

Re-discovery of the rare poisonous mushroom *Podostroma cornu-damae* in Indonesia

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Abstract. *Podostroma cornu-damae* is considered one of the most poisonous mushrooms in the world. The first report of *P. cornu-damae* in Indonesia was provided by Boedijn in 1934, located at Buitenzorg (Bogor), West Java. Our preliminary work based only on the macroscopic features in 2020 indicated the occurrence of *Podostroma* cf. *cornu-damae* in Central Kalimantan (Borneo). Hence, the current research aims to ensure the taxonomical identity of the macrofungi. *Podostroma cornu-damae* is confirmed based on morphological (macro-and microscopic) and molecular evidence. A brief description of the morphology of *P. cornu-damae* and the phylogenetic analyses inferred from the ITS sequence are provided. This study is the first comprehensive report of *P. cornu-damae* for Indonesia after nearly 100 years of hiatus.

Keywords: Ascomycota, macrofungi, morphology, phylogeny, toxic.

Introduction

Podostroma cornu-damae is well known as a rare species of Hypocreaceae. The genus *Podostroma* is recognized by stipitate, clavate, and bright-colored ascospores (Chamberlain et al. 2004). There are only 20 species, subspecies, and a variety of *Podostroma* all over the world, based on Index Fungorum (2022a). Most species of *Podostroma* were initially described as *Hypocrea* and then transferred to several genera (Chamberlain et al. 2004), namely *Podocrea*, *Podostroma*, and *Trichoderma*. The prior reports confirmed that *P. cornu-damae* is frequently reported in Japan, Korea, Taiwan, and China (Gonmori et al. 2011, Choe et al. 2018). This mushroom was also reported to cause many poisonings and death in those countries (Graeme 2014, Lee et al. 2018).

In Indonesia, for 50 years, from 1900 to 1950, a mycologist named Boedijn collected and investigated many macrofungi, especially on Java Island. He was the first researcher to report *P. cornu-damae* from Buitenzorg (Bogor), West Java, in 1934. The following information about this fungus is not available for an extended period in Indonesia and Southeast Asian countries. Recently, some local reports from Indonesian foragers revealed that there are many locations with the occurrence of this macrofungi to be rediscovered in Indonesia. Our pre-work (Putra 2020) indicated that *Podostroma* cf. *cornu-damae* was spotted in Indonesia after nearly 100 years of hiatus since Boedijn. The macrofungi were found to grow in the forest of Borneo Island. However, the initial identification was only based on the macroscopic characteristics. Therefore, the present study gives the morphological (macro-and microscopic) features and phylogenetic placement of *Podostroma cornu-damae* based on ITS sequence DNA.

Materials and methods

Materials collection and morphological examination

Three fruiting bodies were collected at Tamiang Layang forest, Central Kalimantan Provinces (2°06'46.0"S 115°08'25.5"E; 20 m a.s.l), Indonesia, in January 2021. The ascospores were documented *in situ*. Macro-and microscopic characters were described based on the fresh samples. Macroscopic parameters observed include the shape of the

fruiting body, size, color, surface ornamentation, and the level of wetness. Further observation was conducted in the mycology laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia. The microscopic features examined were stroma, ascus, ascospore shape, color, and ornamentation. The morphological identification followed Boedijn (1934) and Chamberlain et al. (2004). The specimens were deposited at Herbarium Bogoriense, Indonesia, with the collection number BO24623.

DNA extraction, PCR, and Sequencing

The DNA extraction was performed using cetyltrimethylammonium bromide, following the protocol of Hermawan et al. (2020). The amplification of the Internal Transcribed Spacer (ITS) region was carried out using ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers (White et al. 1990). PCR amplification was performed in a 40 µL total reaction containing 12 µL ddH₂O, 2 µL of 10 pmol of each primer, 20 µL PCR mix from 2X Kappa Fast 2G, and 4 µL 100 ng template DNA. Amplification used a thermoline PCR. The PCR condition was set as follows: initial denaturation at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72 °C for 1 minute. The final extension was set at 72°C for 10 minutes. The amplicons were estimated on 1 % agarose gels and visualized by the Gel Doc™ XR system. PCR products were sent to the 1st Base Malaysia for sequencing. The edited ITS sequence was deposited at GenBank (OM149710).

