KAVAKA 60(3): 37-43 (2024) DOI: 10.36460/Kavaka/60/3/2024/37-43

Unveiling the Hidden Star: *Astraeus asiaticus* in the Sal Forest of Chhattisgarh, India

Chandrahas Dewangan and Sushil Kumar Shahi*

Bio-resource Product Research laboratory, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur (CG) - 495 009, India.

**Corresponding author Email: sushilkshahi@gmail.com*

(Submitted on May 03, 2024; Accepted on July 29, 2024)

ABSTRACT

This study unfolds the fascinating world of star-shaped fungi, focusing specifically on specimens collected from the Kondagaon forest, located in the southern region of Chhattisgarh, India. By adopting traditional wisdom on edible mushrooms with scientific rigor, the research sheds light on identifying *Astraeus species*. Using an integrated taxonomic approach, the investigation definitively confirms the collected specimen as belonging to the *Astraeus asiaticus* species. The main differentiating factors, particularly basidiospore size and other morpho-anatomical features enabled to distinguish *A. asiaticus* from closely related species. Conclusively, future studies could delve deeper into exploring the genetic structure, nutritional values and bioprospection potential of *A. asiaticus* populations across its geographic range.

Key words: Anatomy, Basidiomata, Basidiospore, Boletales, Epigeous, Morphology.

INTRODUCTION

The *Astraeus* species are a small round fruiting body of a fungus that looks like a small potato, grows about 0.5-4.0 cm under the ground in sandy soil and forms ectomycorrhizal collaboration with roots of the host trees species of Dipterocarpaceae, Fagaceae, and Pinaceae (Phosri *et al.,* 2004; Fangfuk *et al*., 2010; Phosri *et al*., 2013; Karun and Sridhar, 2014; Pavithra *et al.,* 2015; Sultani Ahmadzai *et al.*, 2023). Taxonomically, *Astraeus* is categorized under Diplocystidiaceae, Boletales, Agaricomycetes and Basidiomycota. There are 12 world-wide recognized species included under this genus in Index Fungorum [(indexfungorum.org) April, 2024] out of which, only five species have been identified in Asia as an *A. hygrometricus, A. asiaticus, A. koreanus, A. odoratus* (Syn.*A. thailandicus* Petcharat) (Petcharat 2003), and *A. ryoocheoninii*(Morgan 1889; Kreisel 1976; Phosri *et al.,* 2004; Phosri *et al*. 2007; Ryoo *et al*. 2017).Three species were described in India as an *A. odoratus, A. hygrometricus* and *A. asiaticus* (Ahmad *et al*. 1950; Hembrom *et al*. 2014; Vishal *et al*. 2021). Although *Astraeus* is superficially identical to *Geastrum* but it differs due to the lack of certain characteristics particularly columella and peristomein *Astraeus* but it consists of larger basidiospores than *Geastrum* and has highly branched long capillitial hyphae (Phosri *et al.,* 2004).Based on the findings of Cannon and Kirk (2007), it is suggested that convergent evolution could have taken place within the genera *Astraeus* and *Geastrum*.

Astraeus is widely recognized as a highly esteemed variety of edible fungi in numerous regions across Asia (Mortimer *et al.,* 2012). Basidiomata of *Astraeus* species are collected from woodland areas and commercialized in various countries including India, Japan, Thailand, and Laos as documented by multiple researchers (Sanmee *et al.,* 2003; Dell *et al*. 2005; Butkrachang *et al*. 2007; Karun and Sridhar, 2014). They exhibit a widespread presence across the Indian subcontinent, encompassing regions such as Himachal Pradesh, Jharkhand, Karnataka, Kerala, Orissa, Punjab, Uttarakhand, Uttar Pradesh, and West Bengal (Phosri *et al.,* 2004, 2013; Pradhan *et al*. 2010, 2012, 2013; Pyasi *et al.,* 2011;Mohanan 2011;Karun and Sridhar, 2014;Hembrom *et al.,* 2014;Semwal *et al*. 2014).Furthermore, the interaction of *Astraeus* with various species of forest trees in the Western Ghats of Southern India has been documented (Bhagwat *et al.,* 2005; Karun and Sridhar, 2014).The occurrence of *A. hygrometricus* and *A. odoratus* has been recorded in Korea, as well as in the Kanker and Bastar districts of Chhattisgarh, India. (Kumar *et al.,* 2019; Badhai *et al.,* 2021; Kumar and Netam, 2022). Acharya *et al*. (2020), described to a small round boletale as a Bastar Boda.

