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Research Article

A new record of *Astraeus hygrometricus* (Pers.) Morgan (Boletales, Basidiomycota) from Afghanistan

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Abstract

The mycorrhizal fungus, *Astraeus hygrometricus*, which was found thriving beneath *Quercus baloot* and *Cedrus deodara* trees is being documented in Afghanistan for the first time. Despite previous reports confirming the presence of this fungus in other parts of Asia, Europe and America, there is no prior documentation of its presence in Afghanistan. This report includes a protologue of the specimens collected from Afghanistan, showcasing both macro- and micro-morphological characteristics. Additionally, Scanning Electron Microscope (SEM) images are provided for a detailed view of the basidiospores.

Keywords: Baloot Oak, Deodar Cedar, Earth stars, Habitats, Mycorrhizal fungi, Nuristan

1. Introduction

The *Astraeus* species, which are ectomycorrhizal, establish symbiotic relationships with a broad range of forest tree species. These tree species encompass members of Dipterocarpaceae, Fagaceae, and Pinaceae (Hibbett et al. 2000, Phosri et al. 2004, Fangfuk et al. 2010, Wilson et al. 2012, Phosri et al. 2013, Karun and Sridhar 2014, Pavithra et al. 2015). In terms of taxonomy, *Astraeus* is categorized under Diplocystidiaceae, Boletales, Agaricomycetes and Basidiomycota. According to Index Fungorum (2023), there are 12 recognized species under this genus. These include *Astraeus asiaticus* Phosri, M.P. Martín & Watling (Phosri et al. 2007),

A. hygrometricus (Pers.) Morgan (Morgan 1889), *A. koreanus* (V.J. Staněk) Kreisel (Kreisel 1976), *A. macedonicus* Rusevska, Karadelev, Tellería & M.P. Martín (Crous et al. 2019), *A. morganii* Phosri, Watling & M.P. Martín (Phosri et al. 2013), *A. odoratus* Phosri, Watling, M.P. Martín & Whalley (Phosri et al. 2004), *A. pteridis* (Shear) Zeller (Zeller 1948), *A. ryoocheoninii* Ryoo (Ryoo et al. 2017), *A. sapidus* (Massee) P.-A. Moreau (Paz et al. 2017), *A. sirindhorniae* Watling, Phosri, Sihan., A.W. Wilson & M.P. Martín (Phosri et al. 2014), *A. smithii* Watling, M.P. Martín & Phosri (Phosri et al. 2013), and *A. telleriae* M.P. Martín, Phosri & Watling (Phosri et al. 2013). Out of these, only five species have been identified in Asia. They are *A. asiaticus*, *A. hygrometricus*, *A. koreanus*, *A. odoratus* (which is synonymous with *A. thailandicus* Petcharat) (Petcharat 2003), and *A. ryoocheoninii*.

Astraeus hygrometricus was initially characterized as *Geastrus hygrometricus* by Persoon in France (Morgan 1889, Ito 1959). Descriptions of this species have fluctuated, influenced by factors such as size, shape, gleba color, the external texture of the peridium, and the microscopic features of the peridium and basidiospores (Nouhra and De Toledo 1998, Baseia and Galvao 2002, De Roman et al. 2005, Phosri et al. 2007). *Astraeus hygrometricus* and *A. pteridis* are known to form ectomycorrhizal relationships with a range of host tree species belonging to *Acacia*, *Alnus*, *Castanea*, *Cedrus*, *Eucalyptus*, *Pinus*, *Pseudotsuga* and *Quercus*. However, *A. odoratus* and *A. asiaticus* have exclusively been reported in dipterocarp forests (Trappe 1967, Malajczuk et al. 1982, Phosri et al. 2007, Yomyart 2008, Phosri et al. 2013, Pavithra et al. 2016, Hembrom et al. 2014, Ryoo et al. 2017, Kaewgrajang et al. 2019, Suwannasai et al. 2020).

