

## A new species of *Cyathus* (Agaricaceae) from India

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**Abstract:** The Acharya Jagadish Chandra Bose Indian Botanic Garden is a well-known botanical garden. *Cyathus thindii*, a new species of bird's nest fungus, was collected from this garden, and is described and illustrated in the present paper. It is distinct with its sessile basidiomata growing on a white mycelial pad, plicate exterior (partly) and interior wall of the peridium, comparatively large peridioles without tunica, and medium sized spores. Its relation with allied taxa is discussed and a provisional key to the Indian species of *Cyathus* is given in the present paper.

**Key words:** Bird's nest fungi, Agaricaceae, taxonomy, new species, India

### 1. Introduction

The genus *Cyathus* Haller (Agaricaceae) can be characterized as follows: peridium vase-like or infundibuliform to inverted bell-shaped with 3 layers, surface plicate or smooth, often covered with shaggy or tomentose hairs on the outside; peridiole (gleba) lens-shaped and attached to the peridium with a thread-like cord (funiculus); basidiospore hyaline, smooth and mostly with a thick wall, while the shape and size vary (Lloyd, 1906; Brodie, 1975; Miller and Miller, 1988). Two other allied genera are *Nidula* V.S. White and *Crucibulum* Tul. & C. Tul. *Nidula* differs from *Cyathus* by its lack of a funicular cord with the peridiole. *Crucibulum* has a single-layered peridium and white to yellowish peridioles, while those are black to dark brown in *Cyathus*, as mentioned by Miller and Miller (1988), Zhao et al. (2007), and Das and Zhao (2012). *Cyathus* is the genus with the most species of bird's nest fungi and is represented by ca. 45 species worldwide (Brodie, 1967, 1975; Kirk et al., 2008), 14 of which are from India (Brodie, 1974; Thind et al., 1984; Thind, 2005; Das and Zhao, 2012, 2013). The molecular phylogeny of this genus was conducted by Zhao et al. (2007) based on ITS and LSU sequences from type and authentic specimens. The research indicates that the genus is monophyletic and includes three infrageneric groups recognizable by morphological characters.

Located on the west bank of the River Ganges in Shibpur (Howrah), the Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIG) covers an area of

about 110 ha. It preserves more than 12,000 trees, shrubs, and climbers, representing one of the best collections of native and exotic plants, including a rich collection of bamboos. Recently, while undertaking a routine survey of macrofungi during the premonsoon and monsoon periods in this historical garden, the authors came across an interesting member of bird's nest fungi in its bambusetum. A thorough macro- and micromorphological examination of this sample, followed by a literature review, revealed this taxon as an undescribed species. The molecular approach was also tried on this interesting specimen, however, our repeated efforts at sequencing were unsuccessful; its molecular phylogeny could not be determined. A detailed description, coupled with supporting illustrations of the species, is provided in the current paper. We named the species *Cyathus thindii* sp. nov.

### 2. Materials and methods

Macromorphological/field characterization was conducted on fresh basidiomata. Field photographs of these basidiomata were taken with the aid of a Nikon D300s and an Olympus C-5060. Color codes and terms are (mostly) from the *Methuen Handbook of Colour* (Kornerup and Wanscher, 1978). After recording the macromorphological characters, the basidiomata were dried with the help of a wooden drier.

In the laboratory, macromorphological characters were again observed in both fresh and dry samples with the help

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of a stereo zoom dissecting microscope, Nikon SMZ 1500; photographs were taken through the attached dedicated camera. Micromorphological characters were noted with the aid of a light microscope, Olympus CX 41, from the free hand sections of the dry samples mounted in a mixture of 5% KOH and phloxin, 30% glycerol, lactophenol cotton blue, and Melzer's reagent. Spore measurements are recorded based on those of twenty basidiospores. Spore-measurement and quotient indicating length–width ratio ( $Q = L/W$ ) are presented as minimum–mean–maximum. The herbarium name is after Holmgren et al. (1990). A distribution map was created with the aid of Google Earth (www.google.com/earth).

### 3. Results and discussion

*Cyathus thindii* K. Das, Hembrom, A. Parihar & R.L. Zhao sp. nov.

