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Trichoderma cornu-damae (syn. *Podostroma cornu-damae*): –The first report of a rare mushroom with an anamorphic stage occurrence from Sikkim, India.

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Trichoderma cornu-damae (Pat.) Z.X.Zhu & W.Y.Zhuang, formerly *Podostroma cornu-damae* belongs to the class Ascomycete, family Hypocreaceae of the order Hypocreales. It is a rare species of fungus reported to have highly toxic fruiting bodies and is native to Asia.The fruiting body (telomorph)of this fungus was sighted for the first time in Sikkim, India. Morphological descriptions of the telomorphic stage and habitat details of this species have been provided in the present study.Isolation of pure culture to establish an anamorphic stage occurrence was attempted through a spore culture technique, and the culture characterization confirmed its *Trichoderma* anamorph state.

Keywords: *Hypocrea*, Mushroom, *Podostroma*, Sikkim, *Trichoderma cornue-damae*

INTRODUCTION

Trichoderma, or *Hypocrea,* is a genus of soil-borne or wood-decaying saprophytic fungi. Most *Trichoderma* strains do not form sexual stages but instead produce only asexual spores. However, for a few strains, the sexual stage is known, when found, and it is within the Ascomycetes in the genus *Hypocrea*.*Hypocrea*, the teleomorph of *Trichoderma*, was first described by the Tulasne brothers in 1865 **(**Gams and Bissett 2002). Till recently, *Trichoderma* and *Hypocrea* were treated as separate genera.With several species linked as asexual (anamorph) and sexual (teleomorph) respectively, increasing numbers of *Trichoderma* species have been linked to their teleomorphs.

The first record of the sexual morph of *Trichoderma is* from China and dates back to 1895, when *Hypocera cornu-damae* was reported on rotten wood in Sichuan Province and later transferred to the genus *Podocrea*, before being placed under the genus *Podostroma*(Boedijin,1934*)*. Species of *Hypocrea* Fr. that have a stipe, especially those with a clavate to cylindrical stroma, have traditionally been segregated as the genus *Podostroma* P. Karst. Chamberlain *et al*. (2004) synonymized *Podostroma* with *Hypocrea*. Hence the synonyms:*Hypocrea cornu-damae* Pat., *Podocrea cornu-damae* (Pat.) Sacc.&D.Sacc., *Podostroma cornu-damae* (Pat.)Boedijn., *Protocrea cornu-damae* (Pat.) Sacc. & D. Sacc.

With the revision in the International Code of Nomenclature for Algae, Fungi, and Plants (ICN), fungi would no longer bear more than one name. Under this new provision of ICN, the anamorphic name *Trichoderma* becomes valid and supersedes the teleomorphic name *Hypocrea*. ICTF (International Commission on the Taxonomy of Fungi) andthe International Subcommission on the Taxonomy of *Trichoderma* and *Hypocrea*, preferred to maintain *Trichoderma* over *Hypocrea* (Rossman *et al.* 2013).

The fruiting body of *Podostroma cornu-damae or Trichoderma cornu-damae* has been reported to be highly toxic in Korea **(**Ahn*et al.* 2013) and Japan (Saikawa*et al.* 2001)**.**This deadly mushroom is

