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Discovery of a rare stiped puffball *Calostoma junghuhnii* in Neora Valley National Park, India: A new record for West Bengal, India

SHUVADEEP MAJUMDAR[1], BUDDHA SINGH RAI[2], AJIT RAI[2], UJJAL GHOSH[3], SILANJAN BHATTACHARYYA[4], ANIMESH BOSE[5], NISHA GOSWAMI[6], DYUTIPARNA CHAKRABORTY[7], JIT SARKAR[8], SUDIPTA MUKHERJEE[8,9], KRISHNENDU ACHARYA[8], PRAKASH PRADHAN[8,9,♥]

[1]Parimal Mitra Smriti Mahavidyalaya, Department of Botany. Mal, Jalpaiguri, PIN-735 221, W.B., India
[2]Neora Valley National Park, Gorumara Wildlife Division North. Jalpaiguri, PIN-735 101, W.B., India
[3]O/o Additional Principal Chief Conservator of Forests, North Bengal. Upper Nivedita Road, P.O. Siliguri, Darjeeling, PIN-734 403, W.B., India
[4]Department of Zoology, West Bengal State University, Berunanpukuria. P.O. – Malikapur, Barasat, North 24 Parganas, PIN-700 126, W.B., India
[5]Himalayan Nature and Adventure Foundation, Saibal Mansion. 16 Hill Cart Road, opp. SBI, Siliguri, PIN-734 001, W.B., India
[6]O/o Divisional Forest Officer, Burdwan Division. P.O. and District Burdwan, PIN-713 104, W.B., India
[7]Botanical Survey of India. Howrah, PIN-711 103, W.B. India
[8]Department of Botany, Taraknath Shiksha Prangan, University of Calcutta. 35, Ballygunge Circular Road, Kolkata, PIN-700 019, W.B., India
[9]West Bengal Biodiversity Board, Department of Environment, Government of West Bengal. Prani Sampad Bhavan, 5th Floor, Salt Lake City, Sector-III, Kolkata, PIN-700 106, W.B., India. Tel.: +91 7908106464, ♥email: shresthambj@gmail.com

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Abstract. *Majumdar S, Rai BS, Rai A, Ghosh U, Bhattacharyya S, Bose A, Goswami N, Chakraborty D, Sarkar J, Mukherjee S, Acharya K, Pradhan P. 2022. Discovery of a rare stiped puffball Calostoma junghuhnii in Neora Valley National Park, India: A new record for West Bengal, India. Asian J For 6: 9-14*. The varied topography, soil, and climate of West Bengal, India, make conducive conditions for nurturing the magnificent diversity of macrofungi and their habitats. During an exploration of macro-fungal diversity in Neora Valley National Park, West Bengal, India, in 2021, basidiocarps of *Calostoma* (Calostomataceae) were collected and photographed, along with macro-morphological and ecological notes were taken. The specimens were identified through macro and micro-morphological characters following standard literature. Amplified description of *Calostoma junghuhnii* (Schltdl. & Müll. Berol.) Massee, along with necessary figures and photographs, is presented in this paper. A literature survey revealed that *C. junghuhnii* is a new record for West Bengal. The outcome of the present study would enrich data related to the macro-fungal diversity in the state of West Bengal, India.

Keywords: Annual biodiversity assessment camps, Basidiomycota, Calostomataceae, Neora Valley National Park, UNESCO

INTRODUCTION

Neora Valley National Park (NVNP), under the jurisdiction of Gorumara Wild Life Division, Jalpaiguri, West Bengal, India, comprises around 160 km^2 of pristine late-successional forest with a rich repository of biodiversity in the Eastern Himalayan landscape. That place was one of the components of Global Biodiversity Hotspots (Myers et al. 2000). Besides, NVNP has been included in the tentative list of UNESCO World Heritage Sites due to its superlative natural phenomena or areas of exceptional natural beauty, aesthetic importance, and significant natural habitats for in-situ conservation of biological diversity, including those containing threatened species of outstanding universal value from the point of view of science or conservation (UNESCO 2022).

The altitudinal gradient of NVNP is 180 m to 3200 m, incorporating various forest habitat types such as Subtropical Mixed Broadleaf Forests, Lower Temperate Evergreen Forests, Upper Temperate Mixed Broadleaf Forests, and Rhododendron Forest. Besides being a catchment area for tributaries of the Teesta and Jaldhaka Rivers, NVNP nurtures rich biodiversity through its virgin terrain and spatial continuum with some of the national (Pangolakha Wildlife Sanctuary in Sikkim) and the international (Toorsa Strict Reserve of Bhutan) protected areas.