Very less work has been done on Boletales in Chhattisgarh, Indiainspite of being dense forest and

availability of various mushroom species. The primary objective of this research was to establish the existence of *Astraeus* species within the foothill areas of Kondagaon, Chhattisgarh, India.The traditional understanding of the consumption of *Astraeus* fungi and their connection with *Shorea robusta* has served as a catalyst for the current investigation. Consequently, a comprehensive analysis of both the macroscopic and microscopic features of *Astraeus* species is delineated herein.

MATERIAL AND METHOD

Sample collection- A sample was collected according to the conventional wisdom regarding consumable fungi from the Kondagaon Sal forest situated in the foothills of the Southern region of Chhattisgarh (19.973216^{0}N, 81.607103^{0}E) (**Figure 1**).The dense forests of Chhattisgarh are said to have abundant forest wealth.The natural forest sites are characterized by the presence of predominantly gravel, sandy, and loamy lateritic soils. The emergence of *Astraeus* mushrooms occurs with the first monsoon rains in June, when the weather is sunny and humid. The collection of *Astraeus* specimens was conducted during the morning hours in the vicinity of the roots of *Shorea robusta* in the months of July, 2023. Subsequently, a comprehensive macroscopic examination of the acquired samples was carried out on site, involving the recording of essential general information and morphological features pertaining to the specimens. Habitat photographs were also taken and carried out to the laboratory for further studies and analysis.

