Indian J. Applied & Pure Bio. Vol. 36(2), 569-579 (2021).
ISSN: 0970-2091
A web of Science Journal

Wild edible gasteroid fungus *Astraeus* (Diplocystidiaceae) from Jharkhand, India

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Abstract

The gasteroid fungus *Astraeus* is a highly valued wild edible mushroom consumed in various parts of India and other Asian countries. It is often referred to as a "False Earthstar" because of the star-like presence which is formed at maturity. *Astraeus* is an ectomycorrhizal fungus that colonizes the Dipterocarpaceae, Pinaceae, Betulaceae and Ericaceae forest trees species growing in sandy or loamy rich lateritic soil. The mycological survey between June-July, 2019 of Sal forest of the Porahat forest division of Jharkhand state of India resulted in collection of two notable earthstar species namely *A. asiaticus* and *A. odoratus*. *A. asiaticus* is distinguished by a whitish sub-epigeous basidiomes with sub-hygroscopic rays and has been found for the first time in India. *A. odoratus* is distinguished by a mosaic pattern on the surface of basidiomes and represents second report form Indian Subcontinent. These species are superficially similar but distinct in morphological, anatomical and phylogenetic traits. The present work is aimed to contribute to the knowledge of *Astraeus* diversity and distribution in Jharkhand mycoflora based on morpho-anatomical, molecular and phylogenetic studies. Species description, images of basidiomes, SEM images of basidiospore are provided.

Genus *Astraeus* (Pers.) Morgan is a common member of ectomycorrhizal star-shaped gasteroid fungus community which varies considerably in size, shape, glebal colour, morpho-anatomical features of peridium and spore ornamentations. The *Astraeus* sp. have a cosmopolitan distribution and have been reported from all the continent except Antarctica and are more abundant in warm temperate to subtropical and tropical ecosystems[21-24]. They usually grow on a diverse range of habitat like leaf litter, mossy blocks, termite mould, sand dunes, and lateritic soil[16]. The fruitbodies of *Astraeus* and its allies have a persistent endoperidium which cover hymenophore (gleba), spores released passively by bellows mechanism through apical mouth[4]. Christian Hendrik Persoon[18] first reported *A. hygrometricus* and placed it in *Gasteromycetes*. Afterwards, Andrew Price Morgan[15] established the identity as a distinct taxon- *A. hygrometricus* (Pers.). Kreisel[11] placed order Boletales into *Diplocystidiaceae* family of which 19 species of *Astraeus* are archived at Mycobank Database[25] viz., *Astraeus hygrometricus* (Pers.)[15]; *A. hygrometricus f. hygrometricus* (Pers.)[15]; *A. hygrometricus* var. *hygrometricus* (Pers.)[15]; *A. stellatus* (Scop.) E. Fisch. (1898); *A. hygrometricusf. decaryi* (Pat.) Pat. (1928); *A. pteridis* (Shear)[36]; *A. hygrometricus f. ferrugineus*[33] *A. hygrometricus* var. *koreanus*[33]; *A. koreanus*[33]; *A. odoratus* Phosri Watling M.P. Martín & Whalley (2004); *A. thailandicus*[19]; *A. asiaticus* Phosri M.P. Martín & Watling (2007); *A. morganii* Phosri Watling & M.P. Martín (2013); *A. smithii* Watling M.P. Martín & Phosri (2013); *A. telleriae* M.P. Martín Phosri & Watling (2013); *A. sirindhorniae* Watling Phosri Sihan. A.W. Wilson & M.P. Martín (2014); *A. ryoocheoninii* Ryoo (2017); *A. sapidus* (Massee) P.-A. Moreau (2017) and *A. macedonicus* Rusevska Karadelev Tellería & M.P. Martín (2019)[15,36,11,21,19,22-24,27,17,4]. The gasteroid fungus *A. hygrometricus* (Pers.) Ahmad[1] from Himalaya region and *A. odoratus* Hembrom *et al.*[8], are the only documented species from India. The Porahat forest division is located in the southern region of Jharkhand and consists of number of steep rocky hills and intervening valleys representing dry deciduous Sal forest and sandy lateritic red soil. During mycological survey (June-July, 2019) wild edible mushrooms were collected from various locations of the Sal forests. These indigenous wild edible mushrooms locally known as "Rugra" inhabit the rhizosphere of *Shorea robusta* Gaertn. Mushrooms are not only good sources of food but also have high nutraceutical property. They are collected and consumed by tribals, since a long time[28]. It has been suggested that a flash of lightning disinfect vegetative matter and trigger the growth of rugra. They are dug out by locals after thunderstorms[32] (Fig. 1, A-D). In order to protect and conserve these edible mushroom "rugra" from anthropogenic activity, deforestation and habitat loss, a comprehensive study on the diversity and habitat specificity of wild edible mushroom rugra is required. The present work was initiated to identify the *Astraeus* species found in this region on the basis of integrative taxonomic approach combined with morpho-anatomical, molecular and phylogenetic placement of *Astraeus* and its allied species.

