KAVAKA 42: 16-19(2014)

First report of *Astraeus odoratus* from India

Manoj E. Hembrom[1], Arvind Parihar[2], María. P. Martín[3], Roy Watling[4] and Kanad Das[2*]
[1]*Botanical Survey of India, Central National Herbarium, Howrah 711103, India*
[2]*Botanical Survey of India, Cryptogamic Unit, Howrah 711103, India*
[3]*Real Jardín Botánico, RJB-CSIC, Plaza de Murillo 228014, Madrid, Spain*
[4]*Caledonian Mycological Enterprises, 26 Blinkbonny Avenue, Edinburgh, EH4 3HU, UK*
Corresponding author E-mail: daskanadbsi@gmail.com
(Submitted in March, 2014 ; Accepted on July 01, 2014)

ABSTRACT

***Astraeus odoratus* which can be separated from allied taxa by non-hygroscopic nature of the exoperidium, basidiospores with very high ornamentation and the very thick exoperidial layer with irregular and exceptionally thick-walled cells is reported for the first time from India along with its morphological features, photographic illustrations and the barcoding ITS1 nrDNA sequence.**

Key words: *Diplocystidiaceae*, ectomycorrhizae, Jharkhand, taxonomy, India

INTRODUCTION

The genus *Astraeus* Morgan is characterized by epigeous basidiomata; split exoperidium with hygroscopic to non-hygroscopic rays; endoperidium with an irregular apical pore; gleba white when young, reddishbrown, dark brown or chocolate brown and powdery without a columella when mature; basidiospores echinulate or verruculose, very thick-walled, wall with 2-4 layers; capillitium absent; paracapillitium branched, clamped, hyaline; mycorrhizal (Miller and Miller, 1988; Pegler *et al.*, 1995; Calonge, 1998). At present, *Astraeus* is placed under the family *Diplocystidiaceae* Kreisel belonging to the order *Boletales* (Kirk *et al*., 2008). At least nine species have been described all over the World: *Astraeus hygrometricus* (Pers.) Morgan (Morgan, 1889), *A. pteridis* (Shear) Zeller (Zeller, 1948), *A. koreanus* (V.J. Stanek) Kreisel (Kreisel, 1976), *A. odoratus* Phosri, Watling, M.P. Martín & Whalley (Phosri *et al.*, 2004), *A. asiaticus* Phosri, M.P. Martín & Watling (Phosri *et al.*, 2007), *A. morganii* Phosri, Watling & M. P. Martín, *A. smithii* Watling, M. P. Martín & Phosri and *A. telleriae* M.P. Martín, Phosri & Watling (Phosri *et al.*, 2013), and *A. sirindhorniae* Watling, Phosri, Sihanonth, A. W. Wilson & M. P. Martín (Phosri *et al.*, 2014). In India, only *Astraeus hygrometricus* s.l. has been cited (Ahmad, 1950).

The Rajmahal hills are located in the Jharkhand state in the eastern part of India and geographically consist mostly of hills representing tropical deciduous scrub vegetation. During recent (August–September, 2013) mycological trips, some gasteroid macrofungi were collected from different parts of these hills. Thorough morphological examination followed by the literature survey revealed that two of the *Astraeus* collections did not belong to the species *A. hygrometricus* s.l., and could be any of the Asian species described in the past years. The goal of this study is to clearly identify these specimens, by comparing the barcoding ITS nrDNA sequences (Schoch *et al.* 2012) with allied taxa discussed in Phosri *et al.* (2013). Morphological characters are described and illustrated.

