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Contribution To The Macromycetes Of West Bengal, India: 1-7.

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ABSTRACT

West Bengal is a treasure house of fungal diversity due to its geo-climatic conditions. Altogether seven species belonging to the family Clavariaceae (four species), Dacrymycetaceae (two species) and Pterulaceae (one species) were collected from different corners of West Bengal and reported herein with detailed morpho-anatomical features.

Keywords: Calocera, Clavaria, Clavulinopsis, Deflexula, Taxonomy, West Bengal.

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INTRODUCTION

The clavarioid macrofungi belongs to the order Agaricales and are mostly represented by the families of Clavariaceae Chevall. (120 species under seven genera), Pterulaceae Corner (99 species distributed over 12 genera) and Dacrymycetaceae J. Schröt (101 species of nine genera) [1]. The fruit body structure of these fungi varies from pendant-hydnoid, cylindrical, clavate, coralloid, resupinate to lamellate-stipilate type [2]. These fungi possess the efficacy to establish in a wide range of substrates being a saprotroph, mutualist or parasites that enable them to be worldwide in distribution [3]. In comparison to other group of fungi, morphological characters like ornamentation of basidiospores and their reactivity, structure of hyphae, presence or absences of clamp connections etc. could lead to the identification of clavarioid species [2, 3]. Literature review suggests that Thind (1961) [4] were the pioneers who worked towards the exploration of Indian clavarioid fungi. Later Rattan and Khurana (1978) [5] reported quite a number of fungi belonging to this group from Darjeeling district of West Bengal. However, during the last 15 years, our research team has conducted extensive field works and recorded an immense number of macrofungi from different corners of the state. Taxonomic and molecular exploration revealed that many of them are new to science [6-10], new to the record for India [11-15] while remaining are an addition to the macrofungal flora of West Bengal [16-21]. West Bengal is the only state in India that is circumscribed by the stretches of sea (Bay of Bengal) in the south to the subalpine mountains of Eastern Himalayas in the north, and hence offers diverse sets of habitats for the luxuriant growth of macrofungi. In this communication, seven species belonging to the clavarioid group viz. Calocera cornea (Batsch) Fr., Calocera viscosa (Pers.) Fr., Clavaria tenuipes Berk. & Broome, Clavaria fumosa Pers., Clavaria fragilis Holmsk., Clavulinopsis fusiformis (Sowerby) Corner and Deflexula subsimplex (Henn.) Corner, collected from the state, are reported with detailed morphological characters.

MATERIAL AND METHODS

The study materials were collected during subsequent field trips from 2012-2014 from various places of West Bengal, India. The morphological and ecological features of the collected specimens were noted before the specimens get dried. Microscopic features were obtained from dried specimens by mounting freehand sections of basidiocarps in 5% KOH, melzer's reagent and congo red. Specimens were then examined with a Carl Zeiss AX10 Imager A1 phase contrast microscope. Colour terms and codes follow Kornerup and Wanscher (1978) [22]. Q value denotes length/width ratio of the spores excluding ornamentation. For measurement of average Q value (Q_{av}), 30 spores from each of the collected basidiocarps (n=30) were taken. Microscopic line drawings were made with the help of pencil and camera lucida which were finally traced with rotring 0.1 mm pen. The specimens were then identified according to Corner (1950) [2], Thind (1961) [4] and Rattan and Khurana (1978) [5]. Voucher specimens were preserved based on the protocol as described by Pradhan et al. (2015) [23] and deposited in Calcutta University Herbarium (CUH).

Taxonomy

Calocera cornea (Batsch) Fr.



Figure 1: Calocera cornea. a: Habit with fresh fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b-d = 10 µm.



Classification: Fungi, Basidiomycota, Basidiomycotina, Dacrymycetes, Dacrymycetales, Dacrymycetaceae, *Calocera, C. cornea.*

Fruit body 1–1.5 cm long, up to 0.7 cm broad, cylindrical to sub cylindrical, becoming ellipsoid in cross section, overall white (1A1) when extreme young, becoming white (1A1) at base with greyish yellow (4B4) towards apex and half of upper margin, extreme tip darker, often light yellow (1A5) to greyish yellow (1A6) that fades to yellowish white (1A2) towards inner periphery, simply branched, margin not acute, subulate, sometimes wavy, shallowly forked, solitary or base often caespitose with up to 7 fruit bodies, mostly insititious; gelatinous, with faint furrow in the centre, running halfway from apex. **Spores print** light orange to pale yellow.