Morphological studies :

The fresh immature and mature basidiomes were found in solitary or small cluster of 2-4, partially buried (0.5-2 cm) in soil or under the debris of leaf (litter) of *Shorea robusta* Gaertn. Morphological characters such as, the thickness of endoperidium, exoperidium and colour of gleba were studied from the fresh basidiomes in field and lab. The photographs of the field were taken with the help of a digital camera Nikon D3200 (Fig.1, A-F). Dried basidiospore were mounted in a mixture of 5% KOH in lactophenol and cotton blue, and stained with Congo red reagent[29]. About thirty spores were examined and measured (n=30). The description with detailed analyses of ornamentation were recorded by using Scanning Electron Microscope. For SEM, specimen was air-dried & sputter-coated with Gold-Palladium and observed in Carl Zeiss EVO18 scanning electron microscope. Basidiospore abbreviations according to Sousa *et al*[5]. n = no. of randomly measured basidiospores; x = mean ± standard deviation of basidiospore diameter and height (including ornamentation), Qm= mean height/ width ratio. The coordinates for collected specimen, Rug-1a-JH were 22° 50' 56"N, 85° 21' 1" E and Rug-2a -JH were 22° 51' 36" N, 85° 19' 50" E (Fig. 1). All collected specimen were deposited in the Department of Botany, Dr Shyama Prasad Mukherjee University (DSPMU), Ranchi, Jharkhand, India.

DNA extraction, PCR amplification and sequencing :

The genomic DNA was extracted from approximately 10 mg of gleba of mature dry basidiome. EXpure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd (Coimbatore, India) were used for DNA extraction according to the manufacturer's instruction. PCR amplification and 5.8S ribosomal RNA gene cluster of ITS region sequencing were carried out according to Phosri *et al*[24]. Primer ITS1F (5' TCCGTAGG-TGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') were used for amplification and sequencing[35,7]. ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) were used to carry out sequence reaction. Single-pass sequencing was performed on each template using above ITS1 and ITS4 universal primers. Consensus sequence were assembled and edited with BioEdit 5.0.6[9] software. Prior to the alignment, query sequence were compared with homologous sequence in GenBank/DDBJ using the MEGABLAST algorithm of National Centre for Biotechnology Information (NCBI)[13]. The newly generated sequence Rug-1a-JH and Rug-2a-JH have been deposited on NCBI GenBank with the accession number **MN257431 and MN262679** belongs to *A. Asiaticus* and *A. odoratus* respectively.