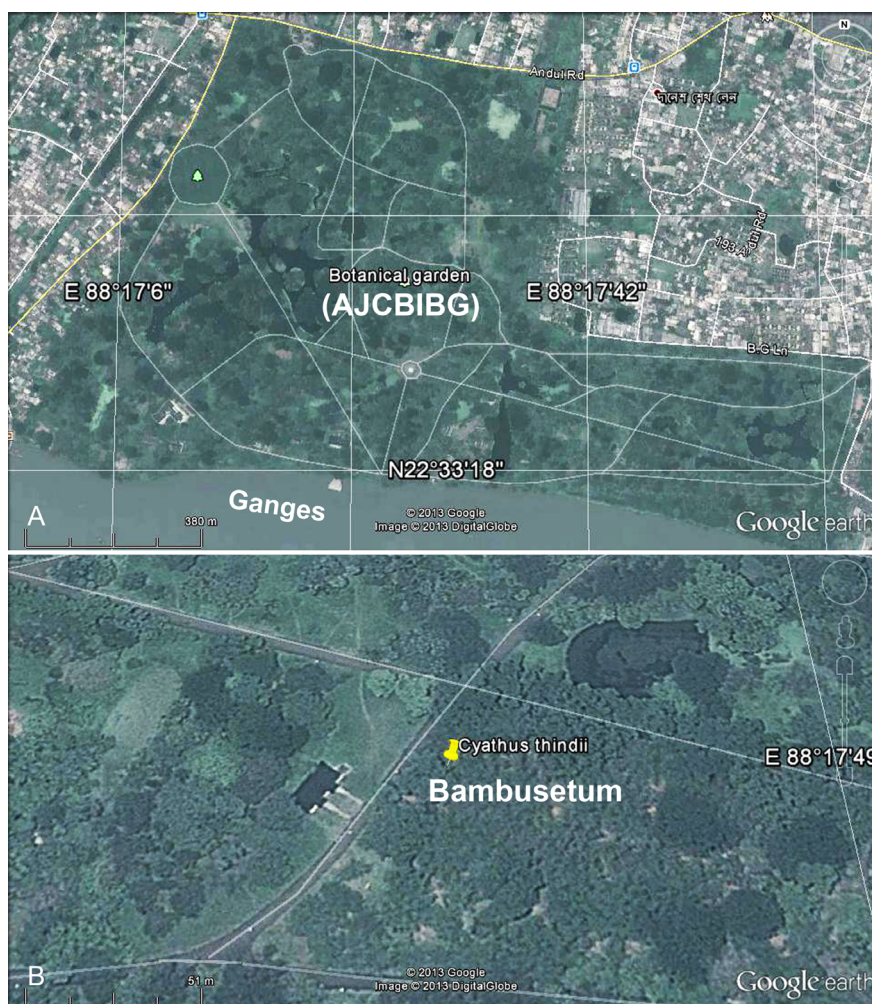
Figure 1, Figure 2, & Figure 3

Mycobank: MB 807040

**Etymology:** in recognition of Prof KS Thind for his invaluable contribution to Indian mycoflora.

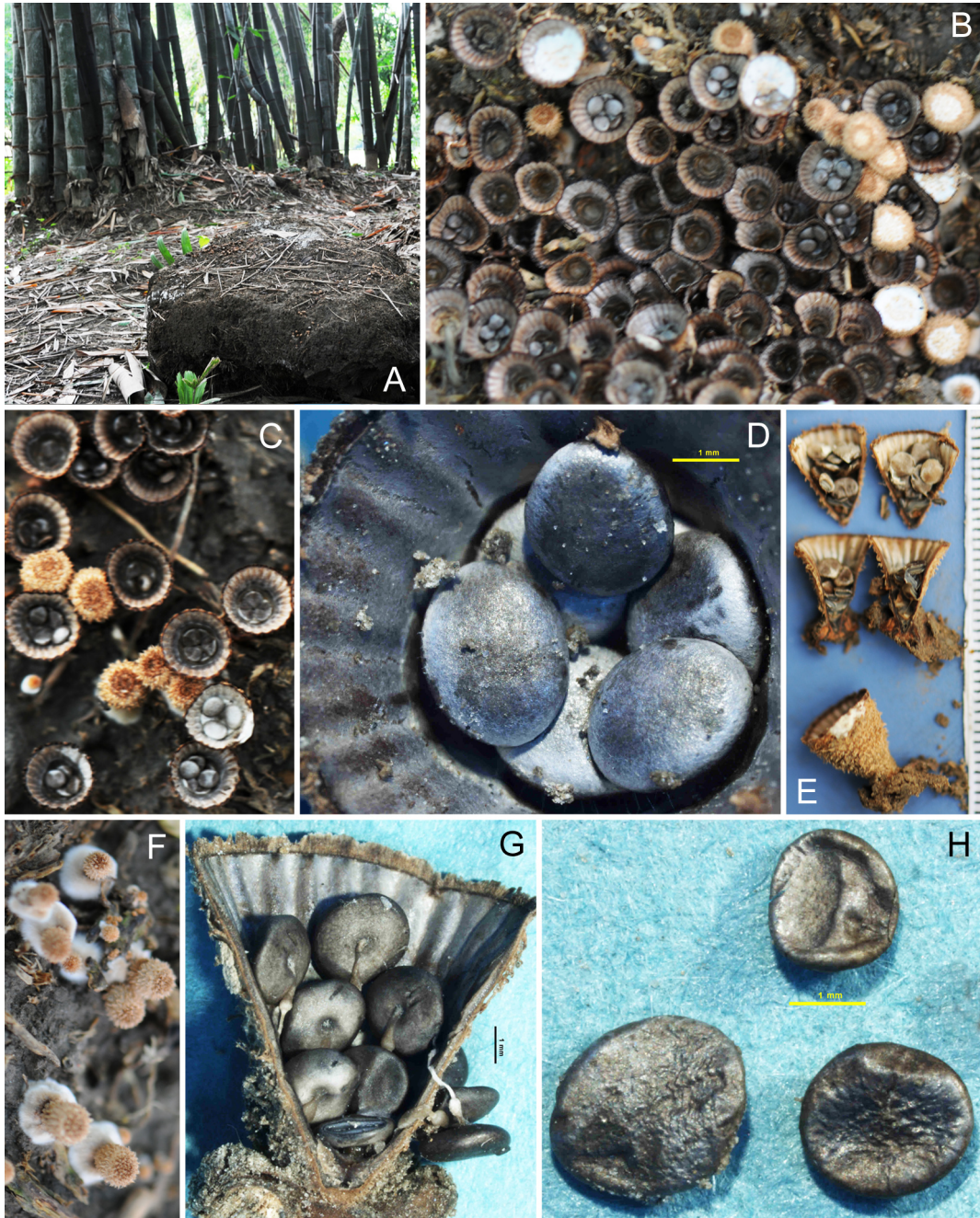
**Type:** India, West Bengal, Howrah, Acharya Jagadish Chandra Bose Indian Botanic Garden, alt. 5 m, 4 June 2013, N22°33'19.80" E88°17'45.42", K. Das, M.E. Hembrom & A. Parihar, KMA 13-011 (**Holotype**, CAL 1147).

**Basidiomata** (peridia) 7–9.5 mm high and 5–9 mm wide across the mouth, sessile, strongly infundibuliform or inverted bell-shaped with a narrow tapering base attached to substratum by a white mycelial pad (basal emplacement) that gradually turns pompeian yellow (5C6) to brown when mature. Exterior wall of the peridium with pyramidal to spinoid hairs in tufts (0.5–1 mm long), plicate up to 1/3 of the length of peridium from mouth, plicae becoming visible when hairs are shed off, apricot (5B6) overall; mouth fimbriate. Interior wall of the peridium longitudinally plicated, smooth, shiny, lead-colored. Epiphragm membranous, disappears with maturity, white (A1). Peridioles 15–17 in number, 1.5–3



**Figure 1.** A. Geographic position of Acharya Jagadish Chandra Bose Indian Botanic Garden; B. Location of *Cyathus thindii* (KMA 13-011) in the bambusetum of AJCBIBG. Map created with the help of Google Earth (www.google.com/earth).