Basidiospores 6.5–9 × 3.5–4.5 μ m, Q_{av} = 1.91, ellipsoid, 1-2 guttate, hyaline, inamyloid, thin-walled, smooth, apiculus small, up to 1 μ m long. **Basidium** 47–54 × 4–4.5 μ m, Y-shaped or biforked, hyaline, oil granule present when viewed with KOH, thin-walled. **Hyphal system** monomitic, 1.5–3.5 μ m broad, branched, hyaline, thin-walled, clamp-connections present.

Habit and habitat: Grows scattered to gregarious, at the base of moist bark of fallen *Cryptomeria japonica* trees; fruit bodies not encroaching wood and preferably growing horizontally in series following cracks in the bark.

Specimen examination: INDIA, West Bengal, Darjeeling, Old Military Road, Sonada, 27° 0' 12.1104"N, 88° 15' 39.6966"E, 22.07.2013, A.K. Dutta & P. Pradhan, CUH AM 025; and Darjeeling, Lolaygaon, 22.08.2012, A.K. Dutta & P. Pradhan, CUH AM 371.

Remarks: *Calocera cornea* is characterized by comparatively shorter fruiting bodies; ellipsoid, 1-2 guttate basidiospores that is smooth and thin-walled; Y-shaped basidia; and monomitic, branched hyphal system with presence of clamp-connection.

Being coralloid in morphology, *C. cornea* often confused with several similar species like *Clavulinopsis laeticolor* and *C. fusiformis*. However, the latter two species have more brittle fruitbodies. *Calocera viscosa* is also to some extant similar to *C. cornea*, but differs by its branched and comparatively larger fruit bodies. Throughout the world, *C. cornea* have been reported to grow on broad leaved and coniferous woods but in West Bengal, the species were found to prefer the coniferous woods only. Based on the coniferous habitat, *Calocera furcata* is similar to *C. cornea* but differs by having basidiospores that is three septate [24]. *Calocera viscosa* (Pers.) Fr.



Figure 2: Calocera viscosa. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b-d = 10 µm.



Classification: Fungi, Basidiomycota, Basidiomycotina, Dacrymycetes, Dacrymycetales, Dacrymycetaceae, Calocera, C. viscosa

Fruit body 3–8 cm high, up to 0.8 cm diam. towards base, ascendant, viscid, vibrant yellowish orange (4B7) to pallid dark orange (5A8), cylindric to ellipsoidal in cross section, branched up to 3 times at upper 3/4th to 3/5th length, branches up to 0.6 cm diam., sub-erect, pliant, tips mostly forked, subulate, base stalked, flesh firmgelatinous. Spore print yellowish.

Basidiospores 7.5-11.5 \times 3.5-5 μ m, Q_{av} = 2.1, ellipsoid, smooth, 2-guttate, becoming 1-septate at maturity, inamyloid, thin-walled. Basidia 24-31 × 3.5-6 µm, cylindrical to subclavate, Y-shaped, hyaline, oil granules present when viewed with KOH, thin-walled with basal septa. Hyphal system monomitic, 2.5-4.5 µm broad, hyaline, thin-walled, clamp connections absent.

Habit and habitat: Grows from the basal cortex of dead and moist bark of fallen Cryptomeria japonica trees, fruit bodies not encroaching wood and preferably growing vertically amongst layer of moss.

Specimen examination: INDIA, West Bengal, Darjeeling, Sonada, 26°58'05.7"N 88°16'51.5"E, 23.07.2013, Arun Kumar Dutta & Prakash Pradhan, CUH AM 372.

Remarks: Calocera viscosa is similar to C. cornea in terms of the presence of Y-shaped basidia that is characteristic for the members belonging to the order Dacrymycetales. However, C. cornea is less robust (up to 15 mm tall), paler, sparingly branched towards extreme apex, and grows in clusters. Calocera viscosa looks similar to Clavulinopsis corniculata but, the later has tetra-sterigmatic basidia and non-viscid in nature. Habitat of C. viscosa have been associated with decaying coniferous woods globally, however its parasitic nature needs to be ascertained [24].

Clavaria tenuipes Berk. & Broome



Figure 3: Clavaria tenuipes. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b-d = 10 μm.

Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Clavariaceae, Clavaria, C. tenuipes



Fruit body 35–40 mm long, 2 mm in diam., cylindrical, white (1A1) towards ground, with translucent greenish grey (1D2) towards lower 1/3rd to 2/6th, greyish yellow (2B3) at the extreme tip, tip blunt, base tapering, up to 1.5 mm diam., context white (1A1), solid. **Taste** mild. **Spore print** white (1A1).