The temperate forests in NVNP are found at elevations where moisture tends to condense and spectacularly remains in the air during the warm moist season. It is one of the species-rich but less explored temperate forests worldwide. These forests are dominated by evergreen broadleaved trees like *Quercus* in the lower reaches from about 2000-2500 m and mixed conifers like *Taxus*, *Tsuga*, and winter deciduous broadleaved species *Acer*, *Betula*, and *Magnolia* in the upper reaches. In addition, the drier south-facing slopes support extensive stands of arboreal *Rhododendrons*. Besides fauna, these forests support a rich diversity of epiphytic, parasitic, ectomycorrhizal, and mycoheterotrophic plants and diverse members of macrofungi and myxomycota (Pradhan 2015; Yonzone 2019; Ghosh et al. 2021).

Regarding biodiversity surveys, Dr. D. Brandis, the then Inspector General of Forests, India (1862), was the first to highlight the importance of conservation of the forest area under NVNP jurisdiction; even Dr. Brandis's proposal was crucial to frame NVNP's existence which came to effect in December 1992. The NVNP, since then, has been explored by organizations of repute like the Botanical Survey of India and the Zoological Survey of

India. Still, the first major systematic study on NVNP was conducted during 2019-2021 through a series of Annual Biodiversity Assessment Camps (ABACs), organized by O/o CCF Wildlife North, Directorate of Forests, Government of West Bengal, with the participation of more than 20 experts on various biodiversity components.

Macrofungi – the group of fungi with visible reproductive structures are an important component of biodiversity in the natural ecosystem. Among multifarious functions, they are the primary agents of decomposition, ectomycorrhizal partnerships with host trees, as well as hardwood, and insecticide parasitism. Macrofungi that produce dust-like spores inside their globose peridial fruitbodies and the spores are released by mature fruitbodies on bursting or external impact in the form of cloud or smoke are informally called gasteroid fungi or puffball fungi. *Calostoma* is a gasteroid genus of 30 species of macrofungi in the suborder Sclerodermatineae (Mycobank 2022). They have a worldwide distribution, including eastern North America, Asia, and Australasia (Massee 1888; Kobayasi 1962). Their somewhat beautifully netted ornamentation of the exoperidium and the presence of bright-colored ostioles resembling human lips wearing lip color have garnered the cognomen of "prettymouths" (World Heritage Encyclopedia 2021). In addition, they are reported to be ectomycorrhizal with the members of Fagaceae (Wilson et al. 2012) and play an important role in the sustenance of temperate deciduous forests.

During the field study of the 5th ABAC (March 2021), specimens of *Calostoma junghuhnii* (Schltdl. & Müll. Berol.) Massee was found between the stretch of the Public Health Engineering (PHE) protection camp and the Alubari protection camp, whose descriptive notes were presented in the present paper.

MATERIALS AND METHODS

Collections were made on 12 March 2021, during the 5th Annual Biodiversity Assessment Camp at Neora Valley National Park, West Bengal, India, en route to the Alubari protection camp from the PHE protection camp. During fieldwork, digital photographs of the samples were taken in their habitat, and their macro-morphological and habitat features were noted. In addition, during the work tenure, a standard identifying protocol, color photographs, and macro-morphological features of each specimen were taken in the field. Next, each collection was wrapped with tissue paper and kept in separate boxes to avoid contamination/ spore mixing. Finally, the collected specimens were carefully withered (separately) in a hot air drier until the moisture was minimal.

Light microscopic features were observed with a Dewinter Smart microscope from thin handmade sections of the dried basidiocarps by staining with Congo Red. In addition, basidiospores were observed in KOH, Melzer's reagent, Congo Red, and Cresyl Blue. Light microscopic photographs were taken with the rear camera of MI A2 mounted upon a microscope bracket clip. Scanning Electron Microscopic studies were conducted in the Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, Kolkata using a Zeiss EVO-MA10 Scanning Electron Microscope.

Forty measurements of basidiospores were taken from each sample to calculate the dimensions of basidiospores. Basidiospore (L & B), Spore ornamentation, and spore wall dimensions include (lowest observed value) 5th percentile value-95th percentile value (highest observed value) following Tulloss and Yang (2021). Mean values are followed by ± standard deviation. Basidiospore Length/breadth ratio denotes the Q value. The mean Q value (Qm) was measured by dividing the sum of the Q value by the total number of spores observed.

