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continued on the back inside cover

Cover: Green Sea Turtle *Chelonia mydas* watercolour by Elakshi Mahika Molur.



Two *Ceratosporella* (Fungi: Ascomycota) species from oak leaf litter in Almora, Uttarakhand, India

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Abstract: Two species of *Ceratosporella* collected on leaf litter of Mohru Oak *Quercus floribunda* in Uttarakhand, India, are described herein and compared with closely allied species. They are *Ceratosporella deviata* and *Ceratosporella cheiroidea*. Among them, *C. cheiroidea* is newly recorded from India. Morphological descriptions, illustrations, and comments are provided for the aforementioned species.

Keywords: Anamorphic fungi, biodiversity, Binser forest, cellotape technique, hyphomycetes, *Quercus*, saprobic, taxonomy.

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Author contributions: The study was conceived and designed by RCG. RCG and MKD performed all work on the project in the field. MKD performed analyses and led the writing. RSU and RCG supervised the research; also reviewed the manuscript's final version.

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INTRODUCTION

The dematiaceous hyphomycete genus *Ceratosporella* Höhn. was established by Höhn (1923), with *C. bicornis* (Morgan) Höhn. as the type species, and subsequently revised by Hughes (1951). *Ceratosporella* is characterized by mononematous, straight, erect, smooth, septate, pigmented (mid to dark brown) and unbranched conidiophores. Conidiogenous cells are percurrently extending, monoblastic, integrated, terminal and produce acrogenous, cheiroid, bifurcate or stauriform, smooth or verrucose, septate, brown to dark brown, solitary conidia with schizolytic secession (Monteiro & Gusmão 2014; Hernández-Restrepo et al. 2017). Besides these traits, the arms extend from a single basal cell of the conidium and branch into rows of cells (1–16), that separate this genus from other morphologically similar anamorphic genera including *Pentaster* Koukol, *Actinocladium* Ehrenberg, and *Triposporium* Rope (Manoharachary et al. 2010; Koukol & Říhová 2013).

Ceratosporella has been reported from temperate and tropical regions worldwide and it is associated with numerous types of plant substrates such as bark, leaves, stems or branches, and the petioles of many plant hosts in both freshwater and terrestrial ecosystems (Ellis 1971; Wu & Zhuang 2005). Most species included in this genus are saprobic in nature, often found growing on decaying plants debris of a wide range of hosts in contrast to the generic type, *C. bicornis*, which is a plant pathogenic fungus on *Zea mays* L. (Hughes 1971; Matsushima 1993; Castañeda-Ruiz et al. 1996; Seephueak et al. 2010). Some species are also known to interact with trees in the families Euphorbiaceae, Betulaceae and Fagaceae such as *Carpinus betulus* L., *Castanea sativa* Mill. and *Hevea brasiliensis* Muell. Arg. In this sense, *Ceratosporella disticha* Kuthub. was isolated from leaf litter of the Palm tree *Arenga westerhautii* Griff in Malaysia (Kuthubutheen & Nawawi 1991).

The Kumaon or Kumaun Himalaya region and its associated forests are considered an important reservoir of Indian biodiversity; nevertheless, the mycobiota of its ecosystems is poorly documented and still remains obscure, predominantly the dematiaceous hyphomycetes that grows on dead and decaying residues of plants. During field work carried out in the subtropical forest area of Almora District, Uttarakhand, India, we found two interesting microfungi associated with decaying Mohru Oak *Quercus floribunda* leaf litter material sharing features of *Ceratosporella*. These two species, namely *C. deviata* and *C. cheiroidea*, have unique

conidia that differ from other taxa in *Ceratosporella* and are reported and described herein.

MATERIAL AND METHODS

Isolate and morphology

Leaf litter of Mohru Oak *Quercus floribunda* Lindl. ex Camus were collected during a mycological field work in Binser forest, Almora, Uttarakhand, India in the June of 2017. The collected samples were preserved in separate zip-locked plastic bags and transported to the laboratory. Once there, they were incubated in moist chambers consisting of sterile Petri dishes at room temperature (about 25°C) and periodically examined within two weeks for the presence of microfungi using a dissecting microscope. The samples were prepared for microscopic examination using a newly developed cellotape technique (Gupta 2016). Microscopic features and fungal structures such as septation, conidiogenous cells, developmental stages, branching pattern, conidial size and shape were measured, photographed and studied by employing standard mycological protocols and relevant literature to enable species identification. The permanent slides and examined specimens were deposited in the fungarium of the Department of Botany, Kumaun University, Almora, India (HKUM).