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native to Asia (Southeast and East) and Oceania (Gonmori and Yoshioka 2005; Gonmori *et al.* 2011).Poisoning by *Podostroma sp.,*is mostly accidental as it resembles some edible mushrooms.The immature stage of this fungus resembles *Ganoderma lucidum* or *Cordyceps militarys* (a health food), and during its fruiting stage it resembles *Clavulinopsis laeticolor* (a coral mushroom) and *Clavulinopsis miyabeana,*due to which it is mistaken as edible andcauses poisoning (Suzuki *et al.* 2002; Kim *et al*. 2016).Symptoms associated with consumption of this fungus include stomach pains, changes in perception, a decrease in the number of leukocytes and thrombocytes, peeling skin, hair loss, and shrinking of the cerebellum, resulting in speech impediment and problems with voluntary movement (Saikawa*et al.* 2001). In another instance, an autopsy revealed multiple organ failures, including acute kidney failure, liver necrosis, and disseminated intravascular coagulation (Koichi *et al.* 2003).Lee *et al*. (2018) reported that it contains a lethal toxin called trichothecene mycotoxin, which is a highly toxic substance**.** In a recent study by Lee *et al*. (2021), it was reported that Roridin E, produced by *Podostroma cornu-damae*, is a mycotoxin with an anticancer activity. This paper briefly describes the characteristics of *Trichoderma cornu-damae* (syn. *Podostroma cornu-damae*), provides information on its ecology,and attempts to link *Hypocrea* and *Trichoderma* morphs in the Ascomycota.

MATERIAL AND METHODS

Sampling site and collection

Lingthem is located in upper Dzongu, North Sikkim, India (27° 31.55' N and 88°30.380' E) at an elevation of 1235m asl, humidity 30-96%, and temperatures ranging from 6-25°C. The climate is subtropical forest type with an average annual rainfall of 2663mm (2010-2020- Meteorological Station Gangtok, Sikkim, India). Fig.1 depicts a map of the sampled area.

The sampling site is located in the transition zone of the Kanchenjunga Biosphere Reserve in the Eastern Himalayan region, which has a high level of biodiversity but has been understudied in terms of fungal richness. Distinctly coloured (red) mushroom stroma was discovered in the same region between 2020 and 2022 from end of July and until the middle of August. All of the important physical traits, morphological features, and ecological data were documented in the field. A few fresh specimens were collected in sterile paper bags for further analysis in the laboratory.

Morphological (Teleomorph)characterization

Macromorphological characters of teleomorph like diameter, height, colour, shape and surface texture of the stroma, spore print, surface texture, colour, rhizomorph presence, moisture contentwere recorded. Microscopy is the key technique to obtain micromorphological characters and cellular structures, for which specimens are cleaned carefully to remove soil and plant debris before slide preparation.Vertical and horizontal sections of ascomata give essential taxonomic information, including perithecial shape, length, and width of internal tissue; shape and size of the ostiole, or peridium, shape; length and wall thickness of cells of the outer, middle (situated immediately below the outer region) and inner region (situated below perithecia) of the stroma; ascus length and width (Foster *et al.* 2011). Sectioning was done by free hand and mounted in Lacto-phenol cotton blue stain. Microscopic observations were made under a compound microscope(model Magnus CH20i). The macroscopic and microscopic characters of the specimen were used to confirm the identificationafter consulting relevant research articles, taxonomic keys, and monographs on Ascomycetes, *Hypocrea*, and *Triochoderma* (Rifai, 1969; Chamberlain *et al*. 2004;Senanayake *et al*. 2020; Gams and Bissett,2002; Samuels and Lodge, 1996; Jaklitsch, 2009,2011).The samples were dried, preserved and deposited in the Department of Botany, Mycology and Plant Pathology Laboratory, NBBGC Tadong, Gangtok, Sikkim.

Culture (anamorph) isolation and characterization

Mature and perfect stage of fruiting bodies or stroma was considered for isolation of pure culture form ascospore.Perithecia were crushed in a drop of 0.7% (w/v) sodium acetate, and perithecial contents were picked up with a capillary tube and put onto cornmeal dextrose agar (CMD + 2% dextrose)aseptically(Chamberlain *et al*. 2004; Chomnunti *et al.* 2011).For pure culture isolation, the mushroom mycelia were transferred to fresh PDA medium and incubated at 25°C for 10 days,

exposing to periodic interval light. Diameter, elevation, margins, texture of the colony as well as pigmentation of the colony from the top and reverse of the plate, extent of sporulation (low, medium and heavy), and presence or absence of radial sulcation were recorded.Hyphae and conidial morphology like shape, size, colour and, appearance of chlamydospores were also recorded after 7-10 days (Gams and Bissett, 2002; Samuels *et al*. 2002; Jaklitsch *et al.*2005, 2008). An interactive key provided by Samuels and his coworkers at http://nt.arsgrin.gov/taxadescriptions/keys/FrameKey.cfm?gen=Trichoderma was also used for morphological identification of *Trichoderma* isolates.