Phylogenetic analysis :

Dataset of Internal Transcribed Spacer (nr ITS) of 27 homologous sequences were retrieved from NCBI GenBank database to establish phylogenetic relationships among *Astraeus* species. *Pisolithus* alb.& Schwein. was used as outgroup[3]. Multiple sequence alignment of the dataset was performed by using MUSCLE 3.8.31[6] with additional manual adjustments to alignment performed in MEGA v.10.1.18[12]. The alignment gaps and ambiguous nucleotides were indicated as "-" and "N" respectively. For nrDNA ITS dataset, maximum parsimony (MP) and Bayesian inference were carried out. In the MP analysis, heuristic searches with tree-bisection-reconnection branch swapping and collapsing branches of maximum length was zero employed to construct the MP tree in PAUP 4.0a[34] with default setting to stop the analyses when reaching 100 trees. For nonparametric bootstrap (MPbs) support, fast stepwise-addition approach was tested for each clade based on 10,000 replicates. Bayesian analysis was performed using Mr Bayes v. 3.2.6[14,26] with default parameters (Nst=6 with 2 runs, 4 chains per run, each run searching for 1M generations sampling every 1K generation). The 50% maturity-rule consensus tree and the posteriorprobability (PP) of the nodes were calculated. Phylogenetic tree was viewed with FigTree v1.31.1 (http://tree.bio.ed.ac.uk/software/figtree/). The data matrices and trees are available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S27831).

Molecular phylogeny :

The Phylogenetic analyses were carried out on ITS dataset of twenty-nine sequences to evaluate the uniqueness of collection in relations to existing *Astraeus* Isolates. *Pisolithus* were used as an outgroup for rooting purpose. The alignment of ITS dataset resulted into 1331 unambiguously aligned nucleotide position consists of 704 constant, 122 parsimony-uninformative and, 505 parsimony-informative. The 100 most parsimonious tree give a length of 1055 steps, CI =0.7848, HI = 0.2457, RC =0.6863. The ML tree and 50% Bayesian majority rule combined consensus tree (not shown) exhibited similar topologies. The *Astraeus* isolates were resolved as monophyletic in highly supported clade (MPbs = 100%, MLbs = 100%, PP = 1.00). The phylogenetic tree generated three main clade (I-III) and seven sub-clades (A-G) based on basidiospore size and shape, together with external features of peridium, (Fig. 2). The clade -I is represented by *A. macedonicus*, a southeast European species from Republic of North Macedonia, while clade -II is represented by *A. sirindhorniae,* an Asian species from Thailand, while, clade-III has seven sub-clade A-G consist of Asian, North-American and European species of *Astraeus*. The sub-clade A and B has Asian species of *A. asiaticus* and *A. odoratus* from Thailand, Sri Lanka and India which forms a strongly supported group distinct from the other major *Astraeus* clade (MPbs = 100%, MLbs = 100%, PP = 1.00). The sub-clade C had only one species from South Korea *i.e.*, *A. ryoocheoninii*, was previous described as a *A. hygrometricus var. koreanus*[27]. Additionally, the sub-clade D has single French species, *A. hygrometricus* which forms sister group with North American and European *Astraeus* species (sub-clade E, F and, G). The sub-clade E is represented by *A. pteridis* of European origin, (MPbs = 99%, MLbs = 100%, PP = 1.00) while sub-clade F and G belong to North American species of *A. hygrometricus* also forming strongly supported groups (MPbs = 100%, MLbs = 100%, PP = 1.00), Fig 2.

Taxonomy :

Astraeus asiaticus Phosri, C., Martín, M. P., Sihanonth, P., Whalley, A. J., & Watling, R. (2007). *Mycological Research*, *111*(3), 275-286, sp. nov.

Mycobank no.: MB510459
Fig. 3 A-J

Description :

Unexpanded Basidiome sub-epigeous, globose to depressed globose, sessile, 10-17 X 8-15 mm, whitish, encrusted with debris, slight odour; rhizomorph 2-3 mm long, persistent, whitish mycelial often partly buried in soil. **Expanded Basidiome** saccate, 15-25 X 8-12 mm. **Exoperidium** 15-22 mm in diam., split into 6-8 sub-hygroscopic rays which expand or recurved when moist and roll inward again when dry; rays 5-10 mm long, longitudinally cracked in mature basidiome, ovate to elliptical in shape. Mycelial layer, thick, encrusted, whitish becoming brown to smoke grey with age exposing the fibrous layer.