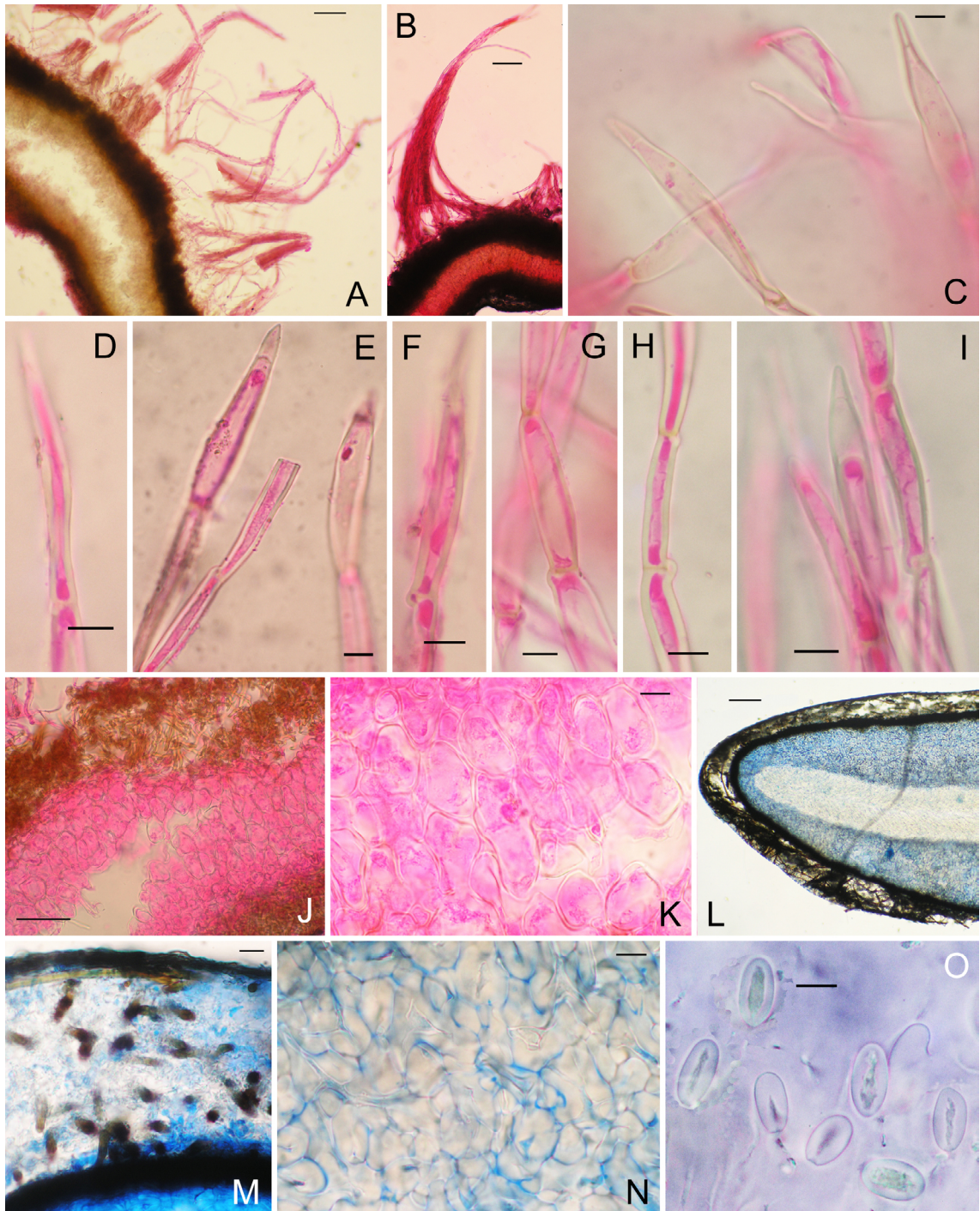
**Figure 2.** *Cyathus thindii* (KMA 13-011): A. Habitat showing soil amongst bamboos; B & C. Mature and immature basidiomata; D. Top view of peridium showing peridioles; E & G. Longitudinal section through peridium showing peridioles and funicular attachments; F. White mycelial pad associated with young basidiomata; H. Gray peridioles. Bars: D, G, & H = 1 mm.