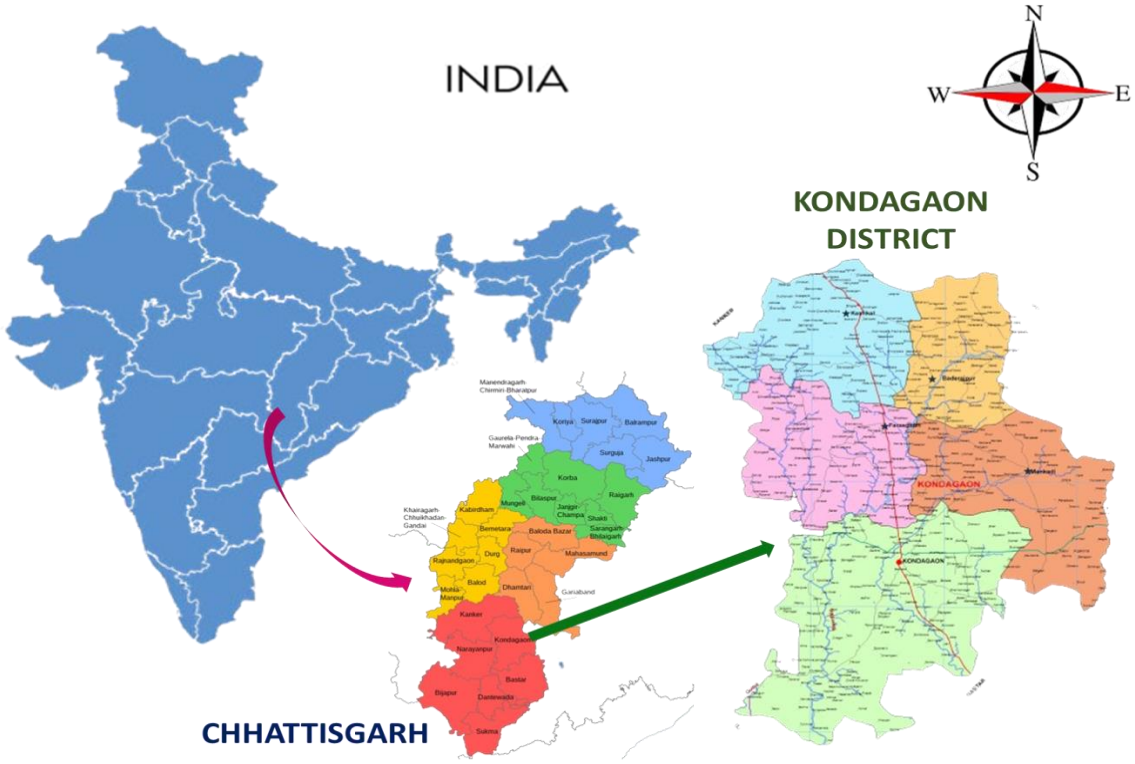

Figure 1: Sample collection area of Chhattisgarh (Kondagaon district).

Morphological analysis

The fresh mature and immature basidiocarps were found in solitary or small cluster of 2-5, partially buried (0.5-4 cm) in soil or under the debris of leaf (litter) of *Shorea robusta* tree. Sometimes, due to heavy rainfall, the water flow causes the basidiocarps to be scattered like small potatoes over the soil surface. Morphological characters such as, basidiocarps shape, odour, peridial and glebal colours and textures, etc. were recorded in the field and in the laboratory (Colour identification chart, Royal Botanic Garden, 1969).To find out the amyloid or non-amyloid nature of the collected sample, Melzer's reagent was applied on the cut portion of the gleba and peridium tissues of the sectioned sample (Largent *et al*. 1977) by checking the color change in gleba and peridium. For further confirmation of the sample, 5% KOH was applied to the inner peridiain 30-minute interval upto 2 hours for changing the color of peridium inner wall. Fundamental as well as specific morphological characters of collected

sample were compared with the key as described by Zeller (1949) and literature found in the monographs (Jordan, 2004; Phillips, 2006; Mohanan, 2011). The collected sample specimen (BPRL0002) was stored in the Bio-resource Product Research Laboratory, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.), India.

Study of anatomical features

To study the anatomical features of the collected sample, fresh basidiocarps were used. Basidiocarps were sliced, using hand-razor blade and mounted on to the glass slide. Thin sections of basidiocarp were first treated with 5% KOH solution for dissolving cellular material, making it easier to observe. After 3-5 minutes, the treated sections were stained with lactophenol cotton blue reagent. The stained sections were observed under microscope [Optika Microscope (SN462759)] and the characteristics of structure obtained microscopically were compared with the literature of Phosri *et al.*, 2004, 2013, Pyasi *et al*. 2011 and Hembrom *et al*. 2014.

RESULTS

Morphological observations

The Basidiocarp was globose to sub-globose and regular in shape. Size measured were 1–3.5 cm and average weight was4-5g (**Figure 2C**). Odour was mild when fresh and unpleasant when it gets old. Peridium was white to brownish yellow in colour but when it was sectioned into two halves then turned brown after half an hour and no latex is exuded from a cut surface (**Figure 2A**). When sporocarp and gleba treated with melzer's reagent then no colour changes appeared which indicated the non-amyloid nature of the sample but when stained with KOH then light green colour appeared around inner peridial membrane. Gleba was white when the fruiting body was immature but turned powdery at maturity, and are not filled with a gel-like material (**Figure 2 A**).

Anatomical observation

Astraeus asiaticus Phosri, M.P. Martín & Watling [**Figure 2 (A-I)**]