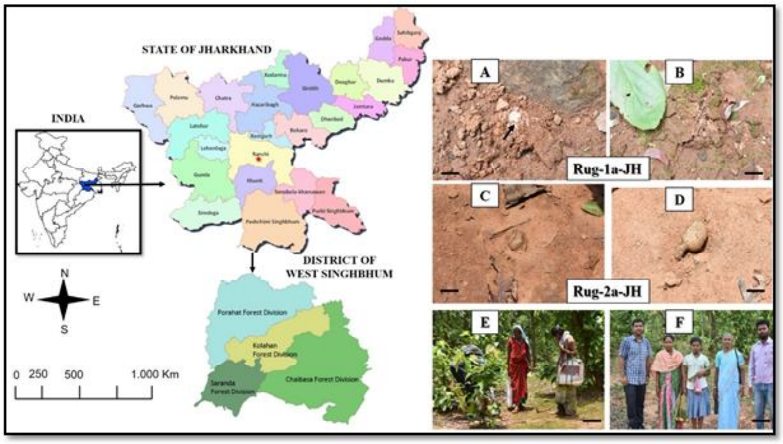


Fig. 1: Study location and map of Porahat forest division, Jharkhand India. (A-B)- ***Astraeus asiaticus*** (Rug-1a-Jh) from the field immature basidiomes partially buried in soil; (C-F) ***Astraeus odoratus*** (Rug-2a-Jh) from the field Immature basidiomes; (E-F) Local tribal resource person who helped in collection of wild edible mushrooms. Scale bar = 20mm.

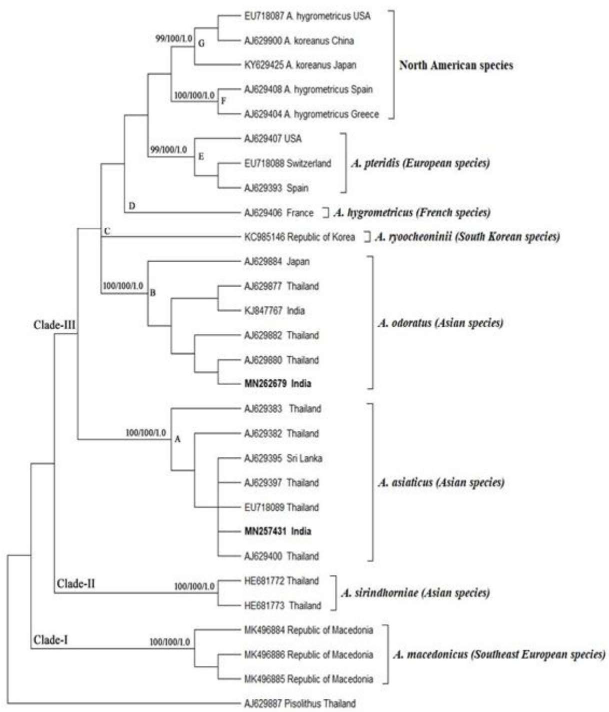


Fig 2: Parsimonious tree inferred from a heuristic search of ITS nrDNA sequence of *Astraeus* spp. Clade I-III and sub-clade (A-G) as mentioned in the text. The sequence of *Pisolithus* sp. used as outgroup. Terminal branches are labelled with appropriate GeneBank accession number and countries of origin. Numbers at the nodes indicate the percentage of bootstrap values obtained from parsimony and maximum likelihood analysis and the posterior probabilities from Bayesian analysis (MPbs/MLbs/PP).

