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ARTICLE

Astraeus: hidden dimensions

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Abstract: The genus *Astraeus* is shown to be even more complex than recent studies have found. There are problems defining what the molecular fingerprint is of the generic type species. The present article, based on molecular and morphological information and the classical literature, attempts to throw further light on these important ectomycorrhizal fungi. Our studies go part way in an endeavour to unravel the taxonomy and systematics of this genus, necessitating the recognition of at least three new species. Potential nomenclatural problems are also outlined.

Key words:
Ectomycorrhizal fungi
gasteromycetes
phylogeny
taxonomy
nomenclature
ITS nrDNA

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INTRODUCTION

Astraeus is a member of *Boletales*, although the morphology is superficially similar to the earthstars of *Geastrum* (syn. *Geaster*), such as *G. multifidum* (syn. *G. coronatum*), and into which *Astraeus* was originally placed. *Geastrum hygrometricum* is the type of *Astraeus* by original designation, and for many years the genus was considered monospecific (Morgan 1889). Similarities with some *Geastrum* species in gross morphology and hygroscopic physiology have led to some confusion with members of that genus from the earliest times. Thus, Coker & Couch (1928) showed that Woodward's (1794) *Lycoperdon recolligens*, which was based on Schmidt's plate defining *G. recolligens* (syn. *G. coronilllum*), is undoubtedly a mixture of a true earthstar (*G. mammosum*, a further synonym of *G. corollinum*) and *Astraeus*, a notion previously adopted by Persoon (1801) and Fries (1829), and an unnecessary "β *anglicum*" was introduced for Woodward's part of the concept. A further example is Bolton's (1788) report of an *Astraeus,* later called *G. boltonii* Willd. 1795 and from Swaines Moor (Halifax West Yorkshire, UK) a most unlikely locality, which R.W. has examined; the figure on which Bolton's record is based is rather poor, but and most probably represents *G. rufescens*, according to J. Palmer (pers. comm., 11 Feb. 1958). Accordingly, any previously published interpretations and associated distributional records of *A. hygrometricus* must be considered with caution. With its so-called ease of identification, *Astraeus* has been recorded from many sites without adequate examination and was said to exhibit a wide host range and distribution. Although a common species, juvenile enclosed gasterothecia have been occasionally confused with entirely hypogeous taxa, or as Lloyd (1902) indicates, macroscopically, when young basidiomes resemble an undeveloped *Scleroderma.*

Dipterocarpaceae, *Fagaceae,* and *Pinaceae* are well-known to form ectomycorrhizal associations; but as the fungus occurs in sandy fields, the hosts are undoubtedly angiosperms. Unfortunately, until recently little attention has been paid to the host association (Wilson *et al*. 2012). The species was previously thought to be cosmopolitan and quite common in warm temperate to subtropical and tropical climates and in all the continents except Antarctica. It is not found in arctic-alpine communities and is less frequent in boreal areas, although it ranges from low to montane regions in the Himalayas. In contrast to North America, it is evidently rare in South America. Perhaps this all-embracing distributional concept is reflected in the epithet "*vulgare"* given to *A. hygrometricus* by Corda (1842). In Thailand, what had been considered to be *A. hygrometricus* had a long history of edibility, and is regularly found on sale in urban and rural markets; however, our previous studies have shown that in Thailand only two species of *Astraeus* are found: *A. asiaticus* (Phosri *et al*. 2007) and *A. odoratus* (Phosri *et al*. 2004).

Massee (1889) described *Geaster lilacinus* from India based on a collection of Gamble (no. 105 in K), differing it is said by the larger basidiospores. Ahmad (1950) examined the material and found the spore-size to fall in line with what was then considered to be the range for "*hygrometricus*". Massee's measurements of microscopic characters are notorious for being erroneous according to the late Derek A. Reid (formerly of the Herbarium, Royal Botanic Gardens, Kew). Ahmad, during his re-examination, indicated that *A. hygrometricus* was common in India, citing material from

Table 1. List of specimens included in morphological and molecular analyses, not studied in Phosri et al (2007). * Specimen identified by Morgan as *Astraeus hygrometricus* (Pers.) Morgan.** Sequences obtained after cloning.

DNA isolation Code	Voucher, Herbarium	Country. Locality	Collection Date	Accession No.
A. hygrometricus				
ASTRAE122	MJ1558 (E, MA-Fungi). Neotype.	France. Provence, Avignon.	2 Jan. 1981	HG000287
ASTRAE129	Isiloglu 2113 (E, MA-Fungi)	Turkey. Aydŷn, Cine, Kuruköy village.	21 Nov. 2004	HG000293
ASTRAE131	Isiloglu 8149 (E, MA-Fungi)	Turkey. Ýzmir, Bayindir, Sariyurt village.	25 Nov. 2006	HG000294
ASTRAE132	Isiloglu 8383 (E, MA-Fungi)	Turkey. Antalya, Gundogmus, Guneycik village.	11-May-07	HG000295
A. morganii				
ASTRAE134*	C.F. Baker (E-00159977). Holotype	USA. Colorado, La Plata co.	29 Mar. 1899	HG000296, HG000296-HG000302**
A. pteridis				
ASTRAE124	MJ9732 (E)	Portugal. Madeira, Valley of Nuns.	21 Jan. 1990	HG000288-HG000290**
ASTRAE126	CORD2123 (E)	Argentina. Punilla valley of Córdoba.	20 Jun. 1996	HG000291
ASTRAE127	CORD2123 (MA-Fungi)	Argentina	-	HG000292
A. telleriae				
ASTRAE121	MJ4705 (GB)	Spain. Barcelona, Maresme.	30 Oct. 1998	HG000286

Dehra Dun, Mussories, and Saharanpur in Uttar Pradesh, and Simla, Dalhousie and the Kulu Hills in Punjab.