Specimens were identified using published literature (Kobayasi 1962, 1965; Chen and Yeh 1975; Massee 1888; Miller and Cotter 1988; Das 2009). Kornerup and Wanscher (1978) were followed for color terminology, while hex color code was used especially for the color of the Ostiole. The voucher specimens were preserved following Pradhan et al. (2015) and were deposited in the Calcutta University Herbarium (CUH), Kolkata, India.

RESULTS AND DISCUSSION

***Calostoma junghuhnii* (Schltdl. & Müll. Berol.) Massee (Figures 1 and 2)**

Basidiomycota, Calostomataceae

Macroscopic observations

Fruitbody gastroid with distinct stipe; solitary or in the group. Exoperidium globose to sub-globose, 9-17 mm × 8.48-16.33 mm, ground color 5A4-5B4, to 8D6 in eroded areas, especially towards Ostiole. With tetra-pentagonal lattice-like reticulations, reticulations are more prominent in the upper 2/3rd exoperidium, smaller warts are present throughout the reticulation ridges, and warts at the center of reticulation are larger and more dehiscent than warts of reticulation ridges. Ostiole burnt sienna-cinnabar (#ea6d56 -#e2604d), with 5-6 sutures, initially covered with warts. Warts 1A4, sometimes with a tint of 29B8-29C8 due to adhesion of bryophytes, pyramidal, more specifically turtle shell-shaped, upper ½ of larger warts with 6C4 coloration. Mesoperidium was not observed. Endoperidium globose, attached to the exoperidium at Ostiole only. Base gelatinous, rhizoidal, 10E7-10D8, moist, made of mycelial interwoven strands, 20-24 mm long, 17-20 mm broad, tapered towards exoperidium attachment in some specimens, while in others swollen in the middle and tapered towards both apex and base.

Microscopic observations

Warts are composed of a hyaline gelatinous matrix, with intermittent hyphae of two types (i) broad, thin-walled congo red (-) hyphae, 12.95-16.49 µm broad (Figure 2A), (ii) slender, filamentous, branched with clamp connections, congo red (+) 3.53-4.71 µm broad (Figure 2B), both hyaline in KOH. Ostiole composed of a hyaline gelatinous matrix with (i) hyaline, branched intermittent hyphae, with swollen base, 3.53-3.77 µm broad (Figure 2C), (ii) hexagonal rose-colored (#de8877-#9c161d) cells, 9.42-

23.55 × 6.59-17.66 µm in size (Figure 2D-F), sometimes with the central vacuole, surface radial, ridges arising from the six angles meeting at the central point. Exoperidium is composed of a hyaline gelatinous matrix (Figure 2G), with intermittent 3.06-3.53 µm broad branched, septate, congo red (+) hyphae with clamp connections (Figure 2H); hyphal granules present in intermitted hyphae but not prominent like that of the stipe. Endoperidium is composed of a hyaline gelatinous matrix, with (i) intermittent hyaline branched, septate hyphae, branches arising near the septum, clamp connections present, hyphal granules absent (Figure 2J), (ii) capillitial threads translucent 3D4-4D4 in color, filamentous, with elongated-curved to elliptical cells 11.30-25.91 µm long × 4.47-4.71 µm broad (Figure 2K). Stipe hyphae are composed of a hyaline gelatinous matrix, with intermittent 3.53-4.71 µm broad, branched hyphae, septate with clamp connections, and brownish granular content (incrustations), septa few, many intermittent hyphae connected in 'H' shape (Figure 2I). Basidiospores (Figure 2L-P) whitish in mass, basidiospore dimension (without ornamentation) (8.54) 9.16-14.48 (16.64) µm × (8.48) 8.81-13.36 (16.33) µm, L_{mean} = 11.73±1.50 µm, B_{mean} = 11.38±1.39 µm, Q = (1) 1.01-1.09 (1.10), Q_{av} = 1.03±0.03, globose to partly subglobose, aguttulate, inamyloid, hyaline to partly refractive in KOH, cyanophilous with Cresyl Blue, metachromatic reaction absent, spore wall more congophilous than ornamentation. Spore ornamentation warty, warts mostly pyramidal, (0.73) 1.01-4.06 (4.22) µm high, mean ornamentation height = 2.88±1 µm, made of 18-30 hyaline longitudinal strands (coremium) arising from spore wall, conjoint at apex, apex obtuse, the base of warts nearly ovate. Spore wall thick when immature, spore wall thickness = (0.46) 0.57-1.69 (1.76) µm, mean spore wall thickness = 1.17±0.34 µm, a wall with the uneven inner wall when a spore is immature and even in mature spores.

