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Morpho-taxonomic and phylogenetic characterization of *Artomyces pyxidatus* (Auriscalpiaceae) from the western Himalayas, India

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Abstract

In this present communication, *Artomyces pyxidatus*, belonging to the family Auriscalpiaceae, is described from the state of Uttarakhand as a new record for the region with molecular phylogeny. It was found in association with *Quercus leucotrichophora* and is characterized by being gregarious, repeatedly branched, smooth, and whitish to pale yellowish at first and sometimes darkening to pale tan or brown; it tastes peppery-acrid. *Artomyces pyxidatus* is presented here as a new record for the Uttarakhand mycobiota, and its identity is based on detailed morphology, anatomy, and nrITS-based phylogeny.

Keywords: *Artomyces pyxidatus*, Uttarakhand, new record, nrITS, Taxonomy, Phylogeny

1. Introduction

Earlier classifications grouped coral fungi into a single taxonomic category, based on the assumption that all coralloid and clavarioid forms shared a close evolutionary relationship[1, 2]. Subsequent molecular phylogenetic studies have demonstrated that these fungi are not monophyletic; instead, coralloid and clavarioid taxa are distributed among multiple orders within the Agaricomycotina[3]. To address this heterogeneity, the genus *Artomyces* included to clavarioid species characterized by simple to pyxidately branched basidiomata, the presence of hymenial gloeocystidia and gloeoplerous hyphae, and small, hyaline, amyloid basidiospores[4, 5]. Molecular phylogenetic analyses of the Russulales by Miller et al.[6] subsequently placed *Artomyces* within the Auriscalpiaceae, where it forms a distinct lineage sister to other genera in the family, including *Auriscalpium, Lentinellus, Gloiodon*, and *Dentipratulum*. Lickey et al.[7] conducted the only detailed phylogenetic and taxonomic analysis of *Artomyces* to date. Their findings corroborated Jülich's view[5] that *Artomyces* represents a distinct genus, clearly separable from *Clavicorona* Doty. In that study, seven new species were formally introduced, one species was transferred from *Clavicorona* through a new combination based on *Clavicorona microspora* Qiu X. Wu & R.H. Petersen, and two additional taxa were provisionally recognized but left unnamed. In summary, Lickey et al.[7] (2003) recognized a total of 15 species in the genus *Artomyces*, using an integrative approach that combined morphological characters, nuclear ribosomal internal transcribed spacer (nrITS) sequence data, and mating compatibility studies. During a fungal foray in Mandal area of Goesphwar, district Chamoli in Uttarakhand, we encountered a clavarioid basidiomycete in the genus that was fruiting on highly decayed wood of *Quercus leuchotrichophora* Sm. This clavarioid fungus, *Artomyces pyxidatus* (Pers.) Jülich was reported for the first time from India. In this present communication, we are presenting this species with micromorphological characteristics along with its phylogenetic analysis.

2. Materials and Methods

2.1 Morphological study

Various macro-morphological features were recorded from fresh fruiting bodies in the field along with the habitat and associated host plants. Macro-chemical test reactions with 10% KOH and 50% ammonia solution were noted down. Colour codes follow Kornerup and Wanscher[8]. Photographs of the fresh basidiomes were captured with a Canon EOS1300D. Micro-morphological characteristics were observed from free-hand sections of dried materials. Sections were mounted in a mixture of 5% KOH, 1% Phloxine, and 1% Congo red, then observed under a compound microscope (Olympus BX43). Line drawings were made with the help of Camera lucida attached to a microscope at 1000x magnification. Microphotographs of various elements were taken using a digital camera attached to an Olympus CX33 compound microscope. A total of 50 basidiospores from each of the specimens were observed. Basidiospore measurements are represented as minimum-mean-maximum length × minimum-mean-maximum width and Q = length/width of basidiospores.