The fungus was the purported to have a wide distribution and broad host range correlated with slight differences in basidiospore morphology from one area to another that indicated to one of us (R.W.) that the picture presently accepted was too simple. The great range in the world exhibited by this fungus indicated that the recognition of a single species, *A. hygrometricus,* required re-investigation. *Astraeus pteridis* from North America and *A. koreanus* from Korea, however, have also been recognised but they appear rarely in the mycological literature.

The complexity of *A. hygrometricus* started to be revealed in some of our more recent studies (Phosri *et al.* 2007), but a wider ranging investigation was shown to be necessary to understand the full diversity of the fungi occurring under this name. This article aims to examine a wide sweep of specimens from a range of localities accross the world including Africa, South-East Asia, the New World, the Himalayas, as well as the classic areas of Europe. We also address issues surrounding the purported wide distribution of *A. hygrometricus*, the number of actual species constituting the genus, and their mycogeography. Some nomenclatorial problems are also considered, emanating from our study, and three new species are described.

MATERIAL AND METHODS

An in depth study of the genus based on cultured (Pibulsongkram Rajabhat University, Thailand) and herbarium material was undertaken by C.P., but a final analysis could only be made by the use of molecular techniques carried, out by M. M., in parallel with a study of the classical literature by R. W.

Morphological study

Additional herbarium specimens under the name *Astraeus hygrometricus* were examined as described in Phosri *et al.* (2004), and compared with data on 160 specimens studied in Martín (1988) and Phosri *et al.* (2004, 2007) located in BCN, E, K, L, OSC, MA-Fungi, and the A. D. Parker Herbarium (Wisconsin, USA).

Amongst the additional specimens examined, was collection E00159977, (Southern Colorado, USA) collected by C. F. Baker in 1899 and identified by Morgan as *A. hygrometricus,* which we are able to include in the molecular analyses (Table 1).

DNA isolation, amplification and sequencing

Samples for DNA extraction were excised from dry basidiomes. To avoid contamination by other fungi, tissues were taken from the inner part of the basidiome. DNA extraction, amplification and sequencing of the ITS regions, including the 5.8S ribosomal RNA gene cluster, followed the protocols in Phosri *et al.* (2009), with the primer pair ITS1F/ITS4 (White *et al*. 1990; Gardes & Bruns 1993), and the cycling protocol described in Martín & Winka (2000). Aliquots of the purified products were mixed separately with the direct and reverse primers before sending to Macrogen (South Korea) for sequencing. When weak PCR products were obtained, the products were cleaned from the gel and cloned; both strands were separately sequenced using vector specific primers T7 and M13. Sequences obtained in this study are included in Table 1.

Consensus sequences where assembled using Sequencher (Gene Codes Corporation, Ann Arbor, MI). Prior to the alignment, sequences were compared with homologous sequences in EMBL/GenBank/DDBJ (Cochrane *et al.* 2011) using the BLASTn algorithm (Altschul *et al*. 1997). Multiple sequence alignment of the consensus sequences obtained in this study and homologous sequences from EMBL/GenBank/DDBJ, mainly presented in Phosri *et al*.