RESULTS

Taxonomy

Ceratosporella deviata Subram., Proc. Indian Acad. Sci. Sect. B. 46: 327, 1957. Image 1A–C

Synonym: *Triposporium deviatum* (Subram.) R.F. Castañeda, Mycotaxon 60: 278, 1996.

Colonies on natural substrate effuse, brown or dark blackish brown, velvety. Mycelium partly superficial and partly immersed in the substratum, composed of irregular branches, smooth, septate, subhyaline to pale or dark brown, 1.5–3.0 µm wide hyphae. Conidiophores simple, erect, straight or slightly curved, smooth, cylindrical, brown or pale brown toward the apex, dark brown toward the base, up to 150 µm long, 4–6 µm wide, often extending percurrently, with up to 10 septa, dark brown basal cell; conidiogenous cells monoblastic, integrated, terminal, smooth, pale brown, cylindrical and truncated at the apex. Conidial secession schizolytic. Conidia acrogenous, smooth, pale to mid brown, with 2–4 (usually 3) divergent, subulate, septate arms, often constricted at septa, the apical (central) arm 30–55 ×

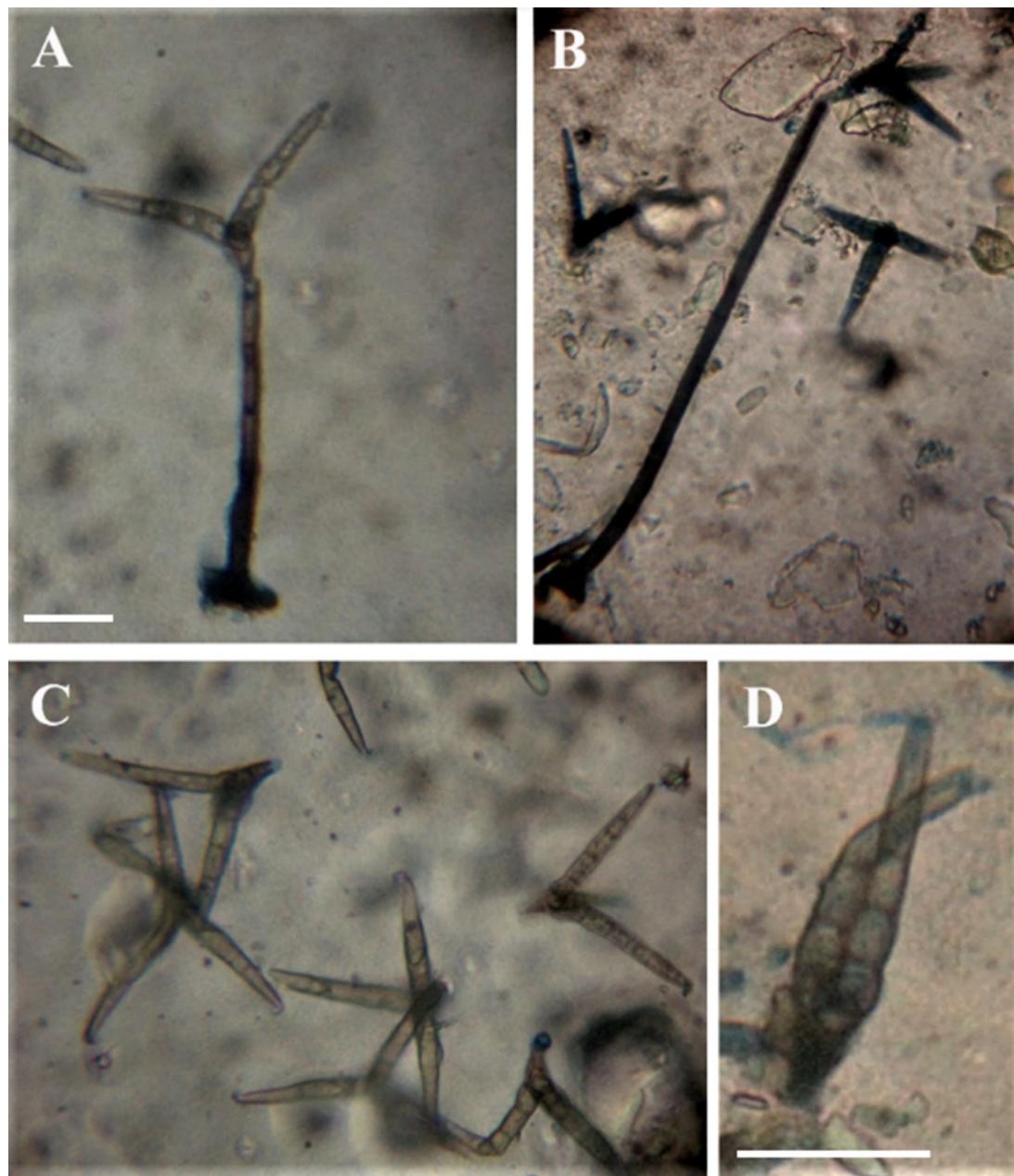


Image 1. A–C—Conidia and conidiophores of *Ceratosporella deviata* | D—Conidium of *Ceratosporella cheiroidea*. Bars A–C = 20 μm | D = 40 μm . © Ramesh Chandra Gupta.

5–7 μm , lateral arms 15–45 \times 4–7 μm , arising from a turbinate or pyriform basal cell, 8–14 μm long, 5–8 μm wide at the broadest part, 3–5 μm at the base attached directly to the conidiophores.

Specimen examined: HKU(M) 2623, 10.vi.2012, India, Uttarakhand, Almora, Binser forest, on dead leaf bases of *Q. floribunda* (Fagaceae), coll. R.C. Gupta.

Note: The collection adequately fits the original diagnosis of Subramanian (1957) for describing *Ceratosporella deviata* on dead leaf bases of *Raphiostyles*

sapida from Chennai (formerly Madras), India. This species is distinct from other representatives of the genus by its two–four (mostly three) divergent conidial arms. This fungus has a close resemblance with species such as *C. stipitata* and *C. bicornis*. However, conidia in *C. stipitata* are smaller and consistently two-armed whereas in *C. bicornis* the two conidial arms diverge more or less 180° (Monteiro & Gusmão 2014). Castañeda-Ruiz et al. (1996) proposed a new combination *Triposporium deviatum* for this species based on the presence of stauroconidia

and its strong resemblance with species of *Triposporium* Corda, particularly *T. elegans* Corda (Ellis 1971).