MATERIALS AND METHODS

1) Morphological studies

Macromorphological/field characters were recorded from the fresh basidiomata. Field photographs of these fresh basidiomata were taken with the aid of Nikon D300s and Olympus C-5060. Colour codes and terms (mostly) are after Methuen Handbook of Colour (Kornerup and Wanscher, 1978). After recording the macromorphological characters, basidiomata were dried in the base camp/laboratory with a wooden drier. Micromorphological features were recorded at the magnification of ×100, ×400 and ×1000 with the aid of a light microscope: Olympus CX 41 from the dry samples mounted in a mixture of 5% KOH and phloxin, in lactophenol cotton blue and in Melzer's reagent. Microphotographs were taken with the help of a dedicated camera Olympus C-5060. Spore measurements are recorded based on twenty basidiospores. Spores are measured in side view. Spore measurements and length/width ratios (Q) are presented as: minimum–**mean**–maximum. Herbarium citation is after Holmgren *et al.*, 1990. Scanning Electron Microscope (SEM) illustrations of basidiospores were obtained from dry spores that were directly mounted on a double-sided adhesive tape pasted on a metallic specimen-stub and then scanned with gold coating at different magnifications in high vacuum mode to observe patterns of spore-ornamentation. SEM studies were carried out with a FEI's Quanta FEG 250 model imported from The Netherlands and installed at the S.N. Bose National Centre for basic Sciences, Kolkata, India.

2) DNA extraction, PCR and sequencing

Genomic DNA was extracted from specimen MEH-13-036 with DNeasy Plant Mini Kit (Qiagen, Germany). The ITS nrDNA region was amplified using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) as described in Winka *et al.*, 1998) following thermal cycling conditions in Martín and Winka (2000). The PCR was performed with one µl of the eluted DNA and one µl of each primer, ITS1F (White *et al.*, 1990)

and ITS4 (Gardes and Bruns, 1993). Before sequencing, 20 µl of the amplificate products were cleaned with 8 µl of 1:10 ExoSAP-IT® (USB Corporation, OH, USA). Consensus sequences were assembled and edited using Sequencher (Gene Codes Corporation, Ann Arbor, MI). BLASTN queries with MEGABLAST option were used to compare the consensus sequence obtained against sequences in the National Center of Biological Information (NCBI) nucleotide database (Altschul *et al.*, 1997). Moreover, the consensus sequence was compared with homologous *Astraeus* sequences analyzed in Phosri *et al.* (2013). Kimura-2-parameter (K2P) pairwise distances were obtained using PAUP* 4.0b10 (Swofford, 2003). The new consensus sequence has been lodged in the EMLB-EBI database with the accession number KJ847767.

RESULTS

Molecular analyses

The ITS consensus sequence obtained was 272 bp, and include the last 5 positions of the SSU nrDNA (5'…CATTA) and almost all the internal transcribed spacer 1. The BLAST search of this sequence showed 97% similarity to the NCBI sequence AJ639876 under *Astraeus odoratus* from Yasothon, Thailand (Phosri *et al.*, 2007). A preliminary analysis (data not shown) confirmed specimen MEH-13-036 is in the clade of *A. odoratus*. In particular, the K2P matrix distance among *Astraeus* sequences included in Phosri *et al.* (2013) and the new sequence obtained in this study gave a value of 0.00000 between the new sequence and sequences of *A. odoratus* (AJ629411, AJ629878, AJ629880 and AJ629882). Thus, the data obtained confirm the presence of *A. odoratus* in India.

Taxonomy

Astraeus odoratus Phosri, Watling, M. P. Martín & Whalley, *Mycotaxon* **89**(2): 458 (2004)

Figs. 1(a – k) & 2(a – f)

Basidiomata solitary, epigeous, fleshy and leathery when fresh, hard and brittle on drying; basidiomata 10–25 mm high, expanded 35–50 mm in diameter; unopened basidiomata /egg not found. Exoperidium 1.3–2.3 mm thick, splitting into 5–6 rays, non-hygroscopic; rays 10–20 × 5–18 mm, acute, few decurved, bifurcated, upper or inner part with mosaic-like breaking pattern extended up to the juncture of endoperidium, grayish brown to yellowish brown (5F3–5F4); outer or lower exoperidium pale straw color. Endoperidium 8–18 × 5–7 mm, subglobose, opening by an irregular pore, olive grey (1F2). Gleba pulverulent, dark brown (6F4), raw umber to coffee color (5F8-5F7).