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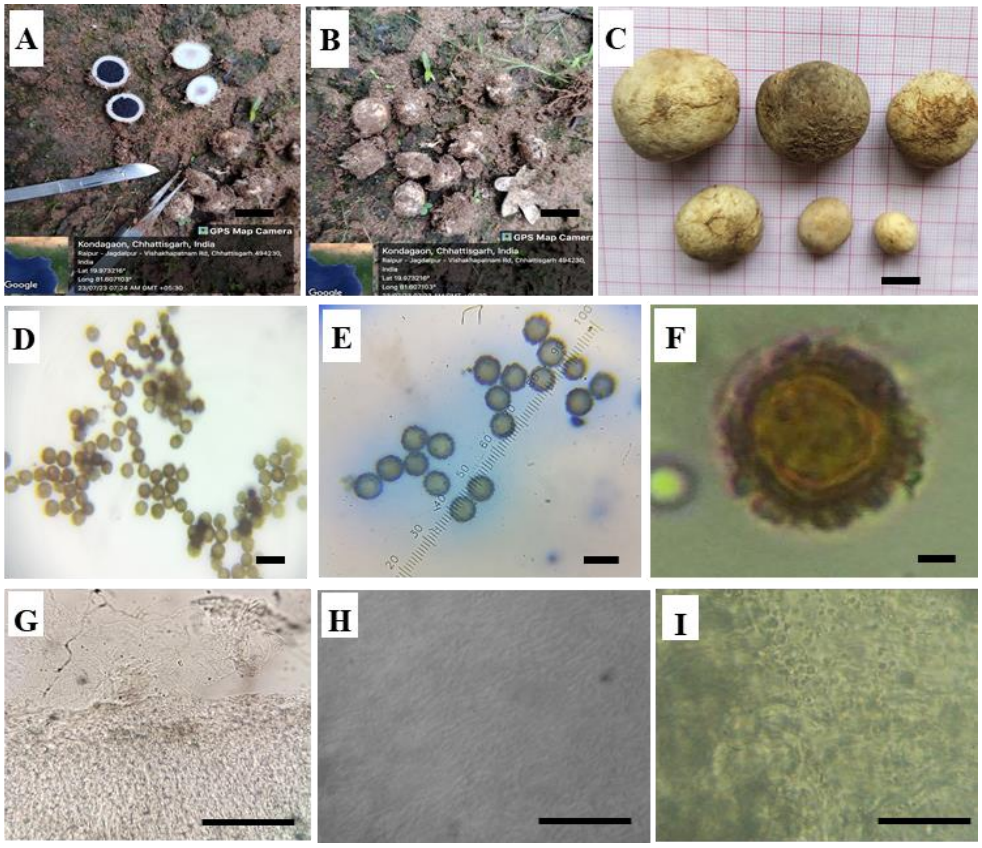

Figure 2: A, T.S of mature and immature Basidiocarps; B, Mature basidiocarps showing star-like structure; C, Basidiocarps colour, shape and size; D-F, Mature basidiospore under different resolution of microscope; G-H, Cross section showing endoperidium; I, Cross-section of exoperidium showing pseudoparenchymatous layer with elongated thick-walled cells (Scale bar: A-C= 1cm; D = 100µm; E = 50µm; F = 10µm; G = 100µm; H & I = 50µm).

Basidiocarp sub-epigeous and some were hypogeous, globose to sub-globose, regular, sessile, 10-35× 8-15 mm, whitish brown, encrusted with debris, surface unpolished, dry, not lubricous, slight odour; rhizomorph 2-6 mm long, persistent, whitish and white mycelial often partly buried in soil. Expanded Basidiocarp size 15- 25× 8-17 mm. Exoperidium split into 6-8 sub-hygroscopic rays which expand and curved when humid and roll inward again when dry, rays 5-12 mm long, longitudinally cracked in mature basidiocarp, ovate to elliptical in shape. Mycelial layer, thick, encrusted, whitish and white becoming brown to smoke grey with age exposing the fibrous layer. Fibrous layer dark brown, fleshy, thin, coriaceous (**Figure 2A, B, & C**). Layer of pseudoparenchymatous was sepia brown, fleshy, thin, glabrous, collar absent. Endoperidium shortly stipitate when young, sessile, sub-globose, 10-15 mm in diameter, whitish to ash grey, glabrous, opens by a slit or tear forming aberrant apical pore, peristome & apophysis absent (**Figure 2G & H**). Gleba white when immature but powderydark brown at maturity (**Figure 2A**).

Basidiospore was globose to sub-globose in shape, 8.1-14.2× 8-14.1 µm on average, Qm = 1.05-3.0,yellowish brown to golden brown, thick-walled, columella absent (**Figure 2D-F**). Capillitium 2-5 µm in diameter, long, branched, interwoven, hyaline, aseptate, encrusted, lumen present. Mycelial layer composed of sinuous-walled hyphae, 1-1.4 µm diameter, lumen present, greenish to hyaline when stainedwith KOH. Fibrous layer composed of sinuous walled hyphae, >1 µm diameter, unbranched, greenish yellow to hyaline in KOH, lumen absent (**Figure 2H & I**).