mm in diameter, biconvex to lenticular, attached to the interior of the peridium with a cord (funicular attachment) originated from a distinct depression in the ventral surface, greenish gray (30D2) to snuff (5F6) or chocolate (6F4) mostly with metallic sheen, sometimes with white patches on outermost peridioles; surface rough to rugulose or irregularly wrinkled; tunica absent; funicular cord 2–3.5

mm long, threadlike, with a distinct swollen bulbous node at the apical half. Pseudostipe short, 1–1.3 mm long.

Peridium wall three-layered: outer, middle, and inner. Outer layer (without hairs) up to 100  $\mu\text{m}$  thick, densely hyphal, brown; hyphae 2.8–4  $\mu\text{m}$  broad, erect to suberect, thick-walled, wall up to 1.1  $\mu\text{m}$  thick, subhyaline to mandarin orange (6B8). Hairs in tufts 280–850  $\mu\text{m}$





**Figure 3.** *Cyathus thindii* (KMA 13-011): A & B. Transverse-section of peridium wall showing tuft of hairs; C-I. Terminal and subterminal thick-walled encrusted cells of peridial hairs; J. Transverse-section of peridium wall showing three layers; K. Middle layer of peridium showing pseudoparenchymatous cells; L. Transverse section of a peridiole showing cortex, subcortex and hymenium; M. Cortex of peridiole in transverse section; N. Subcortex of peridiole in transverse section; O. Hyaline basidiospores. Bars: A, B, & L = 100  $\mu$ m; C-I, K, M-O = 10  $\mu$ m; J = 50  $\mu$ m.

high, hyphal, hyphae 6–11  $\mu$ m wide, repeatedly septate, encrusted, clamped, thick-walled (wall up to 2.5  $\mu$ m thick), brown; terminal cell 20–75  $\times$  6–11  $\mu$ m, fusoid to

lanceolate. Middle layer, up to 150  $\mu$ m thick, composed of densely packed pseudoparenchymatous cells; cells 9–60  $\times$  7.5–45  $\mu$ m, globose to oval, elongated or irregular, wall



slightly thick (up to 0.7  $\mu\text{m}$ ), brown, pink in phloxin. Inner layer hyphal; hyphae up to 4  $\mu\text{m}$  wide, densely interwoven, thick-walled (1–3  $\mu\text{m}$  thick), aseptate, unbranched, subhyaline to brownish. Peridiole three layered: cortex, subcortex, and hymenium. Cortex two layered: exocortex and endocortex. Exocortex 9–10  $\mu\text{m}$  thick, hyphal, dark brown; hyphae mostly parallel, thin-/slightly thick-walled (up to 0.8  $\mu\text{m}$ ), unbranched, aseptate. Endocortex up to 15  $\mu\text{m}$  thick, hyphal, dark brown; hyphae aseptate, thick-walled. Area between exo- and endocortex up to 85  $\mu\text{m}$  thick, with loosely interwoven brown to dark brown branched aseptate hyphae, hyphae up to 6  $\mu\text{m}$  wide and thick-walled (up to 2.5  $\mu\text{m}$ ). Subcortex up to 170  $\mu\text{m}$  wide, convoluted, hyphal, subhyaline; hyphae gelatinous, aseptate, very thick-walled (wall up to 5  $\mu\text{m}$  thick) with narrow lumen. Hymenium up to 180  $\mu\text{m}$  wide, composed of hyphae and spore mass; hyphae hyaline. Spore 10–12.9–16.2  $\times$  7–7.7–10  $\mu\text{m}$ , broadly ellipsoid to cylindrical ( $Q = 1.27\text{--}1.67\text{--}2.10$ ), thick-walled, hyaline, inamyloid.

### 3.1. Specimens examined

India, West Bengal, Howrah, AJCBIBG, alt. 5 m, 4 June 2013, N22°33'19.80' E88°17'45.42', on dry rootlets of Bamboos, Bambusetum, K. Das, M. Hembrom & A. Parihar, KMA 13–011 (Holotype: CAL 1147).