Basidiospores $(5.5-)6-7(-7.5) \times (4.5-)5-6(-6.5) \mu m$, $Q_{av} = 1.14$, globose to subglobose, hyaline, I- guttate (4-4.5 × 3.5-4.5 µm), apiculus short (0.7 µm), wall up to 1.1 µm thick, smooth. **Basidia** 32-43 × 7-9 µm, clavate to subclavate, hyaline, oil granule present when viewed with KOH, thin-walled, 4-spored; sterigmata $3.5-9 \times 1.5-2 \mu m$ long. **Hymenium** 143-179 µm thick, composed of filamentous hyphae. **Sub-hymenium** 71-89 µm broad. **Hyphal system** monomitic, composed of 7-12.5 µm diam., hyaline, thin-walled, septate hyphae, secondary septa present. **Clamp-connection** absent.

Habit and habitat: Growing scattered at the floor of mixed vegetation of *Dendrocalamus* sp., *Artocarpus* sp., *Musa* sp. *Acacia auriculiformis* etc. and upon insect inundated, humus clayey soil along with mosses.

Specimen examined: INDIA, West Bengal, Howrah, Deulpur, 22° 35' 59.3592'' N, 88° 9' 57.4524'' E, 24.08.2014, Arun Kumar Dutta & Soumitra Paloi, CUH AM 374.

Remarks: White coloured solitary fruit body, presence of short apiculus with multiguttate basidiospores and thin-walled, monomitic hyphal system with presence of secondary septa indicates that *Clavaria tenuipes* belong to subgen. Holocoryne. However, the collection made from West Bengal is a bit taller as compared to the original description made by Berk. & Broome based on the collection from England. *Clavaria tenuipes* is allied to *C. fragilis*, but distinguished by its constantly incrassated head and less distinct flexuous stem. *Clavaria gibbsia* is also another closely related species but, narrow spores and smaller size of the basidia differ it from that of *C. tenuipes*. Earlier Massee had placed this species under the genus *Pistillaria*, however this genus has multiguttate basidiospores and clamped basidia. Specimen of *Clavaria tenuipes* described from South Australia [25] differs from our specimen in having longer and narrower spores ($7.5-9 \times 4.5-5 \mu m$) and preference for growth upon dead wood. However, presence of whitish translucent fruit body, secondary septa and absence of clamp-connections are common features between these two specimens.



Clavaria fumosa Pers.

Figure 4: Clavaria fumosa. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b-d = 5 µm.

Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Clavariaceae, *Clavaria, C. fumosa*

Fruit body medium to large, 3–13 cm long, 2–6 mm diam., solitary to caespitose, erect, cylindrical, smooth, without trunk, tapered at base, ground colour varies from white (1A1) to yellowish white (1A2), sometimes

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with a tint of light orange (5A4) at the curved margins, surface turns greenish with FeSO₄ moist but not viscid, apex blunt, unbranched with mostly caespitose at base. **Flesh** becomes hollow at maturity, white (1A1) to yellowish white (1A2). **Spore print** white (1A1).

Basidiospores $(3-)4.5-5.5(-6) \times 3-4.5(-5) \mu m$, $Q_{av} = 1.32$, nearly lacrymoid, hyaline, inamyloid, thinwalled, apiculus 0.7–0.9 μm long. **Basidia** $(36-)39-46(-48) \times (3.5-)4.5-6.5(-8) \mu m$, clavate to sub clavate, hyaline, thin-walled, 4-spored, sterigmata $5-6.5 \times 0.5-1 \mu m$. **Hyphal system** monomitic, composed of 7.5–18 μm diam. hyphae, secondary septate. **Clamp-connections** absent in all hyphae.

Habit and habitat: Growing in small clusters at the sloping floor with decaying leaf litter of Maling bamboo (*Yushania maling*) vegetation.

Specimen examined: INDIA, West Bengal, Darjeeling, 7th Mile Jungle, 27° 0' 54.0576" N, 88° 15' 36.396" E, 01.07.2012, Prakash Pradhan, CUH AM 375.

Remarks: This is the only species of *Clavaria* which has dark fruit body and has cosmopolitan in distribution [26]. *Clavaria fumosa* is easily recognised by its simple, large, greyish-beige, caespitose fruit bodies which lack trunk, having ellipsoid, smooth, thin-walled basidiospores, monomitic hyphal system that is secondarily septate, and absence of clamp-connections in all hyphae. In the global perspective, the species has been reported from Bolivia, Europe, North America, Siberia and Java [27]. Previously there are reports on the occurrence of present species from Mussoorie [4].