2.2 DNA extraction, PCR amplification, and sequencing

Nuclear genomic DNA was isolated from 100 mg of dried fruit bodies using a fungal genomic DNA Mini Kit (RGCB Thiruvananthapuram). The ITS region of the nuclear ribosomal DNA gene was amplified using primer pairs ITS1 and ITS4[9]. PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer 0.2 mM each dNTPs, 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5 M Betaine, 5 pM of forward and reverse primers. PCR amplification was carried out in a PCR thermal cycler programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 sec at 50°C, and a final stage of 4 min at 60°C. The PCR products were purified with QIAquick Gel Extraction Kit (QIAGEN, Germany) and then subjected to Sanger sequencing in an automated DNA sequencer using the same primers. The obtained sequences were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/) and accession numbers of two collections are given in Figure 1.

2.3 Phylogenetic analysis

Phylogenetic analysis was performed using the nrITS dataset comprising our newly generated two sequences along with the sequences retrieved from BLAST searches[10] as well as previously published phylogenies[11, 12]. In this study, a dataset of 56 nrITS sequences of *Artomyces* species including our sequences were used to analyze the data. The nrITS dataset was aligned with MAFFT v.7[13]. Maximum likelihood (ML) phylogenetic analysis inferred from nrITS sequences was performed using MEGA-X software[14]. One-thousand bootstrap (BS) replicates were analyzed to obtain nodal support values. Bootstrap support values (>50%) obtained from ML analysis are shown above or below the branches at nodes. The species of *Lentinus* were used as an out-group for the present phylogenetic analysis.

3. Results

3.1 Phylogeny

The Indian collections are placed within the *Artomyces pyxidatus* clade suggesting their identity as *A. pyxidatus,* SC/PU/24 (which is also evident in the BLAST search and are also well supported by 100% bootstrap values). Our nrITS phylogenetic analysis showed that sequences derived from the Indian collections of *A. pyxidatus* (SC/PU/24) nested within the clade consisting of the sequence of *A. pyxidatus* (PP392806; KR673584) collection from China and Korea, respectively with support value of (MLbs = 100%). Our phylogenetic analysis strongly suggests that the Indian collections are conspecific with Asian *A. pyxidatus* (Fig. 1).