### 3.2. Notes

Present species is fairly common in AJCBIBG. The combination of macro- and micromorphological characters, like sessile inverted bell-shaped basidiomata, a membranous epiphragm that disappears with maturity, a three-layered peridial wall, 15–17 biconvex to lenticular gray to brown (mostly with metallic sheen) peridioles attaching through a funicular attachment to the interior wall of the peridium, and ellipsoid to cylindrical hyaline

basidiospores place this taxon under the genus *Cyathus* Haller (Bottomley 1948; Brodie, 1975).

*Cyathus thindii* is distinguished by a combination of characters like, sessile basidiomata that grow on a white mycelial pad, plicate exterior (partly) and interior wall of peridium, large peridioles (1.5–3 mm) without tunica, elongate ellipsoid, medium sized spores (10–16.2  $\times$  7–10  $\mu\text{m}$ ,  $Q = 1.64$ ). Its association with the rootlets of dead bamboos can also be a possible biological feature of the species.

With plicate peridium and long ellipsoid spores, this new species is quite close to some known *Cyathus* spp. that also have those characters. They are *C. montagnei* Tul. & C. Tul., *C. limbatus* Tul. & C. Tul., *C. renweii* T.X. Zhou & R.L. Zhao, *C. striatus* (Huds.) Willd., and *C. poeppigii* Tul. & C. Tul. However, *C. striatus*, which is the type species of this genus, could be distinguished from *C. thindii* in the field by its much darker colored, long, shaggy hairs at the exterior of the peridium and the absence of a mycelial pad or basal emplacement (Pegler et al., 1995; www.mycoweb.com). *Cyathus montagnei*, *C. poeppigii*, and *C. renweii* have larger spores (15–25  $\times$  10–20  $\mu\text{m}$ ; 25.4–47  $\times$  15–24.13  $\mu\text{m}$ , and 21–31  $\times$  10.5–13  $\mu\text{m}$ , respectively) than those of *C. thindii* (Baseia and Milanez, 2001; Zhou et al., 2004; Cruz et al., 2014). *Cyathus limbatus* is the species that is most similar to this new species in morphology because both of them have brown, reddish brown, dark brown exterior of the peridium, and light colored peridium inner surface. However, the basidiomata of *C. limbatus* are often incurved at the mouth, and the spores (16–21  $\times$  11–15  $\mu\text{m}$ ) are bigger than those of *C. thindii* (Brodie, 1975; Thind et al., 1984). More details about the morphological comparison of those species are given in the Table.

**Table.** Morphological comparison of *Cyathus thindii* sp. nov. with four allied species.

Morphological features	<i>Cyathus montagnei</i> (Baseia and Milanez, 2001 & Trierveiler-Pereira et al. 2013)	<i>C. limbatus</i> (Thind et al., 1984)	<i>C. renweii</i> (Zhao et al., 2004)	<i>C. poeppigii</i> (Baseia and Milanez, 2001 & Cruz et al., 2014)	<i>C. thindii</i> sp. nov.
Basidiomata (Peridium)	7–8 mm high and 6–7 mm wide at mouth; exterior and interior surface plicate. Exterior surface brown, interior surface dull grayish (silvery)	Up to 9 mm high and 8 mm wide at mouth; exterior and interior surface plicate. Exterior surface brown, interior surface gray	8–10 mm high and 5–6 mm wide at mouth; exterior and interior surface plicate. Exterior surface brownish, interior surface gray	6–8 mm high and 4–6 mm wide at mouth; exterior and interior surface plicate. Exterior surface yellowish brown interior surface olive gray to brownish gray	7–9.5 mm high and 5–9 mm wide at mouth; exterior and interior surface plicate. Exterior surface orange-brown (apricot), interior surface lead-colored
Mycelial pad (basal emplacement)	Present, dark brown.	Present, reddish brown.	Absent	Present, light brown.	Present, white becoming yellow then brown.
Peridioles	2–2.5 mm in diameter, lenticular, dark brown to black.	Up to 2 mm in diameter, orbicular, lenticular, deep brown to black.	2 mm in diameter, round, grayish.	2–2.5 mm in diameter, lenticular, dark brown to black.	1.5–3 mm in diameter, biconvex to lenticular, greenish gray to brown (chocolate) with a metallic sheen.
Tunica	Present	Absent	Present	Absent	Absent
Anatomy of peridioles	Not studied/reported	Subcortex up to 190 $\mu\text{m}$ wide, thick walled, lumen very narrow; hymenium up to 300 $\mu\text{m}$ wide	Not studied/reported	Not studied/reported	Subcortex up to 170 $\mu\text{m}$ wide, hyphae highly thick-walled (up to 5 $\mu\text{m}$ thick), with narrow lumen; hymenium up to 180 $\mu\text{m}$ wide
Basidiospores	15–25 $\times$ 10–20 $\mu\text{m}$	16–21 $\times$ 11–15 $\mu\text{m}$	21–31 $\times$ 10.5–13 $\mu\text{m}$	25.4–47 $\times$ 15–24.13 $\mu\text{m}$	10–16.2 $\times$ 7–10 $\mu\text{m}$