Substrate: Sandy lateritic soil covered with litter, fruiting frequently in monsoon season (mid-June to September) (Kumar and Netam, 2022). It is found in dry deciduous forests under the Dipterocarpaceae family, either alone or in small groups (**Figure 2A-B**).

DISCUSSION

The objective of this study was to combine the existing knowledge of mycoflora of Chhattisgarh with the knowledge of diversity and distribution of *Astraeus* in Chhattisgarh. An integrated taxonomic approach was adopted to confirm the identity of the collected star-shaped fungi as belonging to *Astraeus asiaticus*, a first report in Chhattisgarh, India (**Figure 2**). *A. asiaticus* and *A. odoratus* were also reported from the southern region of Jharkhand by Vishal *et al.,* (2021).Molecular and phylogenetic placement of *Astraeus species* in India is uncertain hence an integrated taxonomic approach was used to identify and resolve ambiguities within the genus (Vishal, 2024). *A. asiaticus* belongs to the group of epigeous particular *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 (Phosri *et al*. 2013, 14). *A. koreanus* and *A. morganii* are similar to *A. asiaticus* as they are not having a peristome, columella, and apophysis, but their basidiospores are smaller (9-10.5 µm and 7-10 µm) (Kreisel 1976; Phosri *et al*. 2013).

However, *A. Macedonianus* and *A. asiaticus* both are epigeous in origin and have globose to sub-globose basidiocarps, but *A. macedonicus* has large capillitium (4.2–10µm in diameter) and small basidiospores (7.3–10.1µm in diameter) (Crous *et al.,* 2019). Another very closely related species is *A. hygrometricus*, its outer peridium is brown to date brown, 12–14 acute rays, hygroscopic, basidiospore size varies from 7.5–12.5 µm (Phosri *et al*. 2013; Pavithra *et al*. 2015), characters that well distinguish this taxon from *A. asiaticus*.

The Indian *A. asiaticus* species differs from *A. asiaticus* reported from Thailand (holotype) in spore measurement and peridium thickness.The mycelial and fibrous layer in Indian *A. asiaticus* is composed of sinuous walled hyphae with lumen and diameter >1 µm which is absent in *A. asiaticus* from Thailand as described by Phosri *et al.,* 2007. The Indian *Astraeus aiaticus*also possess pseudoparenchymatous layers with thick-walled, elongated cells of 1-2 × 19-30 µm in size but these are not present in *A. asiaticus* which were described in Thailand.The taxon, *A. asiaticus* can be clearly distinguished from *A. odoratus* as it has smaller basidiocarps, smooth surface of outer peridium, colour of basidiocarp, distinct cells of pseudoparenchymatous layer, numbers of rays and the larger basidiospore than *A. odoratus* (**Figure 2A-I**).

CONCLUSION

This study aimed to identify and document star-shaped fungi collected from the Kondagaon Sal forest in southern Chhattisgarh, India. By combining traditional knowledge, scientific analysis, researches and literature confirmed the

collected specimens as *Astraeus asiaticus*. This research marks the first official record and establishes the presence of this species in the southern region of Chhattisgarh. Further research could explore the distribution and abundance of *A. asiaticus* within the region and their potential ecological roles in the local ecosystem.Since this species has been used as a food supplement by local tribes, analysis of their nutritional composition will shed light on the extent of edibility. If this species can be cultivated artificially throughout the year, it will increase the income of local tribes.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all individuals and organizations who have contributed to the successful completion of this research article. Firstly, I extend my heartfelt appreciation to my research supervisor Prof. Sushil Kumar Shahi for his invaluable guidance, mentorship and support. I would also like to thank the UGC New Delhi and Guru Ghasidas Vishwavidyalaya, Bilaspur whose financial support played a crucial role in enabling the execution of this research. Lastly, I acknowledge the academic community and colleagues for their insightful discussions, constructive feedback and inspiration that enriched the quality of this research work.

CONFLICT OF INTEREST

There is no conflict of interest.

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