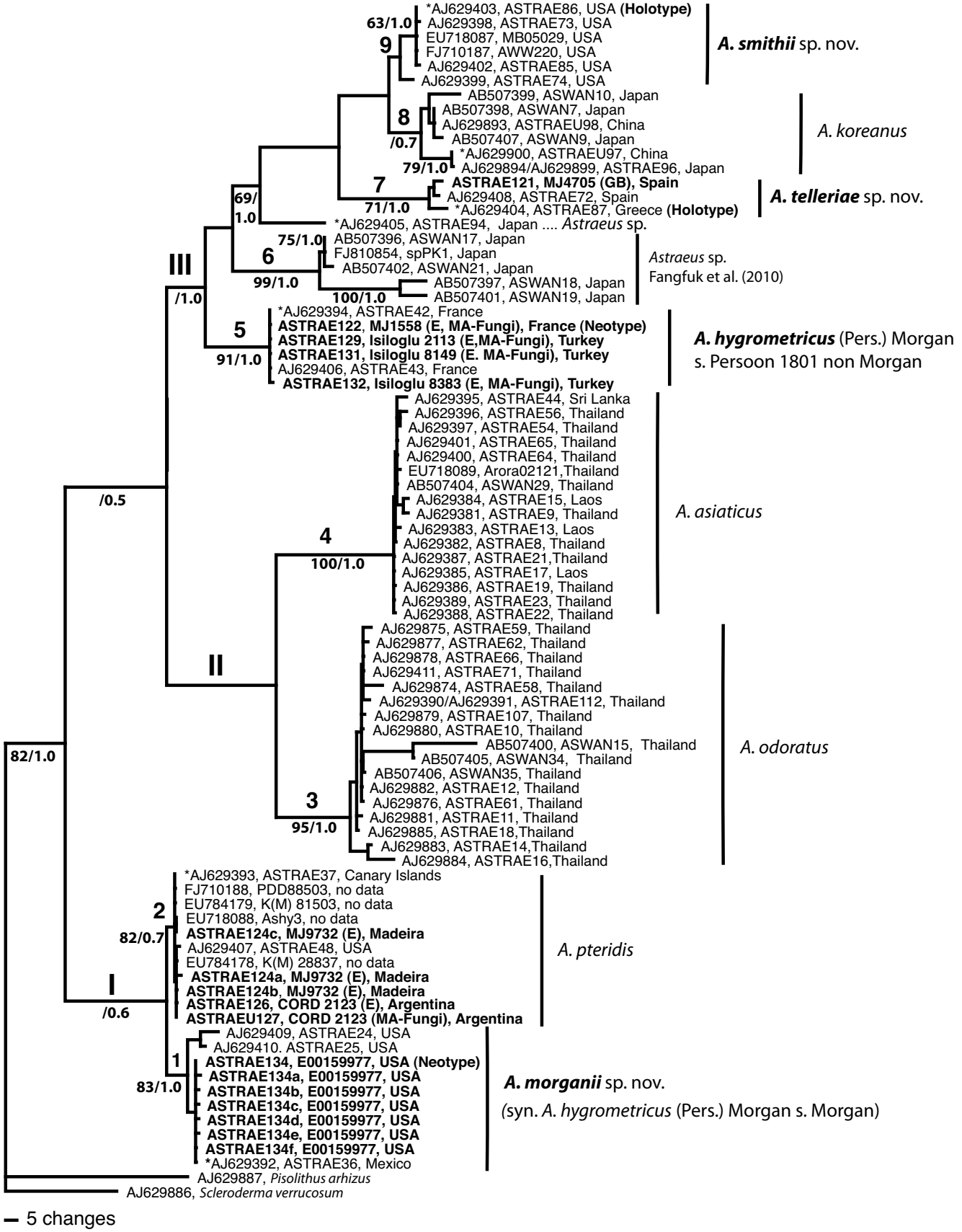

Fig. 1. One of the 100 most parsimonious trees inferred from a heuristic search of ITS nrDNA sequences of *Astraeus* spp. Clades I–III and subclades 1–9 as mentioned in the text. Numbers separated by "/" represent maximum parsimony bootstrap values (BS) and Bayesian posterior probabilities values (PP), respectively. Percentage of bootstrap values < 50 % and Bayesian posterior probability values < 0.5 are not included. Sequences from new specimens included in this study are marked and sequences from type specimens designated here are in bold. (*) before accession numbers from Phosri *et al.* (2007), indicates that SEM spores and basidiomes are illustrated in Figs 2 and 3.

(2007) and Fangfuk *et al*. (2010), were performed using Se-Al v. 2.0a11 Carbon (Rambaut 2002). The alignment was optimized visually. Alignment gaps were indicated as "-" and ambiguous nucleotides were marked as "N". Two sequences were included as outgroup: *Pisolithus arhizus* (AJ629887) and *Scleroderma verrucosum* (AJ629886).

Phylogenetic analyses

The alignment was analysed under a heuristic search, using PAUP v. 4.0b10 (Swofford 2003), and under a Bayesian approach (Huelsenbeck *et al.* 2000, Larget & Simon 1999) assuming a HKY+G model, using MrBAYES v. 3.0 (Huelsenbeck & Ronquist 2001), as described in Telleria *et al*. (2010). The phylogenetic tree was viewed with FigTree v. 1.3.1 (http:// tree.bio.ed.ac.uk/sofware/figtree/) and edited with Adobe Illustrator CS3 v. 11.0.2 (Adobe Systems).

RESULTS

Seventeen new sequences from *Astraeus* specimens were generated, including those obtained from E00159977 (see above). From the Madeira collection (ASTRAE 124, MJ9732 in E), three sequences were obtained after cloning; these sequences show three variable positions (two in ITS1 and one in ITS2 due to transitions T/C). From the Morgan sample, one sequence was obtained after direct PCR and six after cloning; these sequences had six variable positions, five in ITS 1 and one in ITS2, due to one deletion and five transitions (both G/A and T/C).

The ITS nrDNA dataset contained 83 sequences and 891 aligned positions, 468 of them were constant, 170 parsimony-uninformative, and 253 parsimony-informative. Maximum parsimony analysis yielded 100 most parsimonious trees with 779 steps, CI = 0.7125, HI = 0.3790, RI = 0.9276; the strict consensus MP tree (not shown) and the 50 % majority rule Bayesian tree (Fig. 1) had a similar topology.

Astraeus species formed a monophyletic group with high support; the bootstrap value (BS) was 82 %, and the posterior probability (PP) 1.0). At least three main lineages (I, II and III) and nine terminal clades were revealed and are described and related with spore size and morphology here (Fig. 2).

Lineage I consisted of clade 1 (BS = 83 %; PP = 1.0) and clade 2 (BS = 82 %; PP = 0.7). Clade 1 included sequences from Phosri *et al*. (2007) under the name *A. pteridis* and from Mexico and Wisconsin (USA), as well as the seven sequences from the Morgan specimens (E00159977, under *A. hygrometricus*). The spore size ranged from 7.5–10.0 µm, mean ± SD = 9.44 ± 0.86 (Fig. 2A). Clade 2 (BS = 82 %; PP = 0.7) grouped 11 sequences, among them the new sequences from Argentina and Madeira (3 clones), and sequences AJ629392 (Canary Islands) and AJ629407 (specimen OSC49749 from Oregon) under *A. pteridis* in Phosri *et al*. (2007); the spore size ranges from 7.5–12.5 µm, mean ± SD = 10.19 ± 1.24 (Fig. 2B). Sequence AJ629903 from Cornwall, UK (Phosri *et al*. 2007) was not included in the phylogenetic analyses because it was short (465 bp), but it is similar to sequences from clade 2; the Kimura-2-parameter pairwise distance among AJ629903 and nine sequences of this clade gave a value of 0.0000 (e.g. specimens from Argentina, Madeira) or 0.00215 (Canary Islands and Oregon).

