Cyathus striatus: a new record from Arunachal Pradesh and a checklist of Bird’s nest fungi in India

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Abstract
Twenty four species of Bird’s nest fungi belonging to four genera, namely Crucibulum, Cyathus, Nidula and Sphaerobolus have been reported from India with most of the reports were from Northeastern states in the Eastern Himalaya region that is well recognized for its rich biodiversity. Among these genera, Cyathus is the largest genus with 61 species documented so far in the world including 17 species from India. So far, only Cyathus poeppigii has been reported from the Eastern Himalayan state of Arunachal Pradesh. In the present paper, another newly recorded species Cyathus striatus, is reported with its detail taxonomic characteristics. It produces comparatively smaller basidiocarps than the earlier reported collection from Darjeeling (West Bengal). Further, the basidiospores are thin walled but comparatively larger in size. The paper also lists all Bird’s nest fungi from India with detailed information.

Key words – Agaricomycetes – Checklist – Cyathus – Eastern Himalaya

Introduction
The tropical and subtropical forests harbor a great fungal diversity and with integrative taxonomic approach many new taxa have been reported (Accioly et al. 2018). Basidiomycota, which is the second largest phylum after Ascomycota in the Fungal kingdom, has recently been revised in detailed notes and outline (He et al. 2019, Wijayawardene et al. 2020).

One of most beautiful group of Basidiomycota is Bird’s nest fungi which are distributed in six genera, namely Crucibulum, Cyathus, Mycocalia, Nidula, Nidularia and Sphaerobolus (Zhou et al. 2004, Poinar 2014, Cruz & Baseia 2014, Geml et al. 2015, Sharma 2016, Cruz 2017, He et al. 2019). These fungi do not have anamorphic state (Shinners et al. 1997) and were earlier placed in the family Nidulariaceae but have been presently transferred to the family Agaricaceae (Das & Zhao 2013). Except Sphaerobolus, other genera of this group that produce multiple peridioles have been placed in incertae sedis under the order Agaricales, Agaricomycetes (He et al. 2019). Taxonomic key of Bird’s nest fungi has been published by Das & Zhao (2013).

Crucibulum Tul. & C. Tul. (1844) is characterized by interior smooth and tawny yellow cup, simple funiculus and white peridioles. Seven species of Crucibulum have been recorded in the world viz. C. albosaccum, C. crucibuliforme, C. cyathiforme, C. laeve, C. parvulum, C. simile and C. vulgare. From India, only two species C. laeve and C. vulgare have been so far reported.

Genus Cyathus Haller (1768) is saprobic, grows on decaying wood and fertile soil, forming gregarious basidiomata, either cone, funnel or inverted bell shaped, up to 3 cm in height, and contains more than one peridiole, gray to black in colour, connected to a three layered peridium
with a funicular cord (Dorjey et al. 2013). It is the largest genus of Bird’s nest fungi with 61 recorded species (Accioly et al. 2018, He et al. 2019, Góis et al. 2020) with a maximum number reported from Brazil. The world revision of *Cyathus* has been published by Cruz (2017). According the Queensland Mycological Society key to Bird’s nest Fungi, both *Crucibulum* and *Cyathus* have funicular cord whereas *Mycocalia, Nidula* and *Nidularia* do not. Recent phylogenetic analysis placed *Cyathus* closer to *Cystoderma* of the family incertae sedis, Agaricales (Wijayawardene et al. 2020).

*Mycocalia* J.T. Palmer (1961) is a distinct genus of Bird’s nest fungi having globose to sub-globose basidiomata, hyaline to brown peridium, peridioles in hyaline gelatinous matrix, and cylindrical to ellipsoid, hyaline basidiospores. Currently, 7 species of *Mycocalia* have been recorded viz. *M. aquaphila, M. arundinacea, M. denudate, M. duriaeana, M. minutissima, M. reticulata* and *M. sphagnetii* (Index Fungorum 2021).