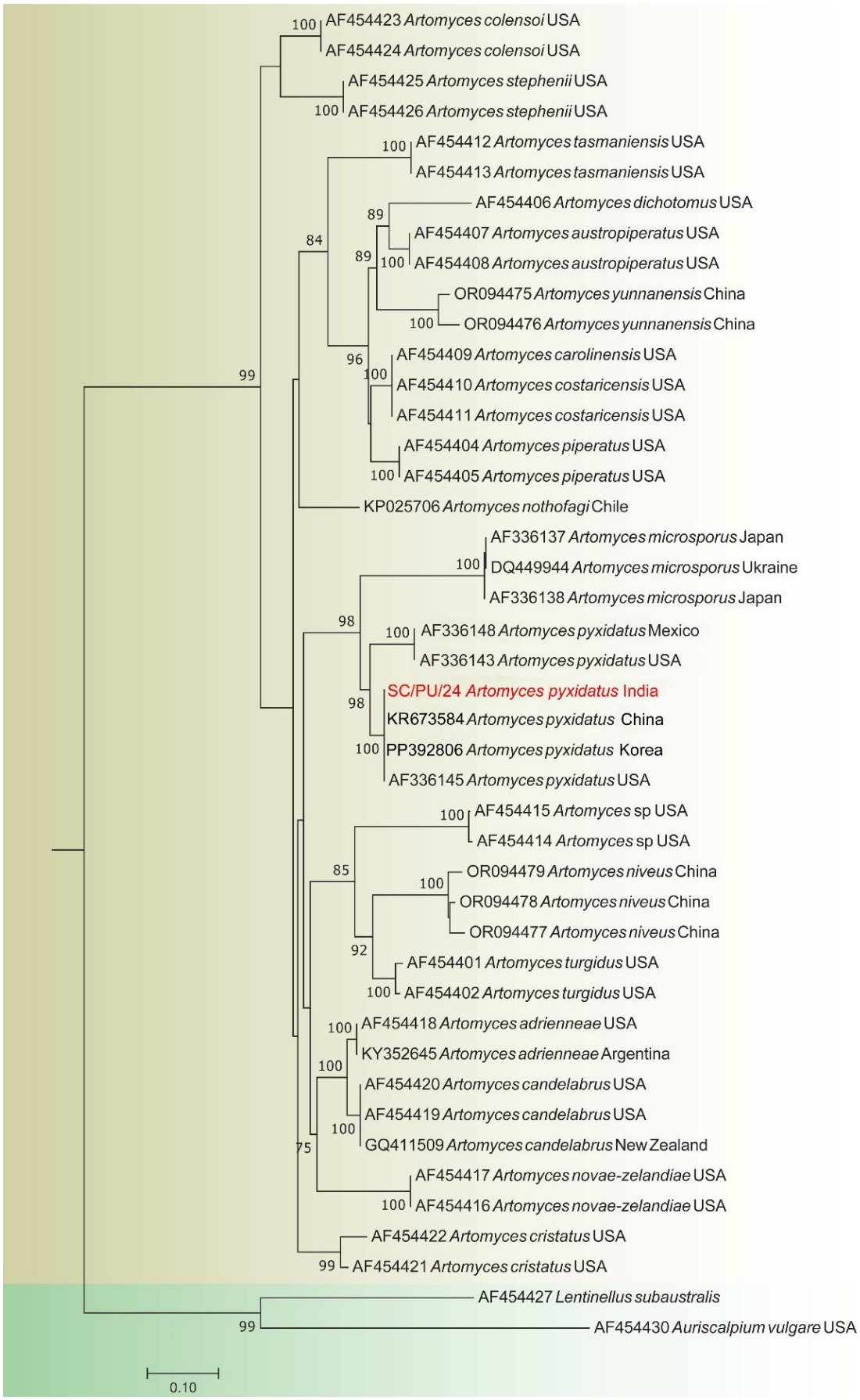

Figure 1. Maximum Likelihood phylogenetic tree inferred from ITS-rDNA sequence data using GTR+GAMMA model of nucleotide evolution constructed in RAxML v.2.0.10. Branches are labelled with ML bootstrap support values (≥50 %). Sequence derived from Indian collection of ***Artomyces pyxidatus*** is shown as bold in the tree

3.2 Taxonomy

***Artomyces pyxidatus* (Pers.) Jülich** (Figs. 2, 3)

Artomyces pyxidatus (Pers.) Jülich (1982) (Syn: *Clavaria coronata* Schwein., 1832; *Clavaria petersii* Berk. & M. A. Curtis, 1873; *Clavaria pyxidata* Pers., 1794; *Clavaria pyxidata* var. *asperospora* S. G. M. Fawc., 1938; *Clavaria pyxidata* var. *pyxidata* Pers., 1794; *Clavicorona coronata* (Schwein.) Doty, 1947; *Clavicorona pyxidata* (Pers.) Doty, 1947; *Clavicorona pyxidata* var. *asperospora* (S. G. M. Fawc.) Lickey et al., 2003; *Clavicorona pyxidata* var. *pyxidata* (Pers.) Doty, 1947; *Merisma pyxidatum* (Pers.) Spreng., 1827)

Description: Basidiomata lignicolous, single or in small groups, reaching up to 80 mm in height and about 25 mm in width at the apex. Typically branched in ranks of 2-5, coronate with pointed tips. Apices coronate-cristate to cuspidate, pale yellow (2B3) to light brown (5A2), becoming dark brown (5A4) on drying. A distinct white basal mycelium is present at the base. Odor peppery, Taste acrid.