Lineage II consisted of the strongly supported clade 3 (BS = 95 %; PP = 1.0) and clade 4 (BS = 100 %; PP = 1.0) from SE Asian specimens, mainly from Thailand. Both clades were reported on previously (Phosri *et al*. 2007) and resulted in the need to recognize two new species, *A. odoratus* and *A. asiaticus,* respectively.

Lineage III included specimens from all around the world grouped in at least five monophyletic clades and a single sequence from Japan (AJ629405; Phosri *et al*. 2007, collection E00159827). The remaining isolates from Asia are distributed in two clades: clade 6 (BS = 99 %; PP = 1.0) consisted of five specimens from Japan (Fangfuk *et al*. 2010, Japanese *Astraeus* group 2), and clade 8 (BS < 50 %; PP = 0.7) grouped six sequences, four from Japan and two from China. The specimens from clade 8 are associated with *Pinus thunbergii*; the basidiomes apparently key admirably to Stanek's *A. hygrometricus* var. *koreanus* (in Pilàt 1958) considered by Kreisel (1969) as be an independent species on the basis of the morphology of both the basidiome and of the basidiospores; the spore size ranges from 5.5–11.5 µm, mean ± SD = 10.15 ± 1.48 (Fig. 2C). The specimens from clade 6 are quite distinct on molecular and morphological grounds from *A. koreanus*, and clearly require formal recognition, although the "species" may even have to be split further in the future along the lines of host, one being associated with *Fagaceae* (AB507396, AB507402, FJ810854) and the other *Pinaceae*, *P. densiflora* (AB507397, AB507401). Also, more specimens should be analyzed to delimit the taxon of specimen E00159827 (AJ629405); the spore size ranges from 7.0–9.0 µm, mean ± SD = 7.92 ± 0.66 (Fig. 2D).

Many isolates considered to be *A. hygrometricus* were grouped in three clades in lineage III. Clade 5 (BS = 91 %; PP = 1.0) consisted of six specimens from France and Turkey; the spore size ranges from 8.5–12.5 µm, mean ± SD = 10.56 ± 1.11 (Fig. 2E). Clade 7 (BS = 71 %; PP = 1.0) grouped three from Spain and Greece; the spore size ranges from 7.5–12.5 µm, mean ± SD = 9.91 ± 1.11 (Fig. 2F). Clade 9 (BS = 65 %; PP = 1.0) comprised six mainly from USA (Wisconsin and Michigan); the spore size ranges from 7.5–12.5 µm, mean ± SD = 10.5 ± 1.24 (Fig. 2G).

DISCUSSION

Now that a division of this species complex is recognised, a major issue is the identity of the original *Astraeus hygrometricus*, first described under the generic name *Geastrum* by Persoon (1801) from material collected around Paris. The well-supported group (clade 5) incorporating collections from France and Turkey, appears, based on distributional data, to represent Persoon's fungus. Unfortunately it has not been possible to extract DNA from any collection made by Persoon.

A second problem arises because none of the collections from the New World so far studied, fall within the circumscription of the European-biased material. *Astraeus hygrometricus* in its strictest sense has not been found in the United States, and collections agreeing with those from North America have

only been found in the UK (sequence AJ629903). This is a rather different picture from the movement of the related *Pisolithus* species around the world with their mycorrhizal hosts, *e.g.* Australian taxa with *Eucalyptus* trees. in Africa (Phosri *et al*. 2012).

An issue then arises as to what Morgan (1889), who demonstrated the differences between *Geastrum hygrometricum* and true earth-stars, had to hand. He correctly felt it necessary to erect a separate genus based on his interpretation of *G. hygrometricum.* Morgan introduced the generic name *Astraeus* to accommodate the single species he had identified as *Geastrum hygrometricum*. On the basis of what we have revealed about the genus, we suggest that he actually had a different species related to, but not the same as, *A. pteridis*. Interestingly, although Morgan described the genus in a paper from Cincinnati he never collected or recorded the fungus from there. However, typification of generic names under the *Code* is based on the types of the names of the included species, whether or not the names were misapplied by the describing author. As *Astraeus* was based on Persoon's specific name, the type of which must be that of, in this case *Geastrum hygrometricum*. Consequently, the generic name *Astraeus* is typified by the European species *G. hygrometricum* (e.g. French specimens in clade 5).

In our proposed scheme, the type of *Astraeus hygrometricus* must have lain in one of clades 1, 2 or 9. Clades 1 and 2 encompass what has been called *A. pteridis* (Shear) Zeller 1948, characterized by basidiomes that are generally larger than those of *A. hygrometricus* of most authors. Lloyd (1901) described *Geastrum hygrometricum* var. *giganteum* from California, and, almost at the same time, Shear (1902) described *Scleroderma pteridis* from material collected in 1899 from the western US as discussed by Guzmán (1970). Lloyd (1902) noted, in addition to California, material from Iowa and Washington. Zeller (1948), after studying the type specimens of *S. pteridis* in NYBG, made the new combination *A. pteridis*. *Astraeus hygrometricus* was also discussed at length by Zeller (1948), who drew attention not only to the differences from what was then considered the true *A. hygrometricus* (i.e. Morgan's fungus), but to the great variability of the species, probably also having taken into account observations on European material deposited in the NYBG. This variability had been reflected over 100 years earlier in Desvaux's (1809) treatment in which where four species were erected; see Pilát (1958). Such a resource was most certainly not available to Morgan when he conducted his anatomical observations. Thus, we adopt the name *A. pteridis* here for the larger Pacific Northwest American species characterising clade 2 (such as collection OSC49749 from Oregon, sequence AJ629407). This species has also been found in the Canary Islands and Madeira, both Atlantic archipelagos, and Argentina, which has strong links with Lusitania. This distribution may indicate either a translocation or that we have sampled extremes of a wide distribution. The remaining closely allied clade 1 brings together the collection made by Morgan and material from Mexico. This is not as surprising as might be first thought as the collecting site in Colorado is in the arid area close to the Texan border. The unique features of specimens analysed from clade 1, clade 7 and clade 9 indicated that it is necessary to recognize three new species: *Astraeus morganii, A. telleriae,* and *A. smithii,* respectively. On the other hand, additional studies are needed to resolve the taxonomic status of specimens from India named as *Geaster lilacinus,* and included in Ahmad (1950) under *A. hygrometricus*, and material from Africa presently under study.