Astraeus hygrometricus is commonly referred to as hygroscopic earthstar, barometer earthstar, or false earthstar (Hayat 2017). As described by Hayat (2017), young and unopened *A. hygrometricus* specimens bear a resemblance to puffballs. As they mature, the outer layer of their fruiting bodies splits open in a star-like pattern, taking on the distinctive earthstar shape. The common names of this species reflect its hygroscopic nature, as it can expand and contract its rays to expose the spore sac in response to increasing or decreasing humidity levels. Although *A. hygrometricus* may appear similar to the true earthstars in *Geastrum*, it is not closely related to them. It differentiates itself by lacking open chambers in the young gleba and a true hymenium. It also has larger, branched capillitium threads and a larger spore size.

Studies on the phylogenetic relationships within *Astraeus*, based on the ITS region, have indicated that *A. hygrometricus* in Southeast Asia is distinctly different from *A. asiaticus* in East Asia in both morphological and molecular features (Phosri et al. 2004, 2007). Nevertheless, *Astraeus* populations in Europe may be partitioned into two unique phylotypes: one from France and the other from the Mediterranean region (Phosri et al. 2007, Fangfuk et al. 2010). An initial investigation conducted by Fangfuk et al. (2010) found that a Japanese species, formerly identified as *A. hygrometricus* var. *koreanus*, was genetically distinct from *A. hygrometricus*. This species was named "A. koreana" by Ryoo et al. (1999), but the designation was deemed invalid as no Latin description was provided and no type specimen was designated. "Astraeus koreana" shows a macro-morphological similarity to *A. hygrometricus* var. *koreanus* (Staněk 1958) and shares micro-morphological traits with the "Japanese Astraeus group 2". Ryoo et al. (2017) reevaluated the taxonomy of *A. hygrometricus* var. *koreanus*, "A. koreana", and "Japanese Astraeus group 2", using phylogenetic analysis to identify crucial morphological characteristics for distinguishing taxonomic boundaries. *Astraeus ryoocheoninii* was proposed as a new species encompassing "Astraeus koreana" and "Japanese Astraeus group 2". The evidence suggests that combining molecular data with traditional morphological characteristics can effectively resolve taxonomic uncertainties within *Astraeus*.

This study was aimed to document the presence of *A. hygrometricus* in Afghanistan. Furthermore, we provide an in-depth examination of the macroscopic and microscopic characters of *A. hygrometricus* samples collected from Afghanistan. This report also includes scanning electron microscope (SEM) images of the basidiospores. This research is the first to identify and report the species *A. hygrometricus* in Afghanistan.

2. Materials and Methods

Fresh specimens of *A. hygrometricus* were collected during field trips conducted from June 2021 to January 2022 in Parun Valley of Nuristan (35° 31′ 59.8 ″N, 70° 86′ 68 ″E) and Chapa Dara Valleys of Kunar (34° 86′ 71.4 ″N, 70° 88′ 33.3 ″E) Provinces, Afghanistan. For the first time, mature mushrooms were spotted and collected in a plot within Parun forest of Nuristan Province. In the subsequent year, fresh and young mushroom specimens were harvested from Galangal Valley in Kunar Province, situated approximately 60 kilometers from Nuristan Province (Figure 1). The collected samples were dried at room temperature (25–30 °C), shielded from sunlight, and preserved at Plant Research Institute Laboratory at Ferdowsi University, Mashhad, Iran.

The collected herbarium specimens were identified using identification keys and authoritative species descriptions (Phosri et al. 2004, 2013). The sample was deposited in Ferdowsi University of Mashhad Herbarium (FUMH) in Iran and was given Herbarium number (E 1341-FUMH). Furthermore, unstained images of the basidiospores were captured using a digital camera (Nikon Eclipse Ni-u) affixed to a stereomicroscope (Nikon, Ds-Fi3 Tokyo, Japan) with magnifications of 400x and 1000x.

For the scanning electron microscope (SEM) analysis, spores extracted from mature fruit bodies were treated with a series of ethanol concentrations (70%, 80%, 90%, 95%, and twice in 100%) and then left to air-dry overnight. The spores were then positioned on stubs using adhesive tabs, coated with a layer of gold, and observed under a scanning electron microscope (Joel, JSM 6380 LA, Japan) at a magnification of 2≥20 µm.