Phylogenetic analyses

Seven selected sequences from BLAST results were used, including *Xylaria bambusicola* as an outgroup. The sequences were aligned using Clustal X Ver. 2.1 (Larkin et al. 2007) saved as PHYLIP format files. The phylogenetic tree of Randomized Axelerated Maximum Likelihood (RAxML) HPC2 on XSEDE was generated on CIPRES (Stamatakis 2014). The phylogenetic tree was produced by bootstrap analyses with 1000 replicates. Only bootstrap (BS) ≥70 was shown on the tree.

Results

Taxonomy: *Podostroma cornu-damae* (Pat.) Boedijn, *Bull. Jard. bot. Buitenz.*, 3 Sér. 13: 274 (1934)

Synonym: *Hypocrea cornu-damae* Pat., in Patouillard & Lagerheim 1895

Podocrea cornu-damae (Pat.) Sacc. & D. Sacc., Syll. fung. (Abellini) 17: 799 (1905)

Trichoderma cornu-damae (Pat.) Z.X. Zhu & W.Y. Zhuang 2014

Ascomata simple (Figure 1), cylindrical to narrowly clavate, or branched dichotomously near the tip, 5-6 cm length, 0.7-1.2 cm width, largest ascomata ca. 1.2 cm broad at basal, some cylindrical to clavate ascomata arising from the same base, light red in young ascomata, buff orange in mature ascomata, stipe with smooth surface, glabrous, brighter orange on the tip appear as the ostiole openings, perithecial elevations not visible. The surface region of the stroma (Figures 3A, B) is ca. 42 μm thick in section, composed of the intertwined thin-walled hyphae, 3.5-4 μm wide. Cells of stroma with some short, free ends. Perithecia (Figure 2) subglobose to elliptical, 211-270 μm \times 132-192 μm . The papilla of perithecia is constructed of round to ellipsoid thin-walled hyphae, papilla protruding via stroma surface. The tissue under perithecia (Figures 3C, D) with intertwined hyphae 5-8 μm wide. Asci cylindrical (Figure 4A), 56-75 \times 3-4 μm , apex thickened, with a ring. Ascospores (Figure 4B) hyaline, with two lipid bodies, finely spinulose, distal part

subglobose to slightly conical, 3.5-3.8 \times 2.8-3.4 μm , proximal part ellipsoidal to subglobose, 2.6-4.3 \times 2.8-3.5 μm .

Habitat

Growing on soil near the tree roots.

Known distribution

Japan, Java (Indonesia), Korea, Taiwan, China (Doi 1973, Boedjin 1934, Gonmori et al. 2011, Graeme 2014, Choe et al. 2018, Lee et al. 2018).

Phylogenetic analyses

The BLAST result showed that *Podostroma* BO24623 has 99.49% similarity with *P. cornu-damae* voucher ASIS24940 and 92% similarity with *Trichoderma brevicompactum* ACCC32847.

The phylogenetic tree (Figure 5) confirmed that *Podostroma* BO24623 was placed under the name of *P. cornu-damae* with an 86% BS value. *Podostroma cornu-damae* BO24623 was a sister clade of *P. alutaceum* and was in a different clade with *P. cordyceps*. In addition, *P. cornu-damae* BO24623 was in a distinct clade of *T. brevicompactum*.



Figure 1. *Podostroma cornu-damae* BO24623 in situ. A) Young ascomata with bright red and orange color; B) Mature ascomata with orange color. Bars: 1cm.

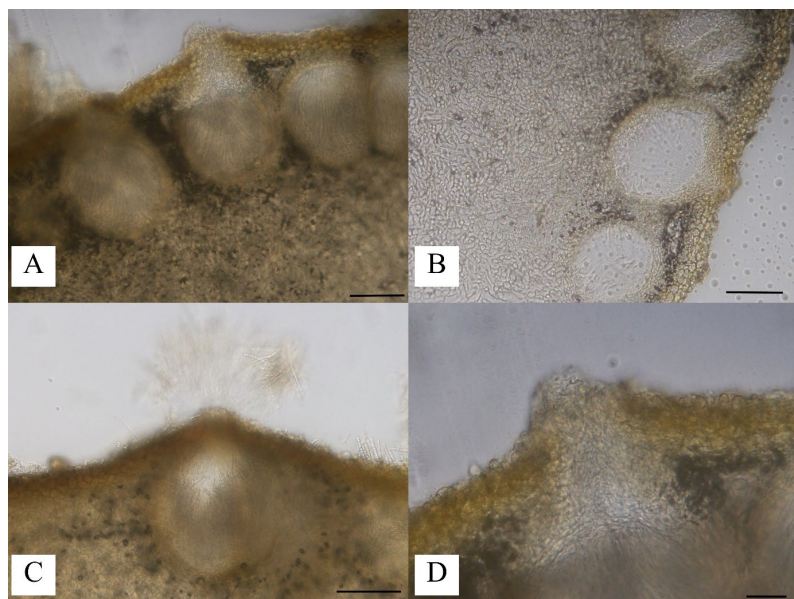


Figure 2. Perithecia characters of *Podostroma cornu-damae* BO24623. A-B) Longitudinal section showing the subglobose to elliptical perithecia; C) Ascus protruding from perithecia; D) A close view of perithecia papilla. Bars= A-C: 100 μm , D: 25 μm .