Known distribution

Nepal, India, S.E. China, Bhutan, Java, Taiwan, Japan, East Indies (Balfour-Browne 1955; Chen and Yeh 1975; Massee 1888; Miller and Cotter 1988; Das 2009).

Material examined

India, West Bengal, Kalimpong District, Neora Valley National Park, Upper Neora Range, Towards Alubari, 27.10545º N and 88.71894º E, elevation 2446 m., Basidiocarps ectomycorrhizal, growing solitary-caespitosely at the floor of a mixed stand of *Quercus lineata* Blume and *Quercus lamellosa* Sm., 12 March 2021, S. Majumdar, P. Pradhan, B.S. Rai, A. Rai, (Calcutta University Herbarium, CUH AM728).

Figure 1. Fruitbodies of *Calostoma junghuhnii* recorded from Neora Valley National Park, India

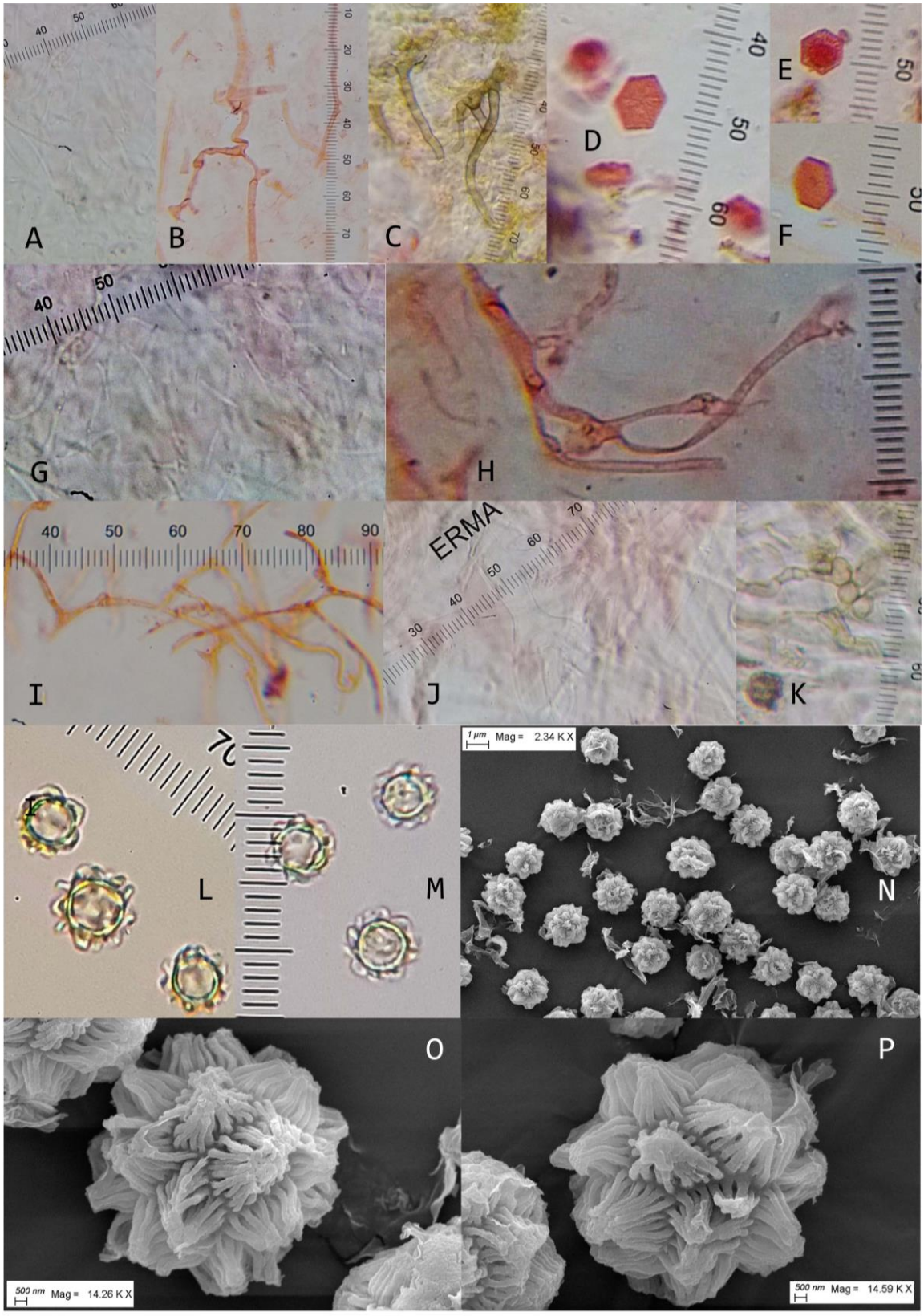

Figure 2. *Calostoma junghuhnii*. Warts (A & B): A = hyaline gelatinous hyphae, B = slender, filamentous, branched hyphae; Ostiole (C-F): C = hyaline, branched hyphae in gelatinous matrix, D,E,F = hexagonal #de8877 - #9c161d coloured cells; Exoperidium (G & H): G = Hyaline gelatinous matrix of exoperidium, H = branched hyphae of exoperidium with clamp connections; Stipe (I): I = branched incrustation hyphae on gelatinous matrix with clamp connections; Endoperidium (J & K): J = Hyaline cells of endoperidium, K = Capillitial threads; Basidiospores (L-P): L-M = micrographs of basidiospores from light microscope; N-P = micrographs of basidiospores from Scanning Electron Microscope. Scale Bar One Optical Division (A-M) = 2.355 µm; Scale Bar (N) = 1 µm; Scale Bar (O-P) = 500 nm

Discussion

West Bengal is an Indian state possessing diverse phytogeographical realms, spreading through its Coastal, Lateritic, Gangetic, and Terai-dooars to Eastern Himalayan montane-subalpine regions. These wide arrays of geomorphology, climatic variations, and vegetation structure make luxuriant conditions for the thriving of a mosaic of life forms, including macrofungi (Pradhan et al. 2012, 2013, 2016; Dutta et al., 2013). Some notable gastroid macrofungi reported from West Bengal are *Calvatia