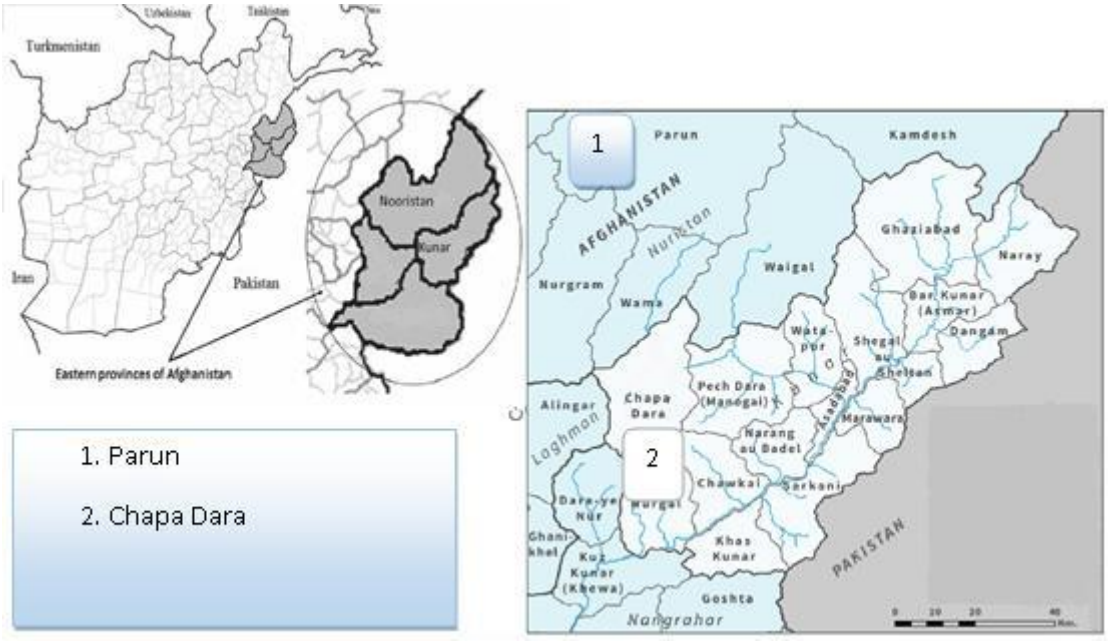

Figure 1. Geographical distribution of *Astraeus hygrometricus* in Afghanistan (Adapted from Gulab et al. 2020).

3. Taxonomy

Astraeus hygrometricus (Pers.) Morgan, J. Cincinnati Soc. Nat. Hist. 12: 20 (1889)

MycoBank number: MB#122650

Morphological characteristics of *A. hygrometricus*

Fruiting bodies of *A. hygrometricus*, varying in shapes and sizes, were observed from Afghanistan. Basidiospores of *A. hygrometricus* ranging from 2.5 to 4.3 cm in diameter were observed during our study (Figures 2–3). The spore sacs were globose or depressed globose, sub-epigeous, sessile, with 18.7–29.7 mm diameter. Young, undehisced spore sacs were covered with thin, white mycelial layer, which tears away at maturity, and the mature spore sacs become star-shaped upon splitting. Outer peridium of spore sac was whitish, thick, rigid, surface granulate, composed of several layers (≥1 mm. when dried), and splits into 5–12 acute rays at maturity. These rays reversibly roll outwards and inwards, respectively, under moist and dry conditions. Inner layer of the rays is whitish, which becomes smoke-grey, cigar-brown or sepia, upon maturation and develops extensive longitudinal cracks upon maturity. Endoperidium is sessile, subglobose, 13–24 mm in diameter, whitish to smoke grey, opening by an apical, irregular mouth lacking defined peristome. Gleba is white at early stages, and turns to purplish chestnut when mature, columella absent; capillitium of long, branched, interwoven, hyaline, slightly encrusted, aseptate threads, 2.5–7.5 µm in diam. Basidiospores are globose, purplish chestnut in color, 8.75–15.2 µm in diameter, very densely ornamented with rounded, narrow, tapered, occasionally coalescent, and having 0.90–1.45 µm long spines. Young spore sacs were white, turning to light grey when matured (Figure 4). Spore sacs were 1.5–2 cm in length, with up to 2 mm thick exoperidium, splitting into 7–13 rays. Light microscope and SEM images microscopes indicated reddish brown colored spores, having 9 µm diameter, and roughly spherical shape, with specified warts (Figures 5–6). The specimens are similar to *A. hygrometricus* and differ from *A. odoratus* morphologically (Table 1). The current study represents the first report of *A. hygrometricus* from Afghanistan.

