C). Excipular

1233 hypha encrusted with granules; (D). Projecting hyphal tips of the excipulum; (E–H) Resin
1234 embedded sections stained in toluidine blue O; (E). Excipulum composed of textura angularis;
1235 (F). Epithecium; (G). Hymenium with asci and paraphyses; (H). Higher magnification of asci
1236 interspersed with interwoven paraphyses; (I). Epithecium of parallel hyphae mixed with textura
1237 intricata; (J). Cylindrical ascus with uniseriate spores, and tapered base; (K). Ascospores; (L–P).
1238 Anamorph; (L). Mitospore mat; (M–O). DIC microscopy; (M). Two conidia produced at the tip
1239 of a conidiophore; (N). Conidiophore branches at septa (arrowhead), sometimes short (arrow),
1240 and conidia at branch tips; (O). Conidia denticles (arrows) and scars; (P). Warty ornaments of
1241 conidia and encrusted conidiophores. –Scale bars (A, B, L) = 2 mm, (C, E, H, I, N) = 20 μ m,
1242 (D) = 25 μ m, (F, G) = 100 μ m, (J, K, M, P) = 10 μ m, (O) = 5 μ m.

1243
1244 Fig 4 – (A–E) *Hydnocystis japonica*. (A). Ascomata; (B–E). Brightfield microscopy. (B).
1245 Excipulum; (C). Paraphyses; (D). Tip of paraphysis; (E). Immature ascus; (F–I) *Paurocotylis*
1246 *pila* (F) Ascoma cut in half (lower) and podocarp fruits (upper); (G–N) Brightfield microscopy.
1247 (G). Excipulum; (H). Projecting granular encrusted excipular hypha; (I). Hyphal ends (arrows) of
1248 excipular cells; (J). Ascospores; (K–N). *Paurocotylis singeri* (Robledo 1858). (K). Tomentum of
1249 encrusted hyphal tips of outer excipular hyphae; (L). Outer excipulum construction of loosely
1250 interwoven, unfused hyphae; (M). Regular hymenium of asci and paraphyses shorter than asci;
1251 (N). Ascus with ascospores; (O–W). *Paurocotylis patagonica* sp. nov. (Type specimen EN59).
1252 (O). Ascoma halves showing gleba (upper left) with hymenium surrounding modified epithecium
1253 (asterisk); (P–W). Brightfield microscopy. (P). Hyphal ends (“hairs”) of outer excipulum; (Q).
1254 Moniliform excipular hyphal tips; (R). Inner excipulum of pseudoparenchyma; (S). Outer
1255 excipulum with unfused interwoven hyphae; (T). Interwoven hyphae in the gleba, above the asci
1256 (modified epithecium); (U). Ascus with ascospores; (V). Forked base of ascus; (W). Ascospores.
1257 – Scale bars (A) = 1 cm, (B, E, K, N, S, T, U, V) = 20 μ m, (C, H, I, J, W) = 10 μ m, (D, F, O, P,
1258 Q, R) = 5 μ m, (G, L) = 30 μ m, (M) = 40 μ m.

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1260 Fig 5 – (A–E). *Paurocotylis watlingii* (FLAS-F-59172) Brightfield microscopy. (A). Excipular
1261 tomentum; (B). Outer excipulum of unfused, interwoven, granule-encrusted hyphae, swollen
1262 between the septa; (C). Outer excipulum with granule-encrusted hyphae; (D). Excipulum; (E).
1263 Hymenium of asci and indeterminate paraphyses; (F–H). *Densocarpa shanorii* (RH1310). (F).

1264 Ascoma halves showing gleba (rt); (G). SEM of opposing hymenia of asci and paraphyses that
1265 grow into the channel between them; (H). Ascospores. – Scale bars (A, G) = 50 μm , (B, C, D, E)
1266 = 20 μm , (F) = 5 mm, (H) = 15 μm .

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