For short term preservation, pure culture discs were made with a sterile cork borer (about 3 mm). Culture discs were placed in 10 mL screw cap tubes with paraffin liquid. The tubes were subsequently stored at 4°C in the Myco-patho laboratory, PG Department of Botany, NBBGC, Tadong, Gangtok, Sikkim.

RESULTS AND DISCUSSION

Descriptionand Identification

Teleomorph-Most *Trichoderma* strains are not encountered in association with sexual stages, and are considered to be strictly mitotic, clonal fungi. Sexual reproduction is known in *Trichoderma* in the sense that the only known teleomorphs of *Trichoderma* are species of *Hypocrea* Fr. and closely related genera, members of the ascomycete, order Hypocreales. The sampleswere found growing on well-decayed wood of angiosperms (decomposing stump and root line of *Alnus nepalensis*). These fungi are conspicuous by their brightly coloured mushroom/fruiting bodies.

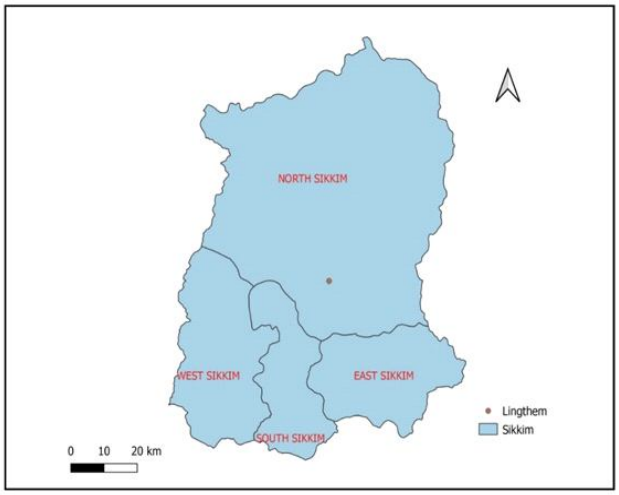

Fig: 1: Map of the study area.

Fig. 2: a. Trichoderma morphafter seven days of incubation, sporulation front and reverse. **b.** Initial pure culture isolation from ascospore. **c.** Hyphae, spore and chlamydospore under 100x magnification.

Fig: 3: a &b- Young *Trichoderma cornu-damae*. **c-** Mature *Trichoderma cornu-damae*. **d-**Fully mature *Trichoderma cornu-damae*e & **f**- Subcortical tissue in section.**g-** An ascus with ascospore. **h &i-** Stained perithecium and unstained perithecium with ascus and ascospore. **j-**Sporeprint.**k-** Culture plate with sporulation.

The stromata are deep pink to red when young fresh and turns reddish orange to brown in colour on maturity. The fruiting bodies of the mushroom are smooth, branched and unbranched, rhizomorph present,approx 7-15cminlength and1 -5cm in width at maturity.The spore print is off white in colour. Cross section clavate stromata have perithecium with ostioles that are globular in shape.Cortical layer are thick and hyaline, loose irregularsubcorticalhyphal tissues arepresent below theperithecium, perithecia embedded in

fleshy stromata formed by pseudoparenchymatous tissue or highly compacted hyphae.The asci are present inside and outside the perithecium.The ascospores are globose to sub-globose (5µ), wall are rough, producing 16 part-ascospores in each ascus. *Hypocera* has eight 1-septate ascospores that disarticulate at the septum.Disarticulation of ascospores is one of the distinguishing characters of the genus. The habit, morphological, anatomical and microscopical details are presentedin Figs. 2&3.