cyathiformis* (Bosc) Morgan, *Lycoperdon perlatum* Pers., *L. pyriforme* Willd., *L. pratense* Pers., *Tulostoma chudaei* Pat from the family Agaricaceae; *Astraeus hygrometricus* (Pers.) Morgan from the family Diplocystaceae; *Geastrum triplex* Jungh. and *G. saccatum* Fr. from the family Geastraceae; *Pisolithus arrhizus* (Scop.) Rauschert, *Scleroderma cepa* Pers. and *S. macrorhizon* Wallr. from Sclerodermataceae; however, there was no earlier record from the family Calostomataceae in West Bengal, India (Pradhan et al. 2011, 2012; Chakraborty et al. 2012, 2013; Dutta et al. 2013; Roy et al. 2014). Among the gastroid macrofungi of West Bengal, the notable ectomycorrhizal partner of *Shorea robusta* Gaertn. (Dipterocarpaceae) forests are *A. hygrometricus*, while *P. arrhizus* is associated with the plantations of *Eucalyptus globulus* Labill. (Myrtaceae) and *Acacia auriculiformis* Benth. (Fabaceae) (Pradhan et al. 2013).

Genus *Calostoma* was established by Desvaux in 1809 and had priority over the synonym *Mitremyces* Nees 1817 (Baseia et al. 2006). *Calostoma* is characterized by typically gelatinous cord-like stipe (pseudo-stipe) and peridium with apical Ostiole. Based on spore morphology, Massee (1888) provisionally grouped *Calostoma* into the western world species with elliptical-oblong spores and the eastern world species with spherical (globose to sub-globose) spores. However, the idea of this artificial segregation of *Calostoma* into western world species and eastern world species has been doubted, as *Calostoma lutescens* (Schwein.) Burnap, a globose-spored species, was reported from the U.S. (Burnap 1897) and as well from Sri Lanka (Massee 1888), while, *Calostoma ravenelii* (Berk.) Massee (Western world species) and *Calostoma japonicum* Henn., both of which have elliptical-oblong spores were reported from Japan (Minakata 1903); further, very recently broadly ellipsoidal *Calostoma sinocinnabarinum* N.K. Zeng, Chang Xu & Zhi Q. Liang was discovered in Central and Eastern China (Xu et al. 2022).