Basidiospores 4–(7.9)–11 × 4–(7.62)–11µm, Q = 1–(1.03)–1.12, mostly globose, golden yellow to yellowish brown (5B7–5B8), thick-walled, consisting of 3–4 layers, spinoid; spines fused and incurved to inrolled apically; under SEM, ornamentation of very high coalescent spines of 1.7–3.0

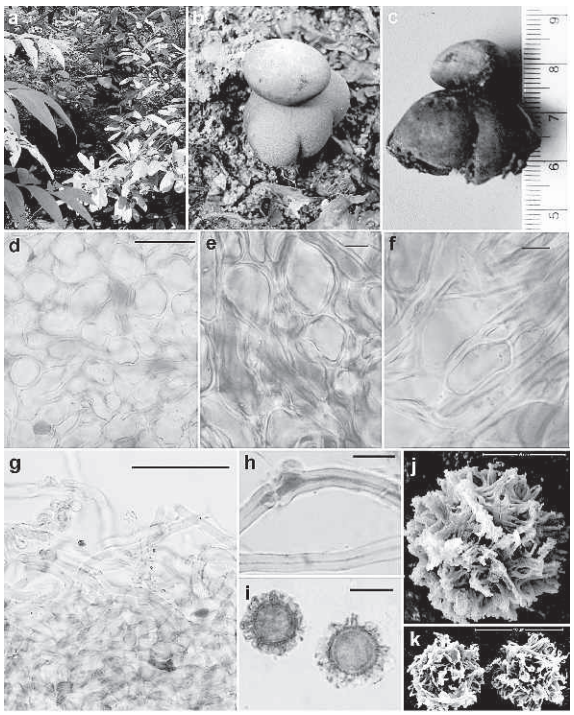

Fig. 1. *Astraeus odoratus* (MEH-13-036). a. Habitat. b & c. Fresh basidiomata. d–f. Cross-section through exoperidium showing pseudoparenchymatous layer with thick-walled cells. g. Cross-section through endoperidium. h. Paracapillitium. i. Basidiospores. j & k. SEM micrographs of basidiospores. Bars: d & g = 50 µm; e, f, h & i = 10 µm; j = 5 µm; k = 10 µm.

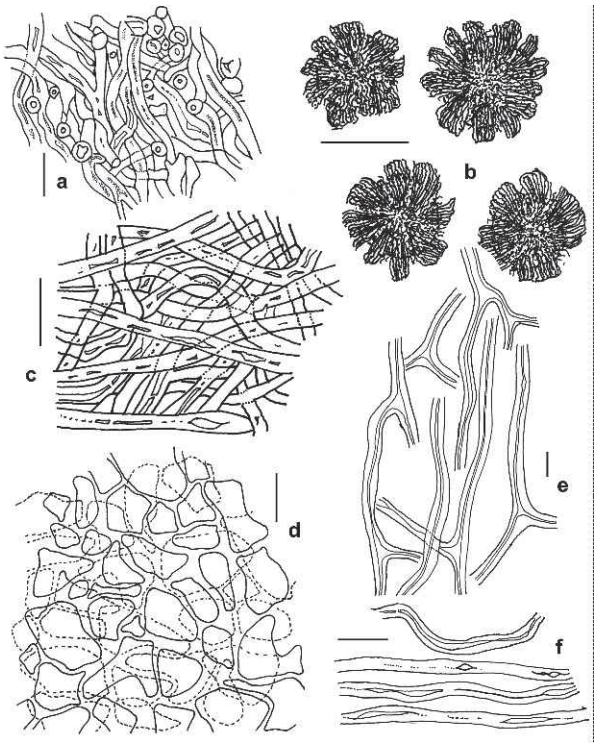

Fig. 2. *Astraeus odoratus* (MEH-13-036) a. Hyphal arrangement of felty layer in exoperidium. b. Basidiospores. c. Hyphal arrangement of endoperidium. d. Pseudoparenchymatous layer of exoperidium. e. Paracapillitium. f. Hyphae from endoperidium. Bars: a–f = 10 µm.