*Nidula* V.S. White (1902) consists of 7 species (Das & Zhao 2013, Poinar 2014), morphologically characterized by urn- to vase-shaped basidiomata containing lenticular brown peridioles, peridium 6-layered, mouth covered by a lid, tunica layer around the peridioles, basidiospores broadly ellipsoid to elongate, hyaline and smooth walled. Three species of this genus have been recorded from India viz. *Nidula candida, N. emodensis* and *N. shingbaensis*.

*Nidularia* Fr. (1817), is the type genus of the family Nidulariaceae. It is characterized by a soft, early deliquescent, pulverulent basidiomata surface, and peridium composed of spinose hyphae (Baseia & Milanez 2001). Like *Mycocalia, Nidularia* also lack epiphragm. Three species have so far been reported viz. *N. confluens, N. farcia* and *N. pulvinata* but none from India.

*Sphaerobolus*, described by Tode (1790), is a unique genus with a minute basidiomata containing single brown peridiole. Due to a distinct spore-dispersal mechanism, it is popularly called artillery fungus. Under this genus (currently placed under Geastraceae, Geastrales, Agaricomycetes; He et al. 2019), four species, namely *S. iowensis, S. ingoldii, S. stellatus* and *S. jaysukhianus* are accepted, out of which later two species are reported from India (Vasava et al. 2020).

In India, 17 Bird’s nest fungi distributed in three genera have been enlisted by Das & Zhao (2012) including 1 species of *Crucibulum*, 14 species of *Cyathus* and 2 species of *Nidula*. Thereafter, many species were added (Table 1) to these three genera and two more species to *Sphaerobolus* viz. *S. jaysukhianus* and *S. stellatus* (Das & Zhao 2013, Vasava et al. 2020).

**Table 1** Checklist of Bird’s nest fungi of India

| Name of the species | Substratum | Site | Reference |
|---------------------|------------|------|-----------|
| *Crucibulum laeve* (Huds.) Kambly 1936. | Unknown | Unknown | Das & Zhao (2012) |
| *C. vulgare* Tul. & C. Tul. 1844. | Unknown | Nilgiris (Tamila Nadu) | Butler & Bisby (1931) |
| *Cyathus. Colensoi* Berk. 1855. | Dead twigs and soil | Shimla hills (Himachal Pradesh), Siliguri (West Bengal), Phey village (Leh, Ladakh) | Yangdol et al. (2018) |
| *C. ellipsoideus* H.J. Brodie 1974. | Unknown | Chikmaglur (Karnataka) | Sharma (2016) |
| *C. gracilis* H.J. Brodie 1973. | Twigs | Sevoke (Siliguri, West Bengal) | Sharma (2016) |
| *C. griseocarpus* Brodie & B.M. Sharma 1980. | Dead twigs and soil | Ukhrul (Manipur) | Brodie & Sharma (1980) |
| *C. hookeri* Berk. 1854. | Unknown | Kollong rock (Khashia hills, Meghalaya) | Sharma (2016) |
| *C. intermedius* Tul. & C. Tul. 1844. | Unknown | Sibpur (near Calcutta, West Bengal), Manali (Himachal Pradesh) | Sharma (2016) |
### Table 1 Continued.

