



Cryptocoryneum polyramosum sp. nov., associated with *Camellia sinensis* in Sichuan Province, China

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Abstract

Most species of *Cryptocoryneum* have been reported on dead wood, with some species rarely reported from leaf litter or arthropod dung. *Cryptocoryneum polyramosum* sp. nov., collected from dead twigs of tea (*Camellia sinensis*), grown in high mountain areas in Sichuan Province in China, is described and illustrated. The new species is distinguished by having up to 24 cylindrical, brown, smooth conidial arms, a significantly higher number compared to other known *Cryptocoryneum* species, which typically possess fewer than 16 arms. The phylogenetic analyses based on a combined sequence dataset of LSU, SSU, ITS, *tef1-a*, and *rpb2* indicate that *C. polyramosum* forms a distinct clade, sister to other species within the genus. Comparative morphological analysis with other *Cryptocoryneum* species highlights the unique features of this taxon. A detailed list of morphological variations is provided to enhance the understanding of the genus *Cryptocoryneum* and its species diversity.

Key words: 1 new taxon, asexual morph, Dothideomycetes, multi-locus, phylogeny, taxonomy

Introduction

Camellia sinensis, commonly known as tea, originated in Southeast Asia and has distributed worldwide through foreign trade and cultural exchanges (Liu *et al.* 2015, Drew 2019, Xia *et al.* 2020a). Tea is widely cultivated in tropical and subtropical areas, and considered to be one of the most important commercial crops globally (Manawasinghe *et al.* 2021, Wang *et al.* 2024). As the homeland of tea, China is the largest producer and consumer, with tea playing a pivotal role in the country's economy (Pan *et al.* 2023, Tao 2023). Development of the tea industry in China has not only generated significant economic value but also contributed to rural economic growth and employment (Liu 2023). Over the past three decades, Sichuan Province has emerged as one of China's major tea production regions (Xiao *et al.* 2018). By 2021, the comprehensive output value of the tea industry in Sichuan exceeded 100 billion RMB, establishing it as an essential pillar of the regional economy (Liang 2024).

Unfortunately, *Camellia sinensis* is susceptible to numerous diseases caused by bacteria, fungi, insects, nematodes and viruses (Zhang *et al.* 2015, Qi *et al.* 2016, Pandey *et al.* 2021). Among the biological threats to tea cultivation, fungal pathogens are the most significant contributors to disease outbreaks (Liu *et al.* 2019, Pandey *et al.* 2021). Accurate identification of diseases affecting various parts of the tea plant is critical to improving tea production and quality. Many pathogenic fungi have been isolated and identified from tea (Yan *et al.* 2018, Tsai *et al.* 2020, Pandey *et al.* 2021, Lin *et al.* 2022), including *Colletotrichum* spp., *Exobasidium vexans* (blister blight), *Macrophoma theicola* (stem canker, twig dieback), *Pellicularia koleroga* (black blight, thread blight), *Pestalotiopsis* (brown blight), *Pseudopestalotiopsis theae* (grey blight), and *Tunstallia aculeate* (thorny stem blight) (Liu *et al.* 2017, Yang *et al.* 2018a, b). In China, more than 100 fungal species have been reported to cause tea plant diseases on the most commercially significant plant parts, including buds, leaves, and shoots (Gao *et al.* 2016, Jayawardena *et al.* 2016b, Liu *et al.* 2016a, b, Li *et al.* 2019, Manawasinghe *et al.* 2021).

As part of an on-going study of exploring the fungal diversity associated with tea plants cultivated in high-

altitude regions of Sichuan Province, a novel species was isolated from dead twigs of *Camellia sinensis*. This study describes the new species based on morphological, molecular and phylogenetic evidence, contributing to the growing understanding of fungal diversity and its ecological interactions with tea plants.

Materials and methods

Specimen collection, isolation and morphological identification

Specimens were collected from dead twigs of tea grown in high mountain areas in Sichuan Province during October 2023 and were taken to the laboratory. Morphological observations were made using a Nikon ECLIPSE E200 stereo microscope, following the methods described in Senanayake *et al.* (2020). Digital images were recorded with a Nikon ECLIPSE Ni-U compound microscope fitted with a Nikon DS-Ri2 microscope-camera system. Measurements were made with the Tarosoft (R) Image Framework program v. 0.9.7, and Adobe Photoshop 2022 (Adobe Systems Inc., San Jose, CA, USA) was used for the processing of photographic plates following Liu *et al.* (2010). Pure fungal cultures were obtained by single-spore isolation as described in Chomnunti *et al.* (2014). Germinating spores were transferred to potato dextrose agar (PDA), and the cultures grown in an incubator at 25 °C. Colony characteristics were periodically observed and recorded while the colony colour was determined according to Rayner (1970).

The holotype and an isotype specimen were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS) in Kunming, China, and the Herbarium of the University of Electronic Science and Technology (HUEST), Sichuan Province, China. Living cultures obtained in this study were deposited in the China General Microbiological Culture Collection Center (CGMCC) and the University of Electronic Science and Technology Culture Collection (UESTCC). Names and numbers of the new taxon were registered in MycoBank (Crous *et al.* 2004).