µm high. Paracapillitium 2.5–7 µm wide, branched, septate, clamped, thick-walled (up to 2.2 µm thick), encrusted; lumen up to 1.5 µm wide, retaining the color of cotton blue and phloxin. Exoperidium distinctly separated into felty, fibrous and pseudoparenchymatous layers. Felty layer 110–225 µm thick, hyphal; hyphae 2–8 µm wide, thick walled; lumen 1–2 µm wide. Fibrous layer 700–950 µm thick. Pseudoparenchymatous layer 550–1150 µm thick, hyaline, cellular; cells 3–70 × 2–30 µm in size, highly irregular in shape, slightly thick-walled. Endoperidium 137–350 µm wide, light brown, hyphal; hyphae 2.5–8 µm wide, thin to thick walled, septate, clamped, with bovista type branching, some ampullate, lumen up to 1.5 µm wide, continuous to discontinuous, retaining the color of cotton blue and phloxine.

Specimens examined: India, Jharkahnd, Rajmahal Hills, Sahibganj-district, sacred groove of Mandro fossil park village, alt. 137 m, 20 August 2013, N25°07'31.2"E87°31'20.2", on ground under *Shorea robusta*, M. E. Hembrom, MEH-13-036 (CAL); ibid., forest area of Dalabari village, alt. 122 m, 26 August 2013, N25°07'31.2"E87°31'20.2", on ground under *Shorea robusta*, M. E. Hembrom, MEH-13-090 (CAL).

Notes: *Astraeus odoratus*, originally reported from Thailand, appears to be rare in the lateritic red soil of Rajmahal hills. Macromorphologically, it can be distinguished by non-hygroscopic nature of exoperidium and 5–6 exoperidial rays. Micromorphologically, it differs in the basidiospores with very high ornamentations and very thick, pseudoparenchymatous, exoperidial layer with irregular and slightly thick-walled cells (Phosri *et al*., 2007).

Astraeus asiaticus (reported from *Dipterocarpus* forests) resembles *Astraeus odoratus* in the field (Phosri *et al*., 2004; 2007). But, *A. odoratus* is quite distinct from *A. asiaticus* which has hygroscopic exoperidium with 5–12 rays and comparatively large (8.75–15.2 µm including the size of ornamentation) basidiospores with distinctly low (0.9–1.45 µm) spines (Phosri *et al.*, 2007).

Moreover, *Astraeus hygrometricus* s.l. (the only reported species of *Astraeus* from India) can be differentiated from *A. odoratus* by its fully hygroscopic nature of the exoperidium showing large reticulate cracking with many rays (10–14) and basidiospores with lower ornamentation ranging from 0.3–0.7 µm as documented by Pegler *et al*., (1995); 0.5–1 µm by Calonge (1998) and up to 0.7 µm by Bisht, (2008).

Like in other parts of the world after careful analysis, more than just the generally accepted *A. hygrometricus* has been found to be present in the Indian subcontinent. The recognition of *A. odoratus* in India suggests that further collections should be made there and in surrounding countries, especially as it has been suggested that *Geastrum lilacinum* Mass. is probably a species of *Astraeus* and collections of the weather barometer fungus from neighbouring Nepal appear to have a distinct molecular signature (Phosri *et al.*, 2013).

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Botanical Survey of India, Kolkata for providing all the facilities during the study of this material. Thanks are due to Dr. Md. N. Aziz (BSI, Cryptogamy), Dr. P. Lakshminarasimhan (BSI, CNH) and Dr. A. Pramanik (BSI, CBL) for helping the authors in many ways. Assistance rendered by Mr. S.N. Das during SEM study of basidiospores is duly acknowledged. Work in the Molecular Systematics Laboratory of the Real Jardín Botánico-CSIC, Madrid, was supported by SYNTHESYS Project http://www.synthesys.info/.