TAXONOMY

Astraeus Morgan, *J. Cincinnati Nat. Hist. Soc.* **11**: 20 (1889).

Type species: *Astraeus hygrometricus* (Pers.) Morgan 1889.

Astraeus hygrometricus (Pers.) Morgan, *J. Cincinnati Nat Hist. Soc.* **11**: 20 (1889).
(Figs 2–3)

Basionym: *Geastrum hygrometricum* Pers., *Syn. Meth. Fung.* **1**: 135 (1801).

Synonyms: *Lycoperdon stellatus* Scop., *Fl. Carniol.,* edn 2: 63 (1760).

Geastrum stellatum (Scop.) Wettst., *Verh. Zool.-bot. Ges. Wien* **35**: 576 (1885); as "*Geaster stellatus*".

Astraeus stellatus (Scop.) E. Fisch., *Nat. Pflanzenfam.* **1** (1**): 341 (1900).

Type: **France**: *Provence*: Avignon, Tarascon-sur-Rhone, Abbaye de Frigolet, in dry sandy grassland, 2 Jan. 1981, *M. Jeppson MJ1558* (E – ***neotype designated here***, MBT176569; MA-Fungi isoneotype; ITS nrDNA sequence ex-neotype HG000287).

Description: *Basidiome* globular or slightly flattened laterally, sometimes with a hint of a basal dome, fully enveloping when dry an inner peridium revealed when mature and moist, 20–25 mm diam before opening, splitting into 12–14 distinct rays, with only 2–3 as minor splittings, rays becoming 36–38 mm broad; outermost layer of outer peridium fuscous- to umber-brown, darkening further when moist; inner surface concolorous, cracking deeply, when moist forming an irregular diamond-shaped pattern, areoles silvery grey; inner peridium 13–14 mm diam, a thin pale buff or pale clay-coloured sack when dry, darkening considerably when moist although retaining a pale, narrow zone around the irregular apical aperture; *rhizoids* lacking; *capillitium* very slightly ornamented with irregularly distributed low warts, hyaline to honey coloured, 4.5–6.5 µm broad, brown, formed of clearly branched formed of irregularly anastomosing hyphal elements. *Spores* minutely warted from low, small rounded prominences, globose, 10–12.5(–13.5) µm diameter, pale brown with larger ones generally paler and often with large central guttule.

Habitat: In dry sandy grassland.

Distribution: Southern France and Turkey.