Distribution

Astraeus hygrometricus is widely distributed across various regions around the world. It thrives in dry, sandy grasslands, most notably in Southern France and Turkey. Additionally, it can be found in fields and woodland areas with sandy soil throughout the Central and Southern United States (Phosri et al. 2013). Historically, this species has been reported from several continents. In North and Central America, it has been reported from the United States and Canada, and *albeit* rare, also in South America, specifically Argentina, Brazil, and Mexico (Coker and Couch 1928, Long and Stouffer 1948, Rick 1961, Esqueda-Valle et al. 1990, Nouhra and De Toledo 1998, Phosri et al. 2004, 2007, 2013, 2014, Fangfuk et al. 2010, Pavithra et al. 2015, Vishal et al. 2021). In Asia, it has been recorded in countries including China, Thailand, India, Pakistan, Korea, Japan, Sri Lanka, Uzbekistan, Israel, and the Philippines (Liu 1984, Petcharat 2003, Phosri et al. 2004, 2007, 2013, 2014, Hembrom et al. 2014, Pavithra et al. 2015, 2016, Isaka et al. 2017, Ryoo et al. 2017, Kaewgrajang et al. 2019, Suwannasai et al. 2020, Ganguly et al. 2021, Vishal et al. 2021, Fong-in 2023). In Europe, it can be found in countries such as Spain, Germany, Greece, Switzerland, Italy, Hungary, England, the Canary Islands, and the Czech Republic (Zeller 1948, Calonge and Demoulin 1975, Phosri et al. 2004, 2013, 2014). It has also been reported in Australia, Africa, and New Zealand (Phosri et al. 2004, 2014, Fangfuk et al. 2010, Pavithra et al. 2015, Vishal et al. 2021). Interestingly, the only continent where *A. hygrometricus* has not been reported is Antarctica (Phosri et al. 2013, Vishal et al. 2021).

Notes

In this study, the morphological traits of *A. hygrometricus* align closely with the descriptions provided in the initial scientific description, or protologue, of the species (Nouhra and De Toledo 1998, Phosri et al. 2004, 2007, Hayat 2017). The specimens examined were found in close association with *Quercus baloot* Griff (a species of oak in the Fagaceae family) and *Cedrus deodara* (Roxb.) G. Don (a type of cedar in the Pinaceae family) trees. These specimens flourished in moist soil and amidst decaying leaves. *Astraeus hygrometricus* is being reported from the high-altitude regions and woodland areas of Parun forest, specifically on north-facing slopes around 2500 meters above sea level. The annual rainfall in these regions varies between 700 and 1000 mm, influenced heavily by the Indian monsoon (Breckle and Rafipoor 2010). Consequently, based on our observations, suitable habitats for the growth of *A. hygrometricus* in Afghanistan are found in the Nuristan Province: north of Parun, above Takri Village, at an elevation of 2560 meters. The geographical coordinates

for this location are 35° 31′ 59.8 ″N, 70° 86′ 68 ″E. The specimens from this location were collected by A. Sultani Ahmadzai & Q. Khadim and were assigned the herbarium number 1341 (FUMH).

Figure 2. Photographic representation of freshly collected *Astraeus hygrometricus* specimens in their natural habitat. A) Close-up view of a specimen, scale bar indicates 3 cm. B) Another specimen, with a scale bar set at 4.5 cm for size reference. C) Image of A. Sultani Ahmadzai alongside the collected specimens to illustrate scale and habitat.