Clavaria fumosa is easily confused in the field with the *C. rubicundula* with regard to its morphology. However, the later one may be differentiated by its more fragile fruit body coloured pinkish tint. Based on the similar colouration of the fruit body and absence of clamps, *Clavaria fragilis* is closer to *C. fumosa* but differ by its less sturdy, more fragile fruitbody and has larger spores [28]. The characteristic features of *C. nebula* like longer basidiospores (> 6 μ m) and tendency of the fruitbody to become blackish on drying clearly distinguish it from *C. fumosa* [29]. Regarding the size and fragility of fruit bodies and growth habit in clusters, *C. fumosa* shares characteristics with some species under *Clavaria fuscoferruginea* complex, but fruit bodies of later are darker, tobacco coloured or reddish brown coloured that become much darker on drying [26]. *Clavaria amoenoides* has a fruitbody coloured yellowish with larger basidiospores and *C. fragilis* easily differs from *C. fumosa* by the presence of a trunk.

Clavaria fragilis Holmsk.



Figure 5: Clavaria fragilis. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b-d = 10 µm.



Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Clavariaceae, *Clavaria, C. fragilis*

Fruit body 8-14 cm long, simple, erect, cylindrical with a trunk, smooth, glabrous, white (1A1). **Stem** 7–18 × 1–2 mm, white (1A1) with concolourous apex, translucent, apical portion forked once when mature, solid, tapering upward, acute, becoming club shaped when mature. **Spore print** white (1A1).

Basidiospores $(4.5-)5-6 \times (4-)4.5-5(-5.5) \mu m$, $Q_{av} = 1.1$, sub-globose to sub-ellipsoid, hyaline, inamyloid, thinwalled, I-guttate, apiculus 0.9–1.1 μm long. **Basidia** 25–29(-32) × 7–8 μm , clavate to sub-clavate, hyaline, thin walled, without clamp connection, oil granule present when viewed with KOH, 4-spored, sterigmata 1.5–2.5(– 3.5) × 0.5-1 μm . **Hyphal system** monomitic, 7–12.5 μm diam., interwoven, thin-walled, hyaline, septate, bilayered, secondary septa present. **Clamp connections** absent.

Habit and habitat: Solitary to caespitose or gregarious, humicolous.

Specimen examined: INDIA, West Bengal, Howrah, Deulpur, 22° 36' 1.818'' N, 88° 9' 56.6208'' E 23.06.2013, N. Chakraborty & P. Samanta, CUH AM 377; Darjeeling, Beside Raj Bhavan, 27° 3' 5.1984'' N, 88° 15' 48.5064'' E, 21.07.2012, A. K. Dutta & P. Pradhan, CUH AM 379.

Remarks: *Clavaria fragilis* is characterized by the presence of a trunk coloured white, gregarious and caespitose habit of fruit bodies, I-guttulate, hyaline, thin-walled basidiospores and absence of clamp connections. It has monomitic hyphal system with secondary septa, which is a characteristic of the subgen. Holocoryne. *Clavaria acuta* is close to *C. fragilis* but differs by the presence of clamp connections. The breadth of the basidiospores of our collection is slightly broader as compared to the material described by Corner (3-4 µm). This material looks similar to the previously reported *C. vermicularis* var. *singaporensis* Corner but differs by its much smaller size of the basidiospores and basidia [15]. *Clavaria fragilis* is cosmopolitan in distribution as suggested by its previous reports of occurrences from Europe, North America, China, Japan, Java, South Africa and Australia, and India [4, 27]. It is interesting to note that *C. fragilis* was found to be substrate specific, regardless of the altitudinal variance.

Clavulinopsis fusiformis (Sowerby) Corner



Figure 6: Clavulinopsis fusiformis. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: hyphae. Scale bars: b–d = 10 μm.

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Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Clavariaceae, *Clavulinopsis, C. fusiformis*

Fruit body 6–13 cm long, 0.5–1.3 cm diam., uniformly yellowish white (2A7), fading with age, whitish towards extreme base, unbranched, cylindrical and often flattening, often grooved, moist but not viscid, apex blunt, no colour change with KOH, flesh light yellow (2A5). **Spore print** white (1A1).

Basidiospores $(5-)5.5-6.5(-7.2) \times (4-)4.5-5.5(-7) \mu m$, $Q_{av} = 1.14$, subglobose to sub-ellipsoid, hyaline, inamyloid, thin-walled, apiculus 0.9–1.1 μm long. **Basidia** $(41-)50-55(-61) \times 6.5-7(7.5) \mu m$, clavate to subclavate, thin-walled, basal part becomes yellowish when viewed with KOH, with dense cytoplasmic content, hyaline, 4-spored, sterigmata 5.5–7.5 × 1.5–1. μm . **Hyphal system** monomitic, with septate generative hyphae, 2.5–4.5 μm diam. **Clamp connections** present in all hyphae.