| Name of the species | Substratum | Site | Reference |
|---------------------|------------|------|-----------|
| *C. limbatus* Tul. & C. Tul. 1844. | Dead wood | Nongpoh (Khasi hills, Meghalaya) | Góis et al. (2020) |
| *C. microsporus* Tul. & C. Tul. 1844. | Unknown | Khasi hills (Meghalaya) | Sharma (2016) |
| *C. novae-zelandiae* Tul. & C. Tul. 1844. | Dead wooden log | Jatinga (Haflong, N.C. Hills, Assam) | Sharma (2016) |
| *C. olla* (Batsch) Pers. 1801. | Unknown | Ladakh | Dorjey et al. (2013) |
| *C. poeppigii* Tul. & C. Tul. 1844. | Soil and twigs | Bomdila & Nichifu (West Kameng, Arunachal Pradesh) | Das & Zhao (2012) |
| *C. renweii* T.X. Zhou & R.L. Zhao 2004. | Unknown | Basgo (Leh, Ladakh) | Yangdol et al. (2018) |
| *C. stercoreus* (Schwein.) De Toni 1888. | Soil and wild animal dung | Khasi hills (Meghalaya), Shoolpaneshwar Wildlife Sanctuary (Sagai, Gujarat) | Patel et al. (2018) |
| *C. striatus* (Huds.) Willd. 1787. | Soil and twigs | Darjeelin, (West Bengal) Emchi (Papum Pare, Arunachal Pradesh) | This study |
| *C. thindii* K. Das, Hembrom, A. Parihar & R.L. Zhao 2015. | Unknown | A.J.C Bose College (Howrah, West Bengal) | Cruz (2017) |
| *C. triplex* Lloyd 1906. | Soil and twigs | Baramulah (Agartala, Tripura) | Sharma (2016) |
| *C. montagnei* Tul. & C. Tul. 1844. | Unknown | Dehradun (Uttarakhand) | Sharma (2016) |
| *Nidula candida* Peck 1893. | Dead twigs of *Abies densa* | Between Hilley and Barsey (Sikkim) | Das & Zhao (2012) |
| *N. emodensis* (Berk.) Lloyd 1906. | Dead wood | Lachen (Sikkim) | Butler & Bisby (1931) |
| *N. shingbaensis* K. Das & R.L. Zhao 2013. | Dead twigs of *Abies densa* | Shingba Rhododendron Sanctuary, (North District, Sikkim) | Das & Zhao (2013) |
| *Sphaerobolus jaysukhianus* AM Vasava, RS Patel & KS Rajput 2020. | Cow dung | Ajwa Road (Vadodara, Gujarat) | Vasava et al. (2020) |
| *S. stellatus* Tode 1790. | Dead moss | Botanic garden (Saharanpur, Uttar Pradesh) | Butler & Bisby (1931) |

Note: **Bold** letters indicate new species, * Indicates molecular data available, Name of the province (State) of India has been put within bracket.

### Material & methods

Fresh basidiomata growing on dead and decaying wood were collected from the Doimukh, Papum Pare district, Arunachal Pradesh, and the macro-morphological characters were noted. Its surface was cleaned with 70% ethyl alcohol before microscopic examination. The samples were examined under stereo zoom microscope (Zeiss Stemi 508, Germany) to locate the structures and photographed with attached Axiocam ERc 5s digital camera. Free-hand sections of basidiomata was prepared and mounted in lactophenol-cotton blue solution. Detailed microscopic examination of fungal structures and microphotography was done under Zeiss Axio Lab. A1 microscope equipped with Axiocam Erc 5s digital camera. Size measurements were taken by ZEN-2012 imaging software version 8.0.0. Photographic plates were prepared using Adobe Photoshop version 7.0. Morphological identification was performed by referring to the outline and notes for Basidiomycota (He et al. 2019, Wijayawardene et al. 2020). Herbarium samples were deposited in the fungal herbaria of the department. Facesoffungi number was registered as described in Jayasiri et al. (2015).
Taxonomy