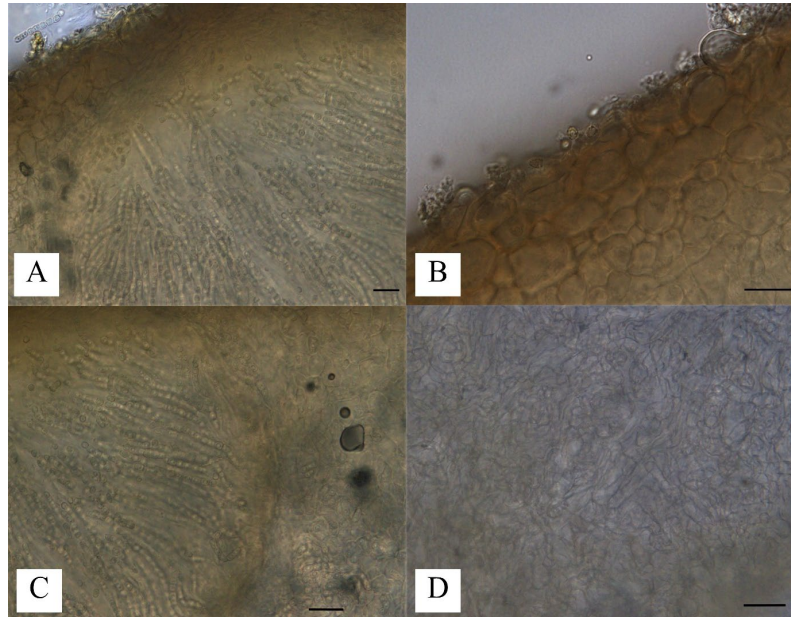


Figure 3. Hyphal stroma of *Podostroma cornu-damae* BO24623. A-B) Surface stroma with oval cells above the perithecia; C-D) The stroma below the perithecia showing interwoven hyphae. Bars= A-C: 20 μ m, D: 25 μ m.

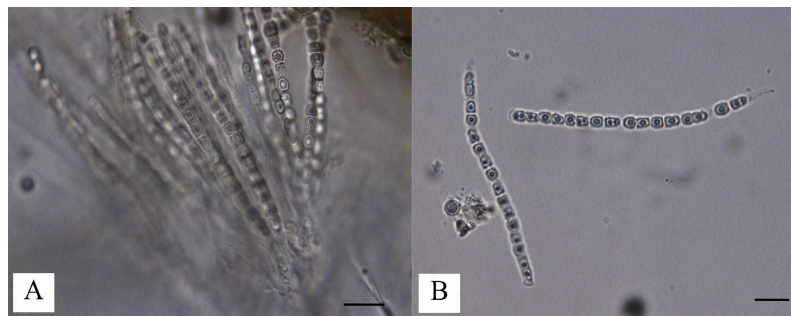


Figure 4. Asci and ascospores of *Podostroma cornu-damae* BO24623. A) Cylindrical; B) Hyaline ascospores with finely spinulose. Bars= 10 μ m.

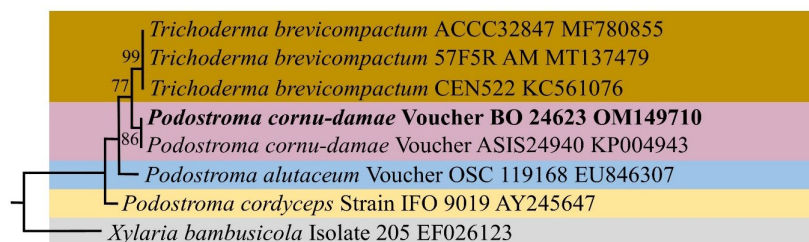


Figure 5. The phylogenetic tree of *Podostroma cornu-damae* BO24623 inferred by the ITS4/ITS5 using RAxML Black Box. The recorded species are indicated in bold. Bootstrap (BS) \geq 70 was displayed on the branches.