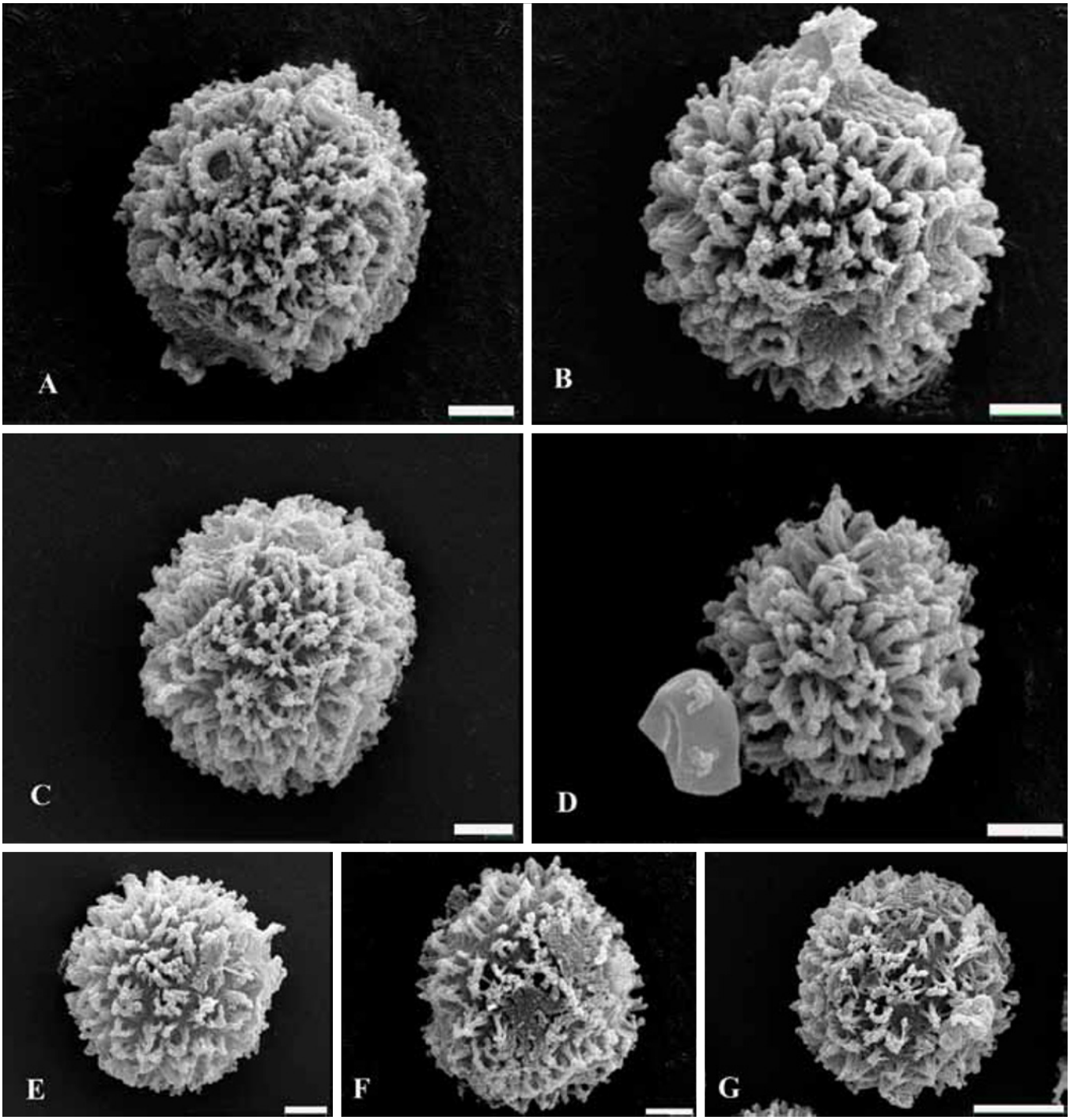

Fig. 2. SEM image of basidiospore ornamentation. **A.** *Astraeus morganii* (AJ629392, ASTRAE36). **B.** *A. pteridis* (AJ629393, ASTRAE37). **C.** *A. koreanus* (AJ629900, ASTRAE97). **D.** *Astraeus* sp. (AJ629405, ASTRAE94). **E.** *A. hygrometricus* (AJ629394, ASTRAE42). **F.** *A. telleriae* (AJ629404, ASTRAE87). **G.** *A. smithii* (AJ629403, ASTRAE73). Bars: A–F = 2 µm, G = 5 µm. Numbers between brackets correspond with those in Fig. 1.

Specimens confirmed by ITS nrDNA analyses: **France**: East of Orange and NE of Avignon, Mollans sur Ouveze, Aug. 1977, *E. A. Ellis* (K(M) 104969; ITS nrDNA sequence AJ629406); Gard, 23 Sept. 1997, *B. W. Brown* (K(M) 50616; ITS nrDNA sequence AJ629394). – **Turkey**: Aydŷn, Cine, Kuruköy village, in *Quercus* forest, 21 Nov. 2004, *M. Isiloglu 2113* (E, MA-Fungi; ITS nrDNA sequence HG000293); Antalya, Gundogmus, Guneycik village, in *Pinus brutia* and *Quercus* forest, 11 May 2007, *M. Isiloglu* 8383 (E, MA-Fungi; ITS nrDNA sequence HG000295); Ýzmir, Bayindir, Sariyurt village, in *Pinus brutia* and *Quercus* sp. forest, 25 Nov. 2006, *M. Isiloglu 8149* (E, MA-Fungi; ITS nrDNA sequence HG000294).

Observations: The earliest name for this species, *Lycoperdon stellatus,* is not to be taken up for this species since Persoon (1801) sanctioned the use of *Geastrum hygrometricum* (Art. 13.1(d)).