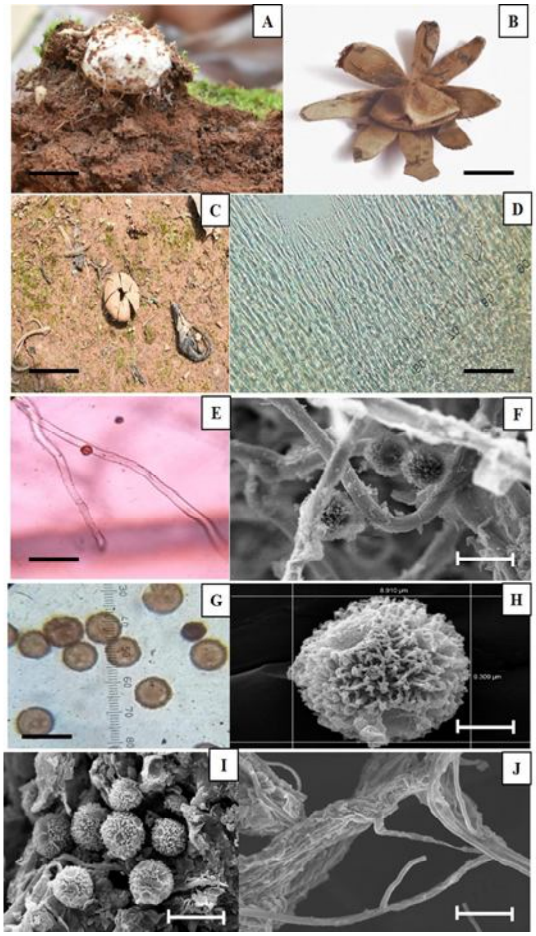


Fig. 3 A-J- *Astraeus asiaticus*: T.S. of (A) Immature Basidiomes (B) Mature basidiomes showing star-like structure; (C) Unexpended dried basidiome rolled again when dry; (D)Cross-section through exoperidium showing pseudoparenchymatous layer with elongated thick-walled cells; (E) Capillitium hyphae with long lumen; (F) SEM of Capillitium hyphae; (G) LM of Mature basidiospore ; (H-I) Spore ornamentation showing spines in groups; (J) SEM of rhizomorph. Magnification at 1,000X. Scale bar: A-C=20mm; D=4µm; E=4µm; F= 2µm; G=4µm; H= 1µm; I= 2µm and J= 20µm.

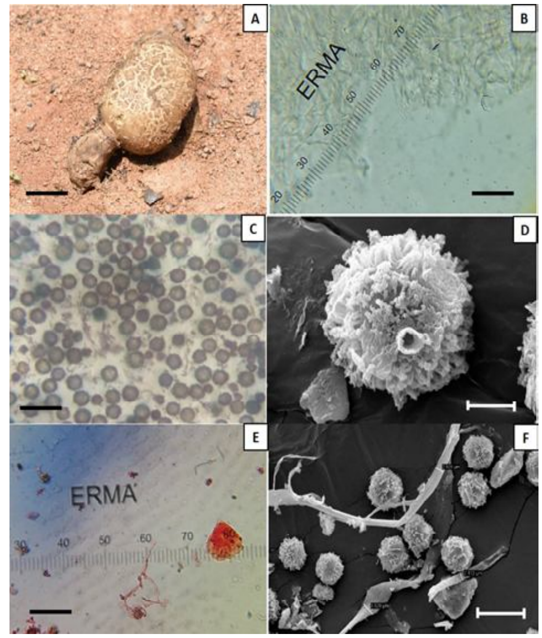


Fig. 4 A-F- *Astraeus odoratus* (A) Immature Basidiomes with mosaic like cracking. (B) Cross-section through exoperidium showing pseudoparenchymatous layer with thick-walled cells. (C) LM of Mature basidiospore. (D) Spore ornamentation showing spines in groups. (E) LM of Capillitium hyphae. (F) SEM of Capillitium hyphae with spores. Magnification at 1,000X. Scale bar: A=20mm; B=4µm; C=4 µm; D =1µm; E= 4µm and F=2µm.