Discussion

Rediscoveries of rare species of fungi are of conceivable interest to the contribution of taxonomic works. In this study, we report the rediscovery of *P. cornu-damae* in Indonesia. *Podostroma cornu-damae* was previously described as *Hypocrea cornu-damae* (Patouillard 1895), renamed to *P. cornu-damae* (Boedjin 1934), and the current name of this

macrofungi is *Trichoderma cornu-damae* (Index Fungorum 2022b). In addition, Chamberlain et al. (2004) placed *Hypocrea* as the synonym of *Podostroma*. In Indonesia, Boedjin (1934) recorded the occurrence of *P. cornu-damae* in West Java. Afterward, only Putra (2020) reported the preliminary work on the occurrence of *P. cornu-damae* without specimen collection. In the present study, the macrofungi were recollected after a hiatus of nearly 100

years.

The rediscovery of unrecorded species over a long haul can improve geographical knowledge (Riddle et al. 2011). The current study and Putra (2020) revealed evidence of a broad range of geographical distribution of this macrofungi, more than 1000 km away from West Java, located in Central Kalimantan (Borneo Island). In addition, several local reports revealed that this macrofungi also had been seen in Bengkulu (Sumatra Island) and Sukabumi (West Java, Java Island). In contrast to Chamberlain et al. (2004), the specimens in this study and Putra (2020) grow on the ground near the living tree, not on the rotten wood.

In the present study, we provide the paratype specimen of *P. cornu-damae* for Indonesia and now deposited at the Herbarium Bogoriense (Indonesia) with the collection code BO24623. The holotype specimen of *P. cornu-damae*, previously described by Boedjin in 1934, was preserved in the UPS Botany, Sweden (Telenius & Shah 2018). Morphologically, *P. cornu damae* is closely related to *P. grossum* but can be distinguished by the warted and bigger ascospores in the latter species (Chamberlain et al. 2004). Traditionally, *P. cornu damae* can be recognized by several characters: stromata buff-orange, cylindrical, narrowly clavate to flabelliform or antler-shaped, dichotomously branched, distal part-ascospores $3.5-4.0 \times 3.0-3.2 \mu\text{m}$, proximal part-ascospores $3.2-4.0 \times 2.5-3.5 \mu\text{m}$ (Chamberlain et al. 2004). The morphological features of our specimens were in accordance with the concept of *H. cornu-damae* (Chamberlain et al. 2004) and the description of *P. cornu-damae* (Boedjin 1934). However, Boedijn (1934) reported that the ascospores of *P. cornu-damae* are smooth. In contrast, the ascospores of *P. cornu-damae* in the current study are finely spinulose and in line with the description of Chamberlain et al. (2004), which might have appeared smooth to Boedjin.

To increase the reliability of our identification, we coupled the morphological data with molecular analysis. The morphological and molecular data confirmed the taxonomical position of *P. cornu-damae* in our study. The phylogenetic tree showed that *P. cornu-damae* BO24623 was in the same clade as *P. cornu-damae* voucher ASIS24940, which also had the highest similarity in the BLAST result. Prior reports showed that the molecular approach is essential to obtaining an accurate taxonomy of the wild mushroom (El-Fallal et al. 2017, Akata et al. 2019, Dulay et al. 2020). Up to now, there is limited information on the nucleotide sequences of *P. cornu-damae* in GenBank. The ITS sequence of our specimens (OM149710) is now available in GenBank and can be used for future studies of *P. cornu-damae*.

As one of the most poisonous mushrooms globally, increasing public awareness of *P. cornu-damae* in Indonesia is crucial. This macrofungi contain trichothecene-type mycotoxin (Choe et al. 2018) and satratoxins (Ohta et al. 2020). They have been reported to cause poisonings and death in many East Asia regions (Graeme 2014, Lee et al. 2018). To date, there has been no poisoning case of *P. cornu-damae* in Indonesia, probably due to its rare occurrence or lack of data on Indonesian macrofungi. Some mushroom foragers cannot distinguish between *P. cornu-damae* and the clavarioid species in Indonesia. In addition, in 2019, the Indonesian Institute of Science reported that only 2.273 species of fungi (macro- and microfungi) were recorded until

2017 in Indonesia (LIPI 2019). Hopefully, the rediscovered *P. cornu-damae* in this study, with a brief description, can contribute to the mycological knowledge development in Indonesia and increase public awareness of this poisonous macrofungi.

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