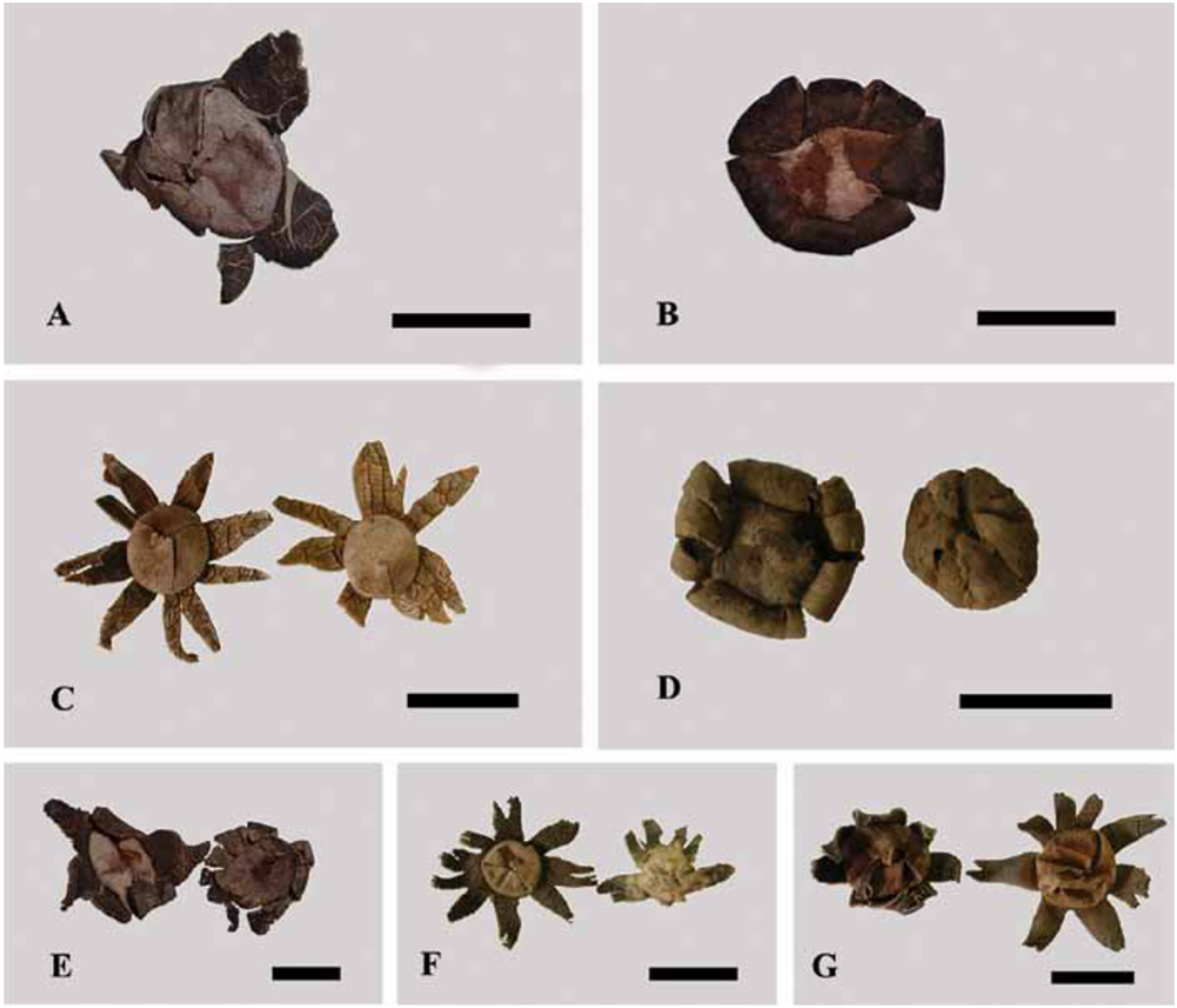

Fig. 3. Basidiomes. **A.** *Astraeus morganii* (K(M) 19550; AJ629392, ASTRAE36). **B.** *A. pteridis* (AJ629393, ASTRAE37). **C.** *A. koreanus* (AJ629900, ASTRAE97). **D.** *Astraeus.* sp. (AJ629405, ASTRAE94). **E.** *A. hygrometricus* (AJ629394, ASTRAE42). **F.** *A. telleriae* (AJ629404, ASTRAE87). **G.** *A. smithii* (AJ629403, ASTRAE73). Bars = 2.0 cm. Numbers between brackets correspond with those in Fig. 1.

Astraeus morganii Phosri, Watling & M.P. Martín, **sp. nov.**
MycoBank MB803905
(Figs 2–3)

Etymology: Named after A. P. Morgan (1836—1907) who first demonstrated the significance of the development of *Astraeus* and how it fundamentally differed from the true earthstars.

Diagnosis: This new species is closely related to *A. pteridis,* but differs in the smaller basidiome, larger spores, and its unique ITS nrDNA sequence.

Type: **USA**: *Colorado*: La Plata Co., Hormosa, north of Durango, 29 Mar. 1899, *C. F. Baker* [Plants of Southern Colorado no. 13; identified by Morgan] (E-00159977 – holotype; direct ITS nrDNA sequence HG000296; and sequences HG000297-HG000302 after cloning).

Description: *Basidiome* depressed-globose, outermost layer and attached mycelium, deciduous, outer peridium splitting into 7–20 pointed rays, at least 5 or so narrower than the rest, expanding to 50–76 mm, strongly hygroscopic, inner layer of outer peridium cartilaginus-gelatinous, hard and rigid when dry, swelling greatly and flexible when wet, smooth to irregularly rugulose, then becoming increasingly cracked and fissured, retaining hygroscopic qualities, stellate, remaining on the soil surface, spreading out in moist weather and bending inward when dry; inner peridium 20–25 mm depressed-globose, sessile, reticulate, pitted, whitish becoming grey or brownish; *capillitium* "rather thinner than spores *fide* Morgan", hyaline. *Spores* globose, minutely warted, brown, 7.5–10 µm diam.

Habitat: In fields and woods in sandy soil.

Distribution: Central to Southern United States southwards to Mexico.

Observation: The above description represents Morgan's understanding of *A. hygrometricus* on which his new genus was based but this differs from what we believe to be Persoon's original concept (*see above*). The holotype collection was purchased for E in 1900, and the collection was dated the same year as the publication of *Astraeus*. Morgan apparently did not find *Astraeus* in Cincinnati, the state where he published his new observations from, but he considered *A. hygrometricus*, in parallel with many other classical and post-classical mycologists, to be a very common fungus with a worldwide distribution. This is now patently untrue. It is recorded by Morgan for several sites in the US but without molecular data it is impossible to say which might be one of the three groupings now recognized there. His records include: California (*Harkness*), Florida (*Calkins*), Kansas (*Cragin*), New England (*Frost*), New Mexico (*Wright*), New York (*Peck*), Pennsylvania (*Schweinitz & Gentry*), South Carolina (*Aitken* in Ravanel, Exs. no. 471), South Carolina (E), Texas (*Drummond*), and Wisconsin (*Brown* & *Trelease*). Unfortunately the material available from these collections, after several attempts, did not allow the reclamation of good DNA.

The type of *Geastrum fibrillosum,* a species described by Schweinitz (1822) from Carolina (*Synops. Fungor. Carol*. no. 330, FH not re-located), was examined by Coker & Couch (1928) and shown to be a weathered member of the *A. hygrometricus* consortium. The persistent basidiomes become fibrillose and ragged, especially in overwintered material; Lloyd (1908) concurred with this decision. However, from our observations above this cannot be assigned to *A. hygrometricus* as now defined, not to one of the newly described taxa from North America since we can not confirm morphological data and do molecular analyses.