Context composed of two hyphal types: generative hyphae with thin walls, usually encrusted with yellow-brown deposits, measuring 3.0–12.0 µm in diameter and bearing clamp connections; and gloeoplerous hyphae, abundant in the trama, aseptate, 3.4–7.5 µm wide, which terminate in the hymenium as gloeocystidia. Gloeocystidia are frequent, predominantly cylindrical to clavate, occasionally fusiform, flexuous, or subcapitate to capitate, 4–7.5 µm wide, consistently constricted within the subhymenium. Basidia are mostly four-sterigmate narrowly clavate to cylindrical, with conspicuous basal clamp connections, measuring 19.5–22.3 × 4.7–5.5 µm. Basidiospores measure 3.0–4.1 × 2.5–3 µm with Q values of 1.0–1.4 (Qm = 1.3), are globose to subglobose, smooth, hyaline, and strongly amyloid in Melzer's reagent, containing a single large guttule.

Habit and Habitat: Gregarious, on wood log of *Quercus leucotrichophora* Sm.

Specimen examined: INDIA. Uttarakhand: district Chamoli, Gopeshwar, Mandal 30°27'20.84"N 79°16'38.24"E, elev. 1900m, 04 July 2024, Shikha Choudhary and Priyanka Uniyal (SC/PU/24).

Distribution: This species has been reported from China and USA[5, 15] and now also from Himalayan district of Chamoli, India.

Notes: *Artomyces pyxidatus* is a species with wide geographical distribution with diverse hosts and chiefly characterized by the presence of almost white, profusely branched basidiomata with cuspidate tips and elongated basidiospores[7]. Present Indian specimen is in conformity with *Artomyces pyxidatus* based on its morphological and microscopical similarities which is also supported by the results of phylogeny depicted in the phylogram.

Discussion

The present record of *Artomyces pyxidatus* from the Chamoli district of Uttarakhand represents a significant extension of its known geographical range to the western Himalaya. The Indian specimen closely matches published descriptions of *A. pyxidatus* in both macro- and micromorphological characters.

Ecologically, the occurrence of *A. pyxidatus* on decaying wood of *Quercus leucotrichophora* at about 1900 m elevation is consistent with its known preference for hardwood substrates in temperate forest ecosystems, while also adding a new host record. The close morphological agreement, supported by phylogenetic placement, validates the identification and underscores the wide ecological amplitude of the species.

Conclusion

The present study provides the first confirmed record of *Artomyces pyxidatus* from the Western Himalayan region of India. Detailed macro- and micromorphological observations, coupled with phylogenetic support, firmly establish the identity of the species. This new regional record significantly extends the known distribution of *A. pyxidatus* and emphasizes the underexplored diversity of clavarioid fungi in the western Himalaya. Continued systematic surveys integrating morphological and molecular data are essential to improve our understanding of fungal diversity, biogeography, and host associations in this ecologically important region.