### 3.3. A provisional key to the 15 species of *Cyathus* in India

1. Spores mainly smaller than 15  $\mu\text{m}$  in every dimension .....2
1. Spores (all or the longest) larger than 15  $\mu\text{m}$  at least in one dimension .....8
  2. Interior wall of peridium ivory- to silver- or lead-colored .....3
  2. Interior wall of peridium never ivory- to silver- or lead-colored .....6
    3. Interior wall of peridium striate to plicate or fluted .....4
    3. Interior wall of peridium never striate or plicate .....5
      4. Exterior surface/wall of peridium brown .....*C. montagnei*
      4. Exterior surface/wall of peridium ivory-colored to pale pinkish buff .....*C. griseocarpus*
        5. Peridium large (up to 15 mm high, 6–12 mm wide); peridioles large (up to 3.5 mm diam.); mouth strongly flaring .....*C. olla*
        5. Peridium small (up to 7 mm high, 6 mm wide); peridioles comparatively small (up to 2 mm diam.); mouth mostly erect .....*C. colensoi*
      6. Spores always smaller than 5  $\mu\text{m}$  (at least in one dimension); exterior surface of peridium striate; interior surface of peridium neither sulcate nor plicate .....*C. microspores*
      6. Spores either 5  $\mu\text{m}$  or larger in any dimension; exterior surface of peridium never striate; interior surface of peridium sulcate or plicate .....7
        7. Exterior surface of peridium covered with strigose hairs, mouth margin entire, crenately lobed .....*C. hookeri*
        7. Exterior surface of peridium shaggy, hairs conical in tufts, mouth margin entire, even .....*C. novae-zelandiae*
    8. Tunica present .....9
    8. Tunica absent .....11
      9. Both exterior and interior surface of peridium concolorous, strongly plicate; peridioles conspicuously ellipsoid ( $2 \times 1 \mu\text{m}$ ) .....*C. ellipsoideus*
      9. Surface of peridium not as above; peridioles never ellipsoid .....10
        10. Interior surface of peridium brownish gray, strongly plicate, spore-wall very thick (up to 3.5  $\mu\text{m}$ ) .....*C. striatus*
        10. Interior surface of peridium silvery gray, smooth to faintly plicate near mouth, spore-wall comparatively thinner (up to 1.5  $\mu\text{m}$ ) .....*C. intermedius*
    11. Spores (at least in one dimension) more than 21  $\mu\text{m}$  .....12
    11. Spores (in any dimension) not more than 21  $\mu\text{m}$  .....13
      12. Exterior surface of peridium yellowish brown, both exterior and interior surface of peridium plicate to fluted .....*C. poeppigii*
      12. Exterior surface of peridium light brown, both exterior and interior surface of peridium smooth .....*C. stercoreus*
      13. Both exterior and interior surface of peridium plicate .....14
        13. Exterior surface of peridium never plicate, interior surface of peridium plicate .....*C. triplex*
        14. Mouth flaring; peridioles up to 2  $\mu\text{m}$  diam. ....*C. limbatus*
        14. Mouth erect; peridioles 1.5–3 mm diam. ....*C. thindii*

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