The macro-micromorphological and anatomical features corroborate that the specimen belongs to the genus *Podostroma* (*Hypocera/Trichoderma)*which includes species with upright, stipitate stromata. The species was first discovered in Sichuan, China, and was described as *Hypocrea cornu-damae* by a French scientist, Narcisse Theophile Patorllard; it was later renamed *Podostroma cornu-damae* (Boedijn, 1934; Zhao-Xiang and Wen Zing, 2014). In several studies, similarities were noted between the *Podostroma* and *Hypocrea*, which differ only in the stalked stroma of the former (Doi, 1987; Rossman *et al*. 1999). Other characteristics of *Podostroma,* such as the morphology of the stromatal tissue, the perithecia, anamorphs and ecology, are indistinguishable from corresponding features of *Hypocrea*. Recent studies using DNA sequence data suggest that *Podostroma* (Chamberlain, 2004 and *Protocrea* (Overton, 2006) are congeneric with *Hypocrea*. Since *Hypocrea* is the telomorph of *Trichoderma,* hence the sampled specimen was identified to be *Trichoderma cornu-damae* (Pat.)Z.X.Zhu & W.Y. Zhuang.

Anamorph-To establish link between sexual and asexual morphs, it is important to obtain isolates and to confirm that they are the same fungus.Cultures establishment are especially important for some groups of ascomycetes (e.g., Hypocreaceae) in which the anamorph (asexual stage) is required for accurate species identification.Recent systematic research suggests that it is not possible to identify a *Hypocrea* species unless its *Trichoderma* anamorph is known (Chaverri *et al.* 2003; Lu *et al.* 2004; Samuels, 2006). Because the anamorph is the form most commonly connected to information on the economic and ecological significance of the genus *Trichoderma*, it is essential to clarify the biology of *Trichoderma* and *Hypocrea* in a unitary way. To link species to their anamorphs by observation of the stroma and cultural studies, several new species of *Hypocrea* and their anamorphs were described from New Zealand (Dingley, 1957).

In the present study, culture isolation through spore culture was established and morphological observation shows rapid mycelial growth, loosely floccose mycelium which are cottony white, surface hyaline in some area, reverse of the colonies are pale yellowish, reaching up to 9cm in 7-10 days of incubation and green sporulation occur on exposure to light; sporulation sparse,vegetative hyphae are septated, hyaline and smooth-walled.Conidia are pale green in colour; smooth;ellipsoidal to ovoidal. Conidia are one-celled, (4×3 µm, L/W => 1.3) they are typically light green, surfaces smooth, spore wall distinct. Chlamydospores are present,unicellular, spherical/ globose in shape (6-6.5*i*m diameter), sparse, normally found as thick-walled, terminal to intercalary. It suggests that the isolated culture is *Trichoderma* anamorph (Figs.2 & 3). Many members of the *Trichoderma* morph state obtained through the investigation of new geographical regions are currently being studied in connection with commercial applications by researchers around the world.

CONCLUSION

The Indian subcontinent is a biodiversity hotspot and home to different classes of fungi and mushrooms; however, many species of poisonous mushrooms and their toxins have yet to be discovered and characterized. *Trichoderma cornu-damae* syn *Podostroma cornu-damae* is a poisonous fungus with brightly coloured, clavate, stipitate, fleshy, and erect stromata.The fungus was once thought to be only native to Korea and Japan, but recent discoveries have been from other Asian countries like Indonesia (Java), China, Korea, Taiwan, New Guinea, Australia and now Sikkim, India. This is the first report of the discovery of this mushroom from Sikkim, India; nevertheless, no poisoning cases have been reported from the region thus far.There have been numerous reports of toxicity induced by unintended consumption of this fungus, as well as the potential of its mycotoxin (Roridin E) as an anticancer agent. Culture isolation and characterizationestablish a link between the *Podostroma/Hypocera-Trichoderma*sexual and asexual morphs, and this finding suggests rich fungal diversity in the Sikkim Himalayan region.

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