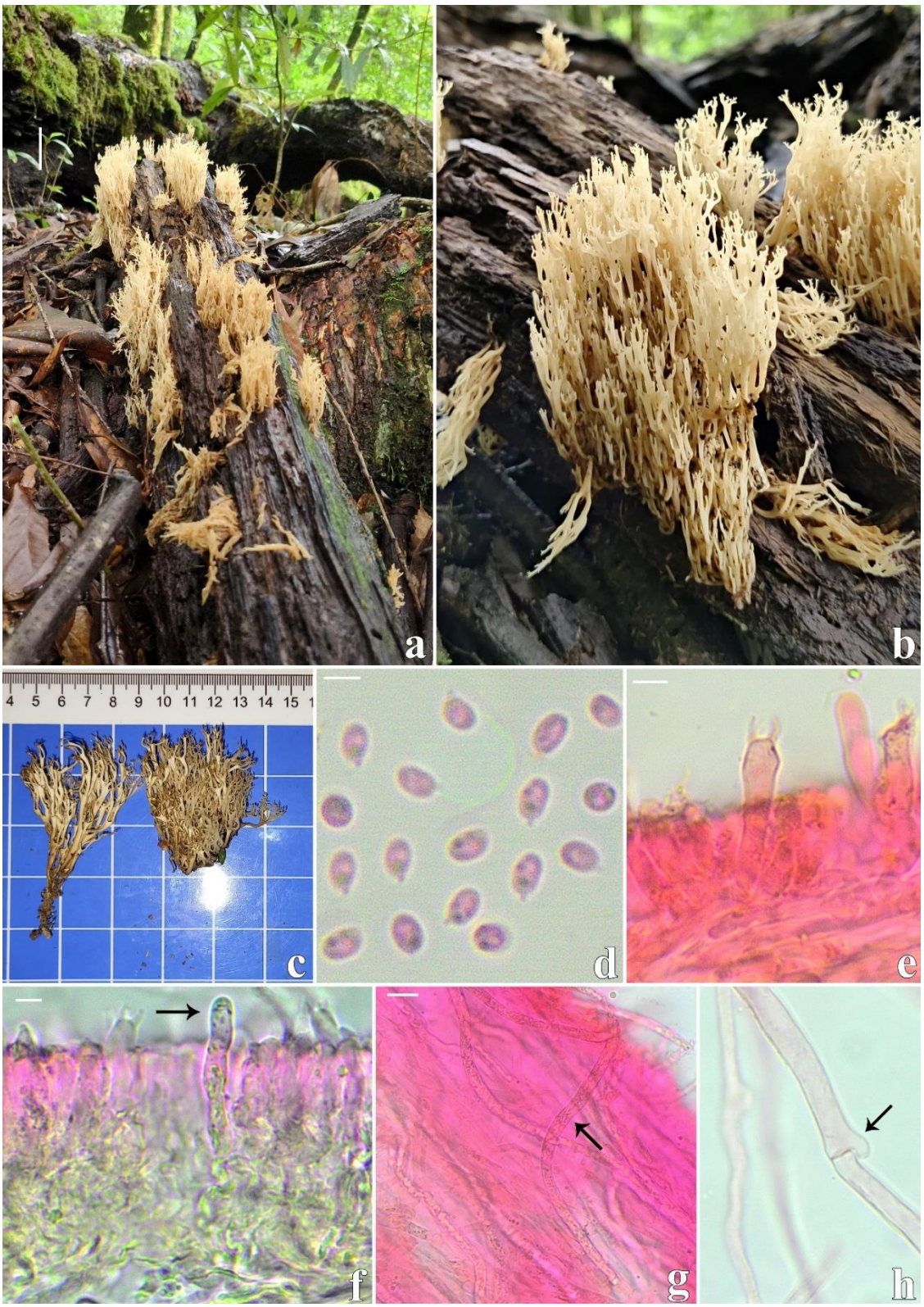

Figure 2. ***Artomyces pyxidatus*.: a–c.** Fresh basidiomata in the field and basecamp; d. Basidiospores; **e.** Basidia; **f.** Gloeocystidia; **g.** Gloeopleurous hyphae; **h.** Contextual hyphae showing clamp connection.Scale bars: **a =** 10 mm, **d–h =** 10 µm