Habit and habitat: Gregarious, upon humus created by loam soil and decaying plant parts of Cryptomeria japonica and Selaginella sp.

Specimen examined: INDIA, West Bengal, Darjeeling, 7th mile, 27°01′54.3″N 88°19′37.2″E, 22.07.2012, A. K. Dutta & P. Pradhan, CUH AM 380.

Remarks: *Clavulinopsis fusiformis* is characterized by its bright yellow and densely fasciculated fruit body, ellipsoid to subglobose ($5-9 \times 4.5-8.5 \mu m$), one to multi-guttulate, slightly thick-walled basidiospores with well-developed apiculus ($1-2 \mu m$); presence of clamp connection at the base of basidia; and clamped, monometric hyphal system. *Clavulinopsis fusiformis* is similar to *C. corniculata, C. laeticolor* and *C. microspora* because of each having Basidia that is eight nuclei, of which four migrate into the basidiospores and the other four, remain in the basidial lumen [29]. *Clavaria amoena* is one of the close species to *C. fusiformis* but it differs in having very short apiculus. The basidiospores size of our collection is smaller as compared to the original description by Corner (1970) [27]. Earlier, the occurrence of the species has been reported from Europe, Japan, U.S.A., Canada, and India [4-5, 27]. Ratan and Khurana (1978) [5] reported the presence of this fungi from 3-Mile, Darjeeling district of West Bengal based on their collection made in August, 1964. Almost 50 years later, we have reencountered the same species at 7th Mile region of the same locality in the month of July, 2012.

Deflexula subsimplex (Henn.) Corner



Figure 7: Deflexula subsimplex. a: Habit with mature fruit body, b: Basidia, c: Basidiospores, d: Skeletal hyphae, e: Generative hyphae. Scale bars: b–e = 10 μm.



Classification: Fungi, Basidiomycota, Agaricomycotina, Agaricomycetes, Agaricomycetidae, Agaricales, Pterulaceae, *Deflexula*, *D. subsimplex*

Fruit body 11–14 mm long, cylindrical, branched, ground colour white (2A1), with tint of orang grey (5B2) to greyish orange (5B3), with 4–20 deflexed branches, 1.5–2 cm long, branchlet simple and divided, apex acute, concolorous throughout, flesh white (1A1). **Spore print** white (1A1).

Basidiospores 10.5–14 × 9.5–12.5 μ m, Q_{av} = 1.05, sub-globose, smooth, subhyaline, I-guttate, apiculus present, ca. 1.43 μ m long. **Basidia** 32–39 × 10.5–11.5 μ m, clavate, hyaline, 4-spored, sterigmata 3.5–4.5 μ m long. **Hyphal system** dimitic; skeletal hyphae 3.5–5 μ m broad, hyaline to sub-hyaline, long, unbranched, wall up to 0.5 μ m thick; generative hyphae up to 3.5 μ m broad, hyaline, branched, septate, thin-walled. **Gloeocystidia** absent. **Clamp-connections** present.

Habit and habitat: Gregarious, growing at the moist bark of dicotyledonous plants, amongst mat of moss, positively geotropic.

Specimen examined: INDIA, West Bengal, Darjeeling, Lloyd Botanic Garden, 27° 2' 39.6708" N, 88° 15' 46.0152" E, 27.06.2012, A. K. Dutta, CUH AM 382.

Remarks: The present species is characterised by its cylindrical, decurved branched fruitbody coloured cream; and thick-walled, ellipsoid basidiospores with a well-developed apiculus. Previously this specimen was reported by Corner (1950) [2] as *D. nivea* (Pat.) Corner from Guadeloupe which was later transferred to *D. subsimplex* [27]. According to Corner (1970) [27], the present species shows its close similarity with *D. nana*, but the latter one differs by the narrower skeletal hyphae and thin-walled basidiospores. Among branched species of *Deflexula*, *D. subsimplex* resembles *D. secundiramea* and *D. sprucei*. However, the latter two differ from *D. subsimplex* in having distinctly horizontal main branches that branch unilaterally to the lower side. Around the globe, there are reports of this species from Cost Rica, Panama, Brazil, Bolivia, Argentina, India, Borneo, and Solomon Islands [30]. The present collection was made from Darjeeling hills and a report from Sikkim Himalayas by Rattan and Khurana (1978) [5] indicative of the preference of this species towards high altitude. Almost 50 years after Ratan and Khurana's collection [5], we have encountered the same species at Lloyd Botanic Garden, Darjeeling.

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