Ceratosporella cheiroidea R.C. Sinclair, Morgan-Jones & Eicker, in Sinclair, Eicker & Morgan-Jones, Mycotaxon 30: 352, 1987. Image 1D

Colonies on natural substrate effuse, light brown to dark brown, velvety. Mycelium partly superficial, mostly immersed in the substratum, composed of septate, branched, pale brown to brown, smooth hyphae. Conidiophores mononematous, macronematous, simple, erect, smooth, thick walled, cylindrical, straight or slightly flexuous, 2–10 septate, 45–110 × 4–6 µm, mostly dark brown with a 4–8 µm wide bulbous base. Conidiogenous cells monoblastic, integrated, terminal, smooth, brown to pale brown, usually cylindrical with two–three successive percurrent extensions, truncated at the apex. Conidial maturation synchronous with conidial ontogeny: conidial secession schizolytic. Conidia solitary, acrogenous, cheiroid, smooth, composed of a 2-celled stalk-like base and usually with two or rarely three cylindrical arms, flexuous, 1–6 septate, usually of unequal sizes, pale brown, 20–55 × 5–7.5 µm, tapering towards the distal cell with primary arms usually longer than secondary arms; basal stalk obconic, versicolor and truncate at base.

Specimen examined: HKU(M) 2640, 11.vi.2017, India, Uttarakhand, Almora, Binser forest, on leaf litter of Mohru oak *Q. floribunda*, coll. R.C. Gupta.

Note: The most striking feature of this isolate is the presence of conidia with 2–3 arms, conidial development pattern and versicolor basal cell. These characteristics were easily observed in the isolated specimens, agreeing with the original description (Sinclair et al. 1987). According to Monteiro & Gusmão (2014), only 12 representatives hitherto described under *Ceratosporella* possess cheiroid conidia— *C. basibicellularia*, *C. basicontinua*, *C. caliculata*, *C. compacta*, *C. cheiroidea*, *C. disticha*, *C. flagellifera*, *C. fertilis*, *C. novae-zelandiae*, *C. hernandezii*, *C. ponapensis*, and *C. stipitata*. *C. cheiroidea* conidia possess 2–3 arms (20–55 µm long in length), with a pattern of conidial development closely similar to the above mentioned representatives of *Ceratosporella*; but the number of arms, septa, size, and pigmented basal cell are the certain features that clearly distinguish this fungus from other mentioned representatives (Sinclair et al. 1987; Monteiro & Gusmão 2014). Synoptic characteristics for all these mentioned *Ceratosporella* species were reviewed and provided by Monteiro & Gusmão (2014).