Figure 3. Illustration of the morphology of *Astraeus hygrometricus* fruiting bodies. The figure depicts the variation in shape and size of the fruiting bodies. (A) Fruiting body with a scale bar indicating 4.5 cm. (B) Range of fruiting bodies with sizes between 2.5 and 4.5 cm. All images were captured by A. Sultani Ahmadzai.

Figure 4. Morphological characteristics of the spore sac in *Astraeus hygrometricus* observed in the field. The figure depicts the varied sizes of spore sacs: (A) Spore sac with a diameter of 4 cm, and (B) Spore sac with a diameter of 3.5 cm. These photographs were taken by A. Sultani Ahmadzai at the collection sites.

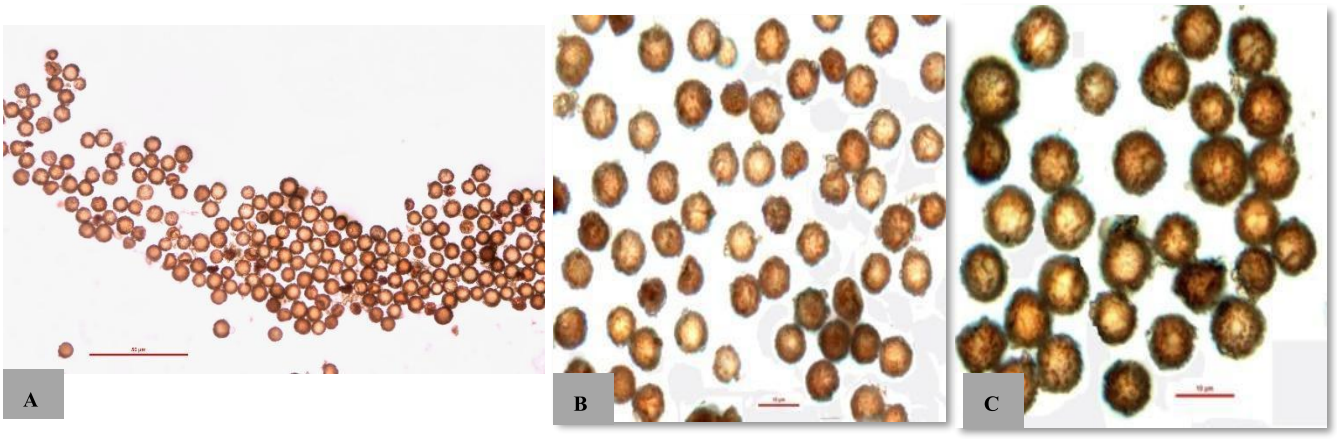

Figure 5. Light microscope images of *Astraeus hygrometricus* basidiospores, unstained. The spore diameter is approximately 9 µm. Panel A shows an image at 400x magnification (scale bar represents 50 µm), while panels B and C provide detailed views at 1000x magnification (scale bar represents 10 µm). These images were captured by A. Sultani Ahmadzai at the Ferdowsi University of Mashhad Herbarium (FUMH) laboratory.