Fibrous layer cigar brown, fleshy, thin, coriaceous. Pseudoparenchymatous layer sepia brown, fleshy, thin, glabrous, collar absent. **Endoperidium** shortly stipitate (when young), sessile, sub-globose, 11-14 mm in diam., whitish to ash grey, glabrous, opens by a slit or tear forming an irregular apical pore, peristome & apophysis absent. **Gleba** powdery dark brown at maturity. **Basidiospore** globose to subglobose, 8.2-14.4 X 8-14.2 µm [x = 11.4 ± 2.2 X 11.2 ±2.1 µm, Q_m = 1.05, n=30], yellowish brown to golden brown, thick-walled, ornamentation includes coalesce to spinoid; spines fused and incurve to in rolled apically, apiculus conspicuous, lack columella, capillitium 2-5 µm in diam., long, branched, interwoven, hyaline, aseptate, encrusted, lumen present. Mycelial layer composed of sinuous-walled hyphae, 1-1.5 µm diam., lumen present, greenish to hyaline in KOH. Fibrous layer composed of sinuous-walled hyphae, >1 µm diam., unbranched, greenish yellow to hyaline in KOH, lumen present. Pseudoparenchymatous layers thick-walled, hyaline in KOH, elongated cells; cells 1-2 X 19-30 µm in size, lumen absent.

Substrate: Sandy lateritic soil covered with litter, fruiting frequently in monsoon season (June-October). It is found as solitary or scattered in small groups under the Dipterocarpaceae *Shorea* dry deciduous forest.

Distribution: Thailand (Phosri *et al.* 2007), Sri Lanka (Phosri *et al*. 2007), Jharkhand, India.

Specimen Examined: India, Jharkhand, West Singhbhum district, Bandgaon Village, Porahat Forest division, alt. 287m, July 18, 2019, 22° 50' 56" N 85° 21' 1" E, on the ground under *Shorea robusta*, V. Vishal and S. S. Munda, Rug-1a (JH); Adjoining forest area of Bandgaon village, alt. 240m, July 24, 2019, 22° 51' 36" N 85° 19' 50" E, on ground under *Shorea robusta*, V. Vishal and S. S. Munda Rug-2a (JH).

Taxonomy :

Astraeus odoratus Phosri, C., A. J., & Watling, R & Martín, M. P., (2007). Mycotaxon 89(2):458(2004).

Mycobank no.: MB488422

Fig. 4 A-F

Description

Unexpanded Basidiome epigenous**,** brownish, particolous, mosaic pattern, globose to subglobose, 15-25 X 20-23 mm, apex rounded, surface not encrusted with debris, coriaceous, rhizomorph, brownish persistent, encrusted. **Expanded Basidiome** saccate 25-35 X 20-25 mm. **Exoperidium** composed of several layer (≤1 mm), split into 5-6 non hygroscopic rays which expand or recurved when moist and roll again inward when dry. Mycelial layer greyish brown to yellowish brown, not encrusted, coriaceous. Fibrous layer sepia brown to smoke grey, fleshy, coriaceous, thick, glabrous. Pseudoparenchymatous pale straw or olivaceous, glabrous, collar absent. **Endoperidium** subglobose, brownish to violaceous black, sessile, apophysis absent, irregular apical mouth, peristome absent, thin papery. **Gleba** pulverulent, dark brown to coffee colour at maturity. **Basidiospore** globose to subglobose, 6.4-12.8 X 6.2-12.9 µm [x = 10.6 ± 1.8 X 10.4 ± 1.8 µm, Q_m = 1.01, n=30], golden yellow to yellowish brown, thick-walled, ornamentation includes coalesce to spinoid; spines fused and incurve to in rolled apically, coalesce, spines 1.5-2.9 µm long, apiculus conspicuous, lack columella, capillitium 2.9- 4 µm diam., hyaline, branched, clamped, thick-walled (≤2 µm thick), lumen up to 2 µm wide. Mycelial layer composed of sinuous-walled hyphae, 1-1.5 µm diam., lumen present, greenish to hyaline in KOH. Fibrous layer composed of sinuous-walled hyphae, >1 µm diam., not branched, greenish yellow to hyaline in KOH, lumen present. Pseudoparenchymatous layers thick-walled, hyaline in KOH, spherical cells; cells 5-7 X 6-14 µm in size., lumen absent. Rhizomorph, thick walled, 4-8 µm diam., wall straight, clamped, encrusted, lumen absent.