**Cyathus striatus** (Huds.) Willd. 1787

Index Fungorum number: IF211223; Facesoffungi number: FoF09811

Saprobic on decaying wood. Teleomorph: **Basidiomata** 6–8 × 5–6 mm diameter at mouth, narrowing towards base, short stalk, scattered to gregarious, infundibuliform with hyaline puffy, basal brown to dark brown apical ends. **Epiphragm** hyaline and rupturing at maturity. **Stipe** attached to wood, cross section revealing three layers, outer one bearing pale brown hair like structures, Exoperidium consisting of wooly trimitic hyphae in which generative hyphae hyaline with or without clamp connections, frequently septate and branched; binding hyphae 2.6–3.3 μm wide, hyaline to pale brown, distantly septate with clamp connections; skeletal hyphae 2.5–3.2 μm, thick walled, brown without septa and rarely branched. **Peridium** >164.5 μm and in the middle >153.8 μm thick, consisting of three different layers. Outer wall conspicuously plicate, tomentose, arranged in regular flexible tufts or fibrose hyaline to pale brown hyphae, 4.7–5.8 μm wide, rarely branched, apical cells ovoid with apiculate ends, middle wall hyaline to pale brown, tightly packed textura intricata hyphae, 2.6–2.8 μm, highly branched, inner wall gray to brown textura, epidermoid tissue. **Peridioles** 1–1.3 × 0.2–0.3 mm, 10–12 per basidioma, circular to elliptical, surface smooth to wrinkled, sticky in nature, coated with thin mucilaginous gel when moist. Funiculus present, **Funiculi** 3.8–5.1 μm, pale yellowish in colour, composed of mycelial cords branching but without septa. Peridiole three layered, covered with thin tunica >15.5 μm thick; Exocortex brown to black, 7.8–12.0 μm, endocortex 40–142 μm wide and hyaline, hymenium 81–86 μm wide consisting of branched hyphae 1.1–2.5μm wide. **Basidiospores** 13.5–19.0 × 9.6–12.0 (13.4) μm (X = 16.4 × 10.8, n=25), ellipsoid, thick and smooth walled (2.4–3.3 μm), apiculated sometimes, hyaline when immature, becoming sub-hyaline at maturity and become smaller, borne on irregularly scattered basidia in hymenium. Anamorph undermined.

Known distribution – Throughout the world.

Material examined – INDIA, Arunachal Pradesh, Papum Pare, Doimukh, 27°08’22.1”N 93°46’07.1”E on 18 September, 2020, on decaying wood, collected by Naniya Takha, identified by Niranjan. M. Specimen voucher number: ARFR-151, deposited in Department of Botany, Rajiv Gandhi University, Arunachal Pradesh.

Notes – Das & Zhao (2012) provided the key to Indian *Cyathus* species that are mainly differentiated by the number of peridial layers. Present specimen contained three layered peridium, peridiole covered with tunica, basidiospores longer than 15 μm, and is similar to *C. ellipsoideus*, *C. intermedius* and *C. striatus*. Further, based on morphological characters, the collected specimens shared similar Basidial and Peridial characters with *C. striatus*. However, in comparison to the previous report (Sharma 2016),the basidiospores of the specimen of *C. striatus* in our collection were slightly larger (13.5–19.0 × 9.6–12.0 (13.4) vs. 11–18 × 9–12 μm) but with thinner wall (2.4–3.3 vs. ~3.5 μm). Therefore, the key morphological characters do ensure that it fits into *C. striatus*, and this species has been recorded for the first time in Arunachal Pradesh.

Discussion

Twenty four species of Bird’s nest fungi belonging to four genera have been recorded so far from India (*Crucibulum*: 2 species, *Cyathus*: 17 species, *Nidula*: 3 species, and *Sphaerobolus*: 2 species). *Crucibulum* has been reported from the state of Tamil Nadu whereas *Sphaerobolus* from Gujarat and Uttar Pradesh. The highest number of species of *Cyathus* has been recorded in India with several species found in Northeastern region of the country that lies within the Eastern Himalayas. *C. striatus* was reported for the first time in Northeastern region from Darjeeling (West Bengal) and second time in the present study from the state of Arunachal Pradesh. Nevertheless, the specimens in our collection are with comparatively smaller Basidioma containing slightly larger but thin walled basidiospores in comparison to the said previous record. Three species of *Nidula* have been recorded, all from the state of Sikkim in the Eastern Himalayas, thus reflecting its narrow geographical distribution in India.
Fig. 1 – *Cyathus striatus* (ARFR-151). a, b Basidiomata. c Endoperidium. d Exoperidium. e Immature basidia. f Stipe associated hyphae. g Stipe section. h-j Peridial layers. k Peridioles with funiculi. l, m Cross section of peridiole. n Funiculus hyphae. o Sub-cortex section of peridiole. p-q Basidiospores. r Basidiospores. Scale bar: c, g = 3000 μm, d-e = 2000 μm, l-m = 40 μm.

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