REFERENCES

Ahmad, S. 1950. Studies in gastromycetes. *Sydowia* **4**: 124–129.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang Z., Miller, W. and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**: 3389–3402.

Bisht, D. 2008. *Gasteromycetes (Lycoperdales & related Fungi) of Uttarakhand*. Unpublished report. Botanical Survey of India. 172 pp.

Calonge, F.D. 1998. *Gastermycetes, I. Lycoperdales, Nidulariales, Phallales, Sclerodermatales, Tulostomatales. Flora Mycologica Iberica*. Vol. 3 Real Jardín Botánico Madrid, J. Cramer 271 pp.

Gardes, M. and Bruns, T.D. 1993. ITS primers with enhanced specify for Basidiomycetes: application to identification of mycorrhizae and rusts. *Mol Ecol.* **2**: 113–118.

Holmgren, P.K., Holmgren, N.H. and Barnett, L.C. 1990. *Index Herbarium. Part 1: Herbaria of the world,* 86th ed. Bronx: New York Botanical Garden, USA. 693 pp.

Kornerup, A. and Wanscher, J.H. 1978. *Methuen Handbook of Colour*, 3rd ed. Eyre Methuen, London. 252 pp.

Kreisel, H. 1976. Gasteromyzetenaus Nepal II. *Feddes Repert. Spec. Nov., Beih.* **87**(1–2): 88.

Kirk, M.P., Cannon, P.F., Minter, D.W. and Stalpers, J.A. 2008. *Dictionary of the Fungi* 10th Edition. CAB International, Wallingford, UK. 771 pp.

Martín, M.P. and Winka, K. 2000. Alternative methods of extracting and amplifying DNA from lichens. *Lichenologist* **32**: 189-196.

Miller, O.K. Jr. and Miller, H.H. 1988. *Gasteromycetes: Morphological and Developmental features with Keys to the Orders, Families and Genera*. Mad River Press, USA 157.

Morgan, A. 1889. North American Fungi. *J. Cincinnati Soc. Nat. Hist.* **12**: 20.

Pegler, D.N., Laessoe, T. and Spooner, B.M. 1995. *British Puffballs, Earthstars and Stinkhorns*. Royal Botanic Garden, Kew 255 pp.

Phosri, C., Watling, R., Martín, M.P. and Whalley, A.J.S. 2004. The genus *Astraeus* in Thailand. *Mycotaxon* **89**(2): 453–463.

Phosri, C., Martín, M.P., Sihanonth, P., Whalley, A.J.S. and Watling, R. 2007. Molecular study of the genus *Astraeus*. *Mycol. Res.* **3**: 275–286.

Phosri, C., Martín, M.P. and Watling, R. 2013. *Astraeus*: hidden dimensions. *IMA Fungus* **4** (2): 347–356.

Phosri, C., Watling, R., Suwannasai, N. Wilson, A. and Martín, M.P. 2014. A new reprsentative of star-shaped fungi: *Astraeus sirhindhorniae* sp. nov. from Thailand. *PlosOne* **9**(5): e71160 doi: 10.1371/journal.pone.00771160.

Schoch, C.L., Seifert, K.A., Huhndorf, S. *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences USA* **109**: 6241–6246. Doi:10.1073/pns.1117018109.

Swofford, D.L. 2003. *PAUP*: Phylogenetic analysis using parsimony (*and other methods),* Version 4.0b10. Sinauer Associates: Sunderland, MA, USA.

White, T.J., Bruns, T.D., Lee, S.B. and Taylor, J.W. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocol. A guide to methods and applications*. Academic press: San Diego, CA.

Winka, K., Ahlberg, C. and Eriksson, O.E. 1998. Are there lichenized Ostropales? *Lichenologist* **30**: 455–462.

Zeller, S.M. 1948. Notes on certain *Gasteromycetes*, including two new orders. *Mycologia*, **40**: 639–668.