Substrate: Sandy lateritic soil covered with litter, fruiting frequently in monsoon season (June-October). It is found as solitary or scattered in small groups under the Dipterocarpaceae *Shorea* dry deciduous forest.

Distribution: Thailand (Phosri *et al.* 2007), North-eastern part of Jharkhand, India (Hembrom et al 2014), Southern India (Pavithra *et al.* 2015), Southern part of Jharkhand, India.

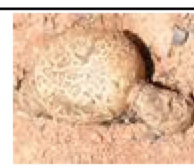
Specimen examined: India, Jharkhand, West Singhbhum district, Bandgaon Village, Porahat Forest division, alt. 287m, July 18, 2019, 22° 50' 56" N 85° 21' 1" E, on the ground under *Shorea robusta*, V. Vishal and S. S. Munda, Rug-1a (JH); Adjoining forest area of Bandgaon village, alt. 240m, July 24, 2019, 22° 51' 36" N 85° 19' 50" E, on ground under *Shorea robusta*, V. Vishal and S. S. Munda Rug-2a (JH).

Aim of this study was to add the existing knowledge of mycoflora of Jharkhand with knowledge of diversity and distribution of *Astraeus* in Jharkhand. An integrative taxonomic approach was taken to confirm the identity of the collected star-shaped fungi belonging to *A. asiaticus* and *A. odoratus*, a first report from southern region of Jharkhand, India (Fig. 1). *A. asiaticus* belongs to the group of epigeous particolous *Astraeus* species that includes *A. sirindhorniae, A. smitthii*, and *A. telleriae* and is distinguished by thickness of peridium, glebal colour, size and ornamentation of basidiospore[23-24]. *A. koreanus* and *A. morganii* are similar to *A. asiaticus* as they lack a peristome, columella, and apophysis, but their basidiospores are smaller (9-10.5 µm and 7-10 µm)[11,23]. Although both *A. macedonicus* and *A. asiaticus* are epigeous in origin, have globose to subglobose basidiomes but *A. macedonicus* has larger capillitium (4.2-10µm in diam.) and smaller basidiospores (7.3–10.1 µm diam.)[4]. Another very closely related species is *A. hygrometricus* with a brown to date brown outer peridium, 12-14 acute rays, hygroscopic, basidiospore size varying from 7.5–12.5 µm[16,23], characters which distinguish well this taxon from *A. asiaticus*. The Indian *A. asiaticus* species differ from *A. asiaticus* reported from Thailand (holotype) in spore measurement (8.75 -15.2 µm), and thickness of peridium. In Indian *A. asiaticus* mycelial and fibrous layer is composed of sinuous-walled hyphae having lumen and diameter >1 µm which is absent in *A. asiaticus* from Thailand as described by Phosri *et al*. (2007). The Indian species also exhibits pseudoparenchymatous layers with thick-walled, elongated cells of 1-2 X 19-30 µm in size. These are not present in Thailand *A. asiaticus*.