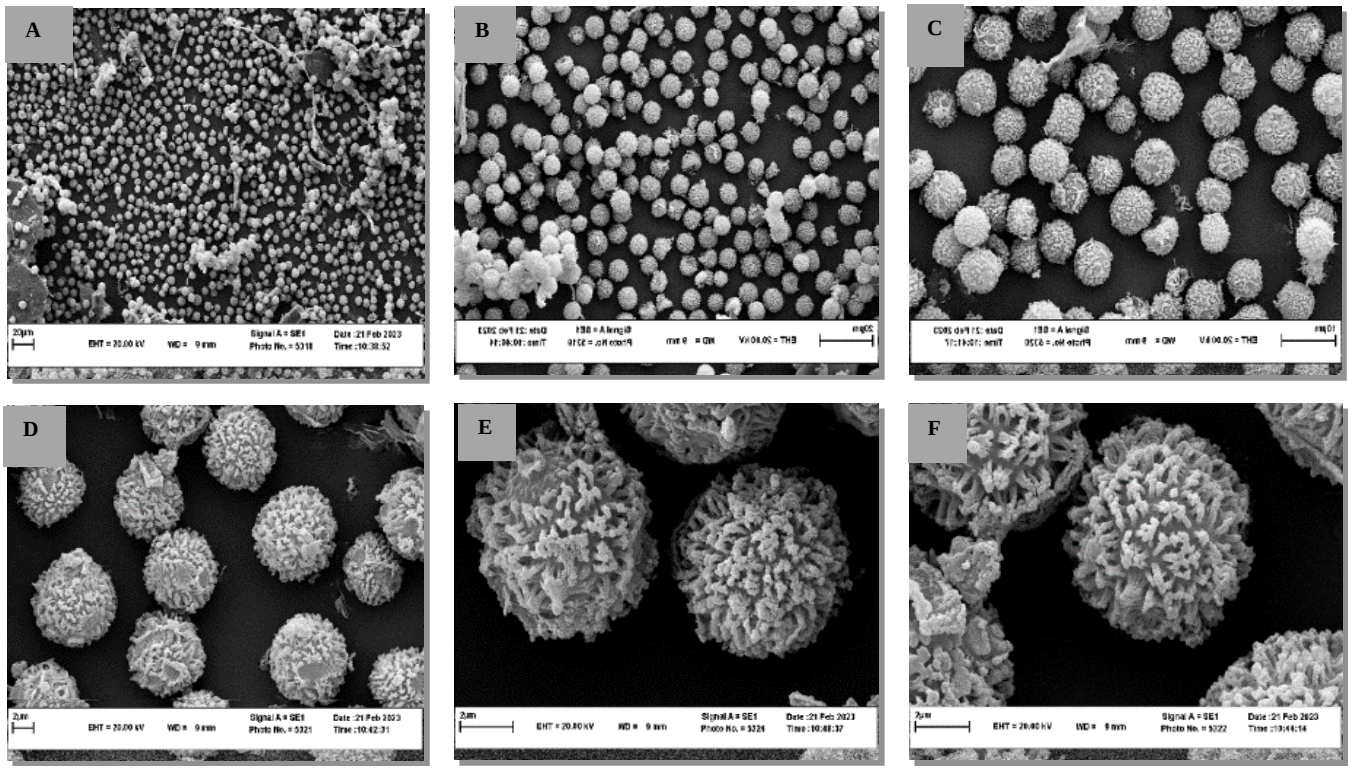

Figure 6. Scanning Electron Microscope (SEM) images depicting the detailed ornamentation of basidiospores, as observed at low and high magnifications at the Central Laboratory of Ferdowsi University of Mashhad (FUM). The scale bars represent different magnifications: 20 µm for sub-figures A and B, 10 µm for sub-figure C, and 2 µm for sub-figures D, E, and F.

Table 1. A comparison of the core characteristics between this new record *A. hygrometricus* and similar taxa

Character / Taxa	*A. hygrometricus* (New record)	*A. hygrometricus*	*A. odoratus*
Outer peridium size	Up to 2 mm	Up to 2 mm	Up to 1 mm
Number of rays	7–13	7–13 acute rays	3–9 broad rays
Endoperidium diameter	13–24 mm	20–25 mm	13–25 mm
Endoperidium Color	Whitish to Smoke grey	Creamish grey	Buff to brownish
Gleba diameter	2.5–7.5 µm	4–6 µm	2.5–6.25 µm
Gleba color	White to purple	Brown to purple	dark brown
Spore size	9 µm	10–12.5 µm	7.5–15.2 µm
Spore color	Reddish brown	Reddish brown	Purple brown
Fruiting season	May-August	May-August	May-Jun

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Author Contributions: Sultani Ahmadzai A. collected and investigated the specimen, and wrote the manuscript; Ejtehadi H. and Farzam M. conceived and designed the research study; Bashirzadeh M. co-wrote and revised the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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