DISCUSSION

During a survey of microfungi aimed to shed light on the microfungi inhabiting leaf litter of Binser forest in Uttarakhand (India), two dematiaceous hyphomycetous fungi belonging to the genus *Ceratosporella* were found. On critical study and comparison with other known representatives of the genus, *Ceratosporella deviata* and *Ceratosporella cheiroidea* were found to be newly recorded species for Uttarakhand and India, respectively. The taxonomic diagnosis of these recorded species is provided herein. Apart from this, the ecology of the recorded species is also described with reference to their substratum and habitat preferences. Our previous surveys also reflect that this region harbors a huge diversity of unknown mycobiota that could be discovered through proper mycological surveys (Dubey et al. 2019b, 2020a,b, 2022a,b, 2023); thus, the protection of the Binser forest is mandatory and of the utmost importance. In recent years, several mycological explorations have been conducted throughout India aimed for the discovery of its largely unknown mycobiota (Dubey et al. 2019b, 2020a,b, 2022a,b). Furthermore, as part of these surveys, several novel fungal species occurring on dead and decaying leaves or other plant parts have been described recently in India (Verma et al. 2021; Singh et al. 2022).

Over the years, 18 taxa have been included and described under the genus *Ceratosporella*, namely, *C. basicontinua* (Matsushima, 1993), *C. basibicellularia* (Matsushima, 1993), *C. bicornis* (Höhn, 1923), *C. cheiroidea* (Sinclair et al., 1987), *C. caliculata* (Lustrati, 1980), *C. disticha* (Kuthubutheen & Nawawi, 1991), *C. compacta* (Castañeda-Ruiz et al., 1996), *C. flagellifera* (Matsushima, 1993), *C. fertilis* (Castañeda-Ruiz, 1985), *C. ponapensis* (Matsushima, 1981), *C. novae-zelandiae* (Hughes, 1971), and *C. stipitata* (Hughes, 1952). However, taxonomic confusion has arisen for some of the taxa due to their similarity and comparison amongst similar genera. Based on the above fact, *Ceratosporella* has been generically delimited and five taxa specifically, *C. deviata* and *C. lambdaseptata*, *C. goidanichii*, *C. longiramosa* and *C. pulneyensis* were excluded and assigned to other similar genera. *Ceratosporella elegans* is a synonym of *C. bicornis*. To date, only 12 species are retained in the genus *Ceratosporella*, with the criteria used for species identification and delimitation being primarily based morphological features such as conidial size, shape, septation, developmental pattern, ornamentation, pigmentation and the absence or presence of appendages (Castañeda-Ruiz et al. 1996; Monteiro &

Gusmão, 2014). Castañeda-Ruiz et al. (1996) provided the key to species of *Ceratosporella*. However, Monteiro & Gusmão (2014) recently revised the key and reviewed the synoptic characteristics for all the accepted species. In this sense, the distinguishing characteristics of all the accepted species were compared with closely resembling species for taxonomic clarity. Similar characteristics are also used for distinguishing other known species in many closely related saprobic dematiaceous hyphomycetes genera, like *Cercosporella*, where little is known about teleomorph relationships and molecular data are sparse (Seifert et al. 2011; Heredia et al. 2014; Xia et al. 2014; Hernández-Restrepo et al. 2017). Even in genera where molecular data are available, morphology alone is often conclusive (Almeida et al. 2014). Therefore, the phylogenetic placement of the representatives in this genus remained highly uncertain until new insights based on molecular data are provided for these fungi. In this regard, ecological and taxonomical observations in this genus are, therefore, largely based on morphology.

CONCLUSION

In the present study, two *Ceratosporella* species were investigated based on their critical morphological characters. These two species possess unique conidia that differ from other species in *Ceratosporella*, and therefore, *Ceratosporella deviata* is described as a new record for Uttarakhand whereas *Ceratosporella cheiroidea* is reported for the first time from India. Overall, this study contributes to the knowledge of the fungal diversity present in Uttarakhand, especially in the oak forest-covered regions, demonstrating the importance to explore other new habitats of the Himalayan region during mycological surveys.