In the Indian sub-continent, the reported species of *Calostoma* are *C. junghuhnii* (Nepal; Bhutan; Sikkim; West Bengal [*from the present study*]), *Calostoma viride* (Berk.) Massee (West Bengal [from Tonglu and Sinchel]); and *C. lutescens*, *C. berkeleyi* Massee, *C. insigne* (Berk.) Massee in Sri Lanka (Massee 1888). The *C. junghuhnii* has been known in the Himalayan region since 1854 (Berkeley 1854), and the first color photograph of *C. junghuhnii* from the Himalayan region (Nepal) was taken by Prof. Hiroshi Hara during the Himalayan Botanical Expedition conducted by the University of Tokyo during 1960 and 1963 (Kobayasi 1965). One striking specimen from Prof. H. Hara's collections from Sikkim was *C. junghuhnii* (collected from Chola 1828.8-2438.4 m), and Kobayasi (1965), during the study of the specimen, mentioned it to appear close to the Kuchibenitake (*C. japonicum*). However, the spores of the sample from Sikkim were found to be spherical rather than ellipsoid, as in Kuchibenitake. The *C. junghuhnii* is distinguished from all other species in the genus by its distinctive exoperidial warts, bright orange-red ostiole/stoma with convergent, pointed teeth, and bluntly warted/tuberculose, globose spores that are 12.5-18 µm in diameter (Massee 1888; Miller and Cotter 1988).

The *C. viride* is a dull green species reported from the Tonglo and Sinchel areas of Darjeeling, which was collected by Sir J.D. Hooker and studied by Berkeley (Massee 1888). Perhaps it is endemic and very rare. The Stark dull green color of the fruit body makes *C. viride* different from *C. junghuhnii*. The *C. lutescens* is similar to *C. junghuhnii* regarding ochraceous exoperidium and globose spores. However, the latter has somewhat larger coarsely tuberculose spores with cinnamon-brown endoperidium and mostly intact exoperidium. In comparison, the former has smaller verrucose spores (7-9 µm), which resemble pitted under Scanning Electron Microscope with yellowish endoperidium and form a collar-like base at maturity due to the separation of exoperidium from endoperidium (Castro-Mendoza et al. 1983). The globose spores of *C. berkeleyi* are minutely verruculose and smaller (7-9 µm) than that of *C. junghuhnii*, which has tuberculate globose spores; further, *C. insigne*, though having creamy white fruitbody and globose spores of the size 14-17 µm, rather have coarsely spinulose spores instead of tuberculose spores (Massee 1888).

Phylogenetically some studies have placed *C. junghuhnii* close to other Asian species like *Calostoma areolatum* Y.H. Ma, B. Zhang & Y. Li, *Calostoma oriruber* Massee [as *'orirubra'*] and *C. sarasinii* Lim (Wilson et al. 2012; Phosri et al. 2014; Liu et al. 2018). Spores of *C. areolatum* are larger (15.5-18.2 µm), and irregularly verruculose separates it from *C. junghuhnii*. The *C. junghuhnii* is close to *C. orirubra* for spore size (14-17~18 µm) and the exoperidium breaking into areas and drying more or less as scales on the endoperidium; however, the former has pale ochraceous exoperidium with a greenish tinge, while exoperidium of the latter is brown (Massee 1888). The spores of *C. junghuhnii* are non-truncate, and exoperidium is pale ochraceous, while *C. sarasinii* spores are partially truncated (Boedjin 1938; Lim 1969), and the exoperidium surface is creamy in color (Wilson and Hibbet 2006).

Habitat-wise, the altitudinal range of *C. junghuhnii* varies from 2380 m in Chipling, Nuwakot District Nepal (Miller and Cotter 1988); 2438.4 m in Sikkim (Massee 1888); 2446 m in the present study; 2500 m in S.E. Tibet (Balfour-Browne 1955); ca. 2700 m in Halhale, Nepal (Kobayasi 1965) and 3000 m in Bakkhim, Sikkim (Kobayasi 1965). It prefers the damp soil of montane dense primary forests mainly composed of hardwoods (Chen and Yeh 1975). In the Central Himalaya (Bagmati Zone,

Nuwakot District, Nepal), *C. junghuhnii* is reported to be associated with *Quercus semecarpifolia* Sm. (Miller and Cotter 1988). In Eastern Himalaya (Sikkim), it is reported to be associated with *Lithocarpus* (Das 2009), while from the present study in Neora Valley National Park, India, it is known to be associated with a mixed stand of *Q. lineata* and *Q. lamellosa*.

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