The mosaic like pattern on the surface of the basidiome, external exoperidial features, number of rays, thick-walled pseudoparenchymatous layers, glebal colour, and smaller basidiospore distinguish *A. odoratus* from other species such as *A. hygrometricus, A. thailandicus, A. ryoocheoninii. A. odoratus* has 5-6 non-hygroscopic exoperidial rays, whereas *A. hygrometricus* has 7-15,[16,23] *A. thailandicus* (4-5)[19] and *A. ryoocheoninii* has 15-19[27] exoperidial rays. Besides, the collected sample *A. odoratus* has smaller basidiospore (6.4-12.8 µm) than *A. hygrometricus* (7.5-15 µm)[16,23], *A. thailandicus* (7.5-13.5 µm)[19] and larger than *A. ryoocheoninii* (5.3-9 µm)[27]. The specimen *A. odoratus* collected from Jharkhand, India differs from Thailand counterpart (holotypes) in spore measurement (7.5-15 µm) with 1.04- 1.66 µm long coalescent spines and presence of thick-walled, pseudoparenchymatous layers composed of spherical cells (5-7 X 6-14 µm) which are absent in the specimen described by Phosri *et al.* (2004). The taxon, *A. asiaticus* can be distinguished from *A. odoratus* by the smaller basidiomes, smooth surface of outer peridium, colour of basidiomes, numbers of rays, distinct cells of pseudoparenchymatous layer and the larger basidiospore (Fig. 3 & 4). In addition, this species can be found in Jharkhand (India) over a more prolonged period from May-August while *A. odoratus* is frequently found during the month of May-June (Table-1). The phylogenetic analyses placed the described Indian collections (*A. asiaticus* and *A. odoratus*) into Asian clade based on molecular and morphological traits (Fig. 2). The genus *Astraeus* has been regarded as monophyletic in origin[21-24]. *Astraeus* form putative ectomy-corrhizal associations with variety of host tree species including *Shorea siamensis* Miq., *S. roxburghii* Don., *S. farinose* Fischer., *D. alatus* Roxb. ex G. Don, *D. intricatus* Dyer., *D. Obtusifolius* Teijsm. ex Miq. and *Hopea odorata* Roxb[10].

Among all the *Astraeus* species, *A. hygrometricus*, *A. odoratus*, *A. asiaticus* have received the most attention in terms of nutritional and nutraceutical potential and consumed in South-East Asia well as in South-west India, South-Western region of West Bengal and Jharkhand[21-22,30]. Furthermore, bioactive compounds extracted from the

Table-1. Structural differences between *A. asiaticus* and *A. odoratus.*

Features	*A. asiaticus*	*A. odoratus.*
Basidiomes Size in diam.	10-17 X 8-15 mm (unexpanded) 15-25 X 8-12 mm (expanded)	15-25 X 20-23 mm (unexpanded) 25-35 X 20-25 mm (unexpanded)
Colour	White	Brown
Peridium Surface	Smooth surface with soil debris	Mosaic pattern with few soil particles.
Number of rays	6-8; sub-hygroscopic	5-6; non-hygroscopic
Pseudoparenchymatous layer	Cell elongated in shape	Cells spherical in shape
Spore size	8.2-14.4 X 8-14.2 µm	6.4-12.8 X 6.2-12.9 µm
Fruiting season	May-August	May-June
		

basidiomes of *A. asiaticus* (Astrasiaone and Astradiate) and *A. odoratus* (Astraororic acids A-D, Astraodorol and Astraeusins A-L) have antituberculosis, antibacterial, cytotoxicity, antioxidant, anticancer, and antidiabetic activity[2,20,31,33]. The gasteroid earthstar mushrooms thus are natural reservoir of potent nutraceutical and pharmaceutical compounds and serve as the new drug discovery interface. These are expensive due to the limitation of natural occurrence and the difficulty of industrial production by cultivation thus suggesting that only a fraction of total fungal wealth has been subjected to scientific scrutiny.

Despite their multitude of importance, identification and documentation of fungi eternally remains a daunting task for mycologist. India has diverse biodiversity and treasure house for mushroom. The recognition of *Astraeus* species in India suggest that, further collection should be made in adjoining states to obtain mushroom wealth based on integrative taxonomic approach could resolve the taxonomic uncertainties in the taxon *Astraeus*.

Declaration on conflict of interest :

The authors declare no conflict of interest.

We would like to express our gratitude to the Department of Botany, DSPMU, Ranchi, for providing the essential facilities, as well as the Department of Nanotechnology, University of Calcutta, West Bengal, for allowing us to use their SEM. We appreciate the efforts and assistance of Mr. Sharath Kumar Nair and Mr. Sushant Kumar Jha of DSPMU, Ranchi, as well as the late Mr. Piyush Lugun. It would have been difficult to find the fascinating Mushroom without the help and assistance of Mr. Stephan Nag, Mrs. Rajni Nag, and Mrs. Julia Horo.

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