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Article

Use of remote sensing and GIS in assessing the impact of *Prosopis juliflora* proliferation on land use, land cover and diversity of native flora at Point Calimere Wildlife Sanctuary, India

– Sourav Gupta, Subhasish Arandhara, Selvarasu Sathishkumar & Nagarajan Baskaran, Pp. 24451–24462

Communications

Two *Ceratosporella* (Fungi: Ascomycota) species from oak leaf litter in Almora, Uttarakhand, India

– Manish Kumar Dubey, Ram Sanmukh Upadhyay & Ramesh Chandra Gupta, Pp. 24463–24468

The genus *Holigarna* Buch.-Ham. ex Roxb. (Anacardiaceae) in the central Western Ghats, Karnataka, India

– Kumbar Mudakappa Manjunath, H.S. Shashwathi, H.M. Rakshitha Jain & Y.L. Krishnamurthy, Pp. 24469–24484

Report of *Bathycoelia indica* Dallas, 1851 (Hemiptera: Heteroptera: Pentatomidae) as a pest of pomegranate *Punica granatum* L. cultivated in Maharashtra State

– P.S. Kudnar, Gaurang G. Gowande & Hemant V. Ghate, Pp. 24485–24495

First documentation of diversity of the Heteroptera of Cotton University Campus, Kamrup (Metropolitan), Assam, India

– Santana Saikia & Anjana Singha Naorem, Pp. 24496–24502

Checklist of hawkmoths (Lepidoptera: Bombycoidea: Sphingidae) in the Central Highlands of Vietnam

– Trang Q. Le & Lien V. Vu, Pp. 24503–24528

Observations on the courtship behaviour of Deocata Pipefish *Microphis deocata* (Hamilton, 1822) (Actinopterygii: Syngnathiformes: Syngnathidae) in an aquarium

– Anu Saikia, Jayanta Kumar Nath & Dandadhar Sarma, Pp. 24529–24534

Freshwater fish diversity and IUCN Red List status of glacial-fed (Bheri) and spring-fed (Babai) rivers in the wake of inter-basin water transfer

– Kumar Khatri, Bibhuti Ranjan Jha, Smriti Gurung & Udhav Raj Khadka, Pp. 24535–24549

Population status and habitat use of White-crested Kalij Pheasant *Lophura leucomelanos* hamiltoni (J.E. Gray, 1829) in the Limber Wildlife Sanctuary, Jammu & Kashmir, India

– Arif Nabi Lone, Bilal A. Bhat & Khursheed Ahmad, Pp. 24550–24556

Assessment of diversity, abundance, and seasonal variations of bird species in Bengaluru District, India during COVID-19 lockdown

– H. Hemanth, Rajalakshmi K.S. Vinanthi & Kuppusamy Alagesan Paari, Pp. 24557–24567

An annotated checklist of the birds in Loharghat Forest Range, Assam, India

– Taniya Talwar, Leons Mathew Abraham, Borojit Rabha & Mrigen Rabha, Pp. 24568–24583

Trade of skulls as novelty and aquarium objects are an additional threat to porcupines

– Jessica Chavez, Kuntayuni & Vincent Nijman, Pp. 24584–24588

Review

Fishes of Cocibolca, the great Central American lake

– Topiltzin Contreras-MacBeath, Byron Josue Rodríguez Pérez, Humberto Mejía-Mojica & Juan Manuel Rivas-González, Pp. 24589–24596

Short Communications

Twice blooming flowers of *Antigonon leptopus* Hook. & Arn. (Magnoliopsida: Caryophyllales: Polygonaceae), a key forage source for insects during wet season in habitats disturbed by humans

– P. Suvarna Raju, P. Srikanth & A.J. Solomon Raju, Pp. 24597–24600

Two new weevil species of the genus *Mylocerus* Schoenherr, 1823 (Coleoptera: Curculionidae: Entiminae) from India

– G. Mahendiran, M.M. Nagaraja & M. Sampathkumar, Pp. 24601–24606

Notes

Additional record of the Black Turmeric *Curcuma caesia* Roxb. (Zingiberales: Zingiberaceae) in Bhutan

– Karma Orong, Namgay Shacha, Kezang Tobgay & Rinchen Namgay, Pp. 24607–24610

A record of Chestnut-and-Black Royal *Tajuria yajna istrodea* De Nicéville, 1887 (Lepidoptera: Lycaenidae) from Arunachal Pradesh, India

– Ruksha Limbu, Ramandeep Achint, Renu Gogoi, Roshan Upadhyaya & Jyoti Gaur, Pp. 24611–24614

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