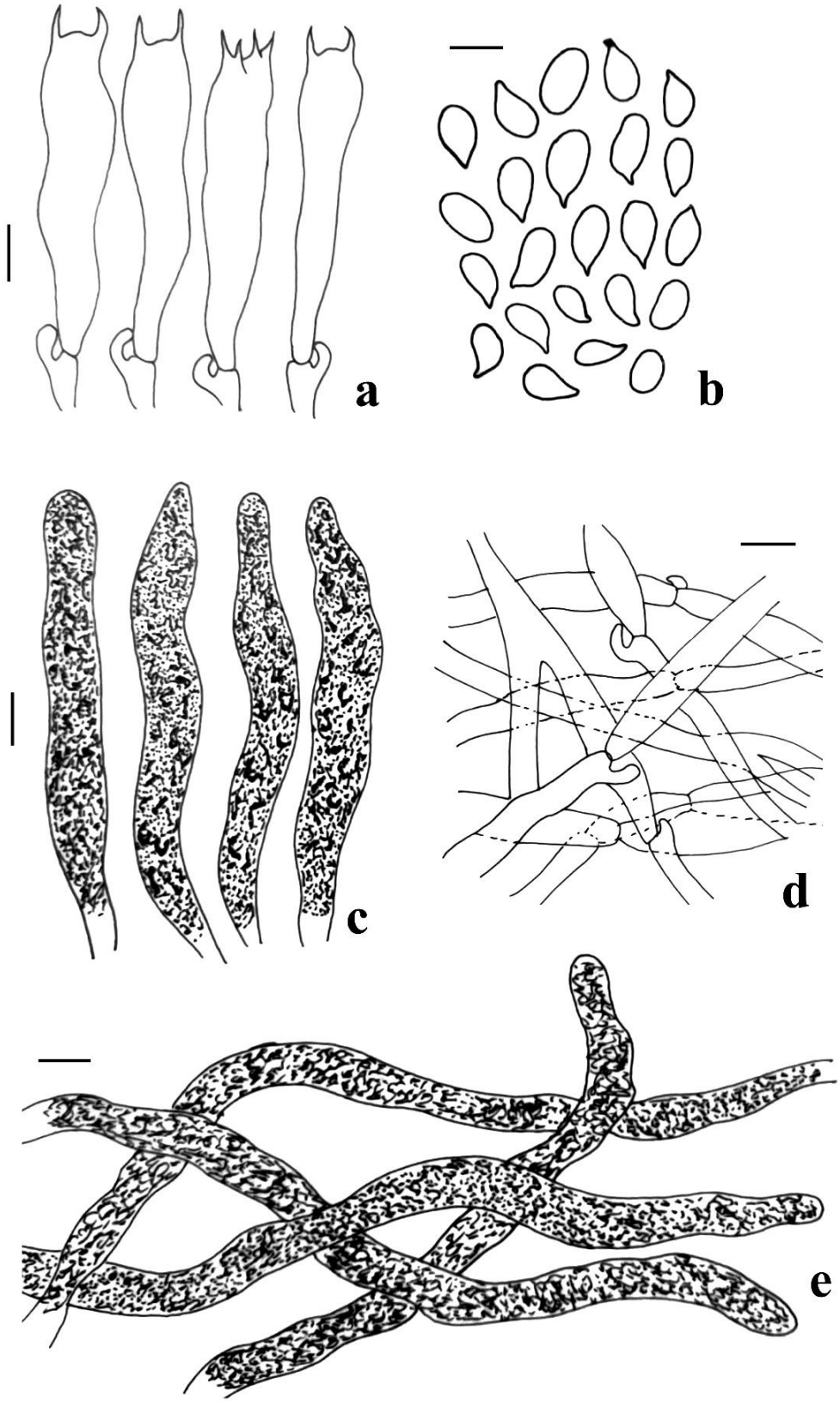

Figure 3. Line drawings of *Artomyces pyxidatus*.: a. Basidia; **b.** Basidiospores; **c.** Gloeocystidia; **d.** Contextual hyphae showing clamp connection; **e.** Gloeopleurous hyphae. Scale bars: **a–e =** 10µm

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Authors' Contributions

SC conducted the experiment and wrote the original draft of the paper. PU conceptualized and supervised the project, while YPS handled the editing and corrections and helped in writing original draft. All authors have read and agreed to the final version of the manuscript.

Data Availability Statement

All data generated is included in this article.

Conflict of interest

The authors declare that there is no conflict of interest regarding this research work.

Ethical Statement

This study involved plant materials only. No experiments were conducted on human participants or live animals. All experimental procedures complied with relevant institutional, national, and international guidelines for plant research and environmental safety. The authors confirm that no ethical approval was required for this study.

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