Specimens confirmed by ITS nrDNA analyses: **Mexico**: *sine loc.,* 10 Mar. 1991*, W. C. Weightman* (K(M) 19550; ITS nrDNA sequence AJ629392). – **USA**: *Wisconsin*: Adams County (Alan D. Parker Herbarium, Fungi of Wisconsin; ITS nrDNA sequence AJ629410); Walworth County, Young Rd. Steinke, *A. D. Parker*, 15 Oct. 1995 (Alan D. Parker Herbarium, Fungi of Wisconsin; ITS nrDNA sequence AJ629409).

Astraeus smithii Watling, M.P. Martín & Phosri, **sp. nov.**
MycoBank MB803906
(Figs 2–3)

Etymology: Named after the late Alexander H. Smith (1904–1986), formerly Ann Arbor, who published on Michigan puffballs and their allies amongst his many other North American macromycete monographs.

Diagnosis: This species is characterised by the inner peridium at maturity becoming matted-fibrillose to reticulate, the dark almost blackish rhizoids, and its unique ITS nrDNA.

Type: **USA**: *Michigan*: Chippewa County, Upper Penninsula, Whitefish Bay, Whitefish Point, on surface of sandy soil in sand-dune system, 25 Aug. 1965, *R. Watling Wat. 874/2023* (E-00159828 – holotype; ITS nrDNA sequence AJ629403).

Description: *Basidiome* 10–20(–30) mm, almost globose or slightly ellipsoid, with dark almost blackish rhizoids some of which persist at the base, splitting into 7–15 rays; outer peridium distinctly layered, surface matted-fibrillose and intermixed or embedded with sand particles, overlying a thin, fugacious layer, which breaks up, although hard when dry, seated on a fibrous-rimose innermost and exposed layer; inner peridium a pale coloured, papery-thin sack, the surface at maturity matted-fibrillose to reticulate, opening by a single tear; gleba white when young, becoming cocoa-colour at maturity; *capillitium* buff to pale brown, encrusted, thick-walled, highly branched hyphal elements. *Spores* 7.5–12.5 µm, globose with a hyaline sheath overlying thickened warty layer composed of pegs.

Habitat: On soil surface margins of woodland, open areas.

Distribution: Central and Northern United States.

Specimens confirmed by ITS nrDNA analyses: **USA**: *Michigan*: Luce County, Upper Peninsula, Lake Superior, Crisp Point, on sandy soil in sand-dune system, 5 Aug. 1965, *R. Watling 709/1137A* (E-00159829; ITS nrDNA sequence AJ629402). *Wisconsin*: Adams County, Castle Rock, 21 Sept. 2001, *A. D. Parker* (Alan D. Parker Herbarium; ITS nrDNA sequence AJ629398); Portage County, Stevens Point, 29 Sept. 1989, *A. D. Parker* (Alan D. Parker Herbarium; ITS nrDNA sequence AJ629399).

Astraeus telleriae M.P. Martín, Phosri & Watling, **sp. nov.**
MycoBank MB803958
(Figs 2–3)

Etymology: Named after Maria Teresa Telleria, Director of the Real Jardín Botánico-CSIC 1994–2006, who has contributed extensively to Spanish mycology especially through the *Flora Mycologica Iberica* project.

Diagnosis: This new species differs from related species in the very pubescent, even minutely woolly, inner layer of the outer peridium, and its unique ITS nrDNA sequence.

Type: **Greece**: Ventuna, Nov. 1908, *M. Wilson 167* (E-00159833 – holotype; ITS nrDNA sequence AJ629404).

Description: *Basidiome* 35–42 mm, globose at first, splitting into 12 small rays; outer peridium distinctly layered, pubescent *s.l.*, without adhering soil particles; inner layer of outer peridium very pubescent even minutely woolly but then concentrically rimose; inner peridium thin, pale buff with central, irregular fissure at apex, cocoa-coloured within; *capillitium* buff to pale brown, some elements encrusted, thick-walled and strongly branched. *Spores* 7.5–12.5 µm, globose and ornamented with small warts.

Habitat: On soil surface, margins of woodland (*Pinus* spp. and *Quercus* spp.), open areas.

Distribution: Mediterranean – Southern Spain to Greece.

Specimens confirmed by ITS nrDNA analyses: **Spain**: *Catalunya*: Barcelona, Maresme, Santa Ischia, Arenys de Munt, 30 Oct. 1998, *M. Jeppson MJ4705* (GB; ITS nrDNA sequence HG000286). *Madrid*: Majadahonda, 23 Feb. 2002, *P. P. Däniels & C. Phosri* (MA-Fungi; ITS nrDNA sequence AJ629408).

CONCLUSIONS

Our study indicates that the name *Astraeus hygrometricus* has previously covered several separate species. Further, in addition to the three new species recognized here, more taxa remain to be categorised. More fresh collections are required for a species originating in India, for which there may be a possible previous name available, and at least one noted from Japan. Much work is required on collections from Japan and North America, and more material is needed from South America where the collections may represent recent introductions. The names "*Astraeus hygrometricus"* and "*A. pteridis*" are both mentioned from Australia (May *et al*. 2003), so in any further study it would be essential to incorporate material from that country. Simple examination of material in E collected by A. Morrison in Western Australia shows great variation including a collection with "pygmy" basidiomes, little more than 15 mm across even when expanded, and spores 6.5–7.0 × 6.0–6.5 µm.

With the integration of studies of type material deposited in herbaria, with molecular data from fresh collections of known provenance, a fuller attempt can be made to resolve the taxonomy of this interesting genus. At least the branches of the tree so far developed clearly demonstrate that *Astraeus hygrometricus,* as previously circumscribed, to be not a single species but made up of a multitude of cryptic species. The present study is a partial solution to this ever-increasing conundrum.

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