DNA extraction, PCR amplification and sequencing

Fungal mycelia grown on PDA plates for 7 days were scraped for genomic DNA extraction using the Trelief TM Plant Genomic DNA Kit (Beijing TsingKe Biotech Co., Beijing, China). The following five partial gene regions were amplified and sequenced: large subunit rRNA gene (LSU), small subunit rRNA gene (SSU), internal transcribed spacer (ITS), translation elongation factor 1-alpha gene (*tef1- α*) and RNA polymerase II subunit B gene (*rpb2*). PCR products were checked by agarose gel electrophoresis and sequencing was carried out by Sangon Biotech Co., Shanghai, China.

Sequence alignment and phylogenetic analyses

Based on BLASTn results and recent publications, related sequences were derived from Hongsanan *et al.* (2018) and Boonmee *et al.* (2021) and downloaded from GenBank (Table 1). The sequences were aligned with the online multiple alignment program MAFFT version 7 (Katoh & Standley 2013). The exports were further trimmed using trimAl (Capella-Gutierrez *et al.* 2009) version 1.2, and the sequence dataset was concatenated by SequenceMatrix 1.7.8 (Vaidya *et al.* 2011). Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) methods following Dissanayake *et al.* (2020). ML analysis was performed by the RAxML HPC v8_XSEDE tool via the CIPRES Science Gateway V3.3 (Miller *et al.* 2010) with rapid bootstrap analysis/search for the best-scoring ML tree (-f a). The BI analysis was conducted with PAUP version 4.0a169 (Swofford 2002) and MrBayes v.3.2.7 (Huelsenbeck & Ronquist 2001). The best model for each locus was determined independently by MrModeltest 2.3 (Nylander *et al.* 2004), and posterior probabilities were evaluated by Markov chain Monte Carlo sampling (MCMC) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002). The phylogenetic tree was visualized by Treeview version 1.6.6 (Page 1996) and drawn in Adobe Illustrator 2024 (Adobe Systems Inc., San Jose, CA, USA). Alignment and tree were deposited in TreeBASE. (www.treebase.org)

TABLE 1. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers. Newly generated sequences are indicated in red and ex-type strains are in bold.

Species	Voucher/Strain /Culture	GenBank accession numbers				
		LSU	SSU	ITS	<i>tefl-a</i>	<i>rpb2</i>
<i>Aquasubmersa japonica</i>	KT 2862	LC061587	LC061582	LC061592	LC194384	LC194421
<i>Aquasubmersa mircensis</i>	MFLUCC 11-0401	JX276955	JX276956	JX276954	–	–
<i>Atrocalyx acutisporus</i>	KT 2436	LC194341	LC194299	LC194475	LC194386	LC194423
<i>Crassimassarina macrospora</i>	KT 1764	LC194344	LC194302	LC194478	LC194389	LC194426
<i>Cryptoclypeus oxysporus</i>	KT 2772	LC194345	LC194303	LC194479	LC194390	LC194427
<i>Cryptoclypeus ryukyuensis</i>	KT 3534	LC194347	LC194305	LC194481	LC194392	LC194429
<i>Cryptocoryneum akitaense</i>	KT 3019	LC194348	LC194306	LC096154	LC096136	LC194430
<i>Cryptocoryneum brevicondensatum</i>	yone 152	LC194349	LC194307	LC096155	LC096137	LC194431
<i>Cryptocoryneum condensatum</i>	CBS 122629	LC194351	LC194309	LC096157	LC096139	LC194433
<i>Cryptocoryneum condensatum</i>	CBS 122633	LC194352	LC194310	LC096158	LC096140	LC194434
<i>Cryptocoryneum congregatum</i>	KT 2892	LC194353	LC194311	LC096159	LC096141	–
<i>Cryptocoryneum japonicum</i>	KT 2961	LC194354	LC194312	LC096160	LC096142	LC194436
<i>Cryptocoryneum japonicum</i>	KT 3300	LC194356	LC194314	LC096162	LC096144	LC194438
<i>Cryptocoryneum longicondensatum</i>	KT 2913	LC194360	LC194318	LC096166	LC096148	LC194442
<i>Cryptocoryneum longicondensatum</i>	KT 3487	LC194361	LC194319	LC096167	LC096149	LC194443
<i>Cryptocoryneum paracondensatum</i>	KT 3241	LC194363	LC194321	LC096169	LC096151	LC194445
<i>Cryptocoryneum polyramosum</i>	CGMCC 3.27592	PQ654501	PQ654503	PQ655488	PQ660441	PQ660443
<i>Cryptocoryneum polyramosum</i>	UESTCC 24.0134	PQ654502	PQ654504	PQ655489	PQ660442	PQ660444
<i>Cryptocoryneum pseudorilstonei</i>	CBS 113641	LC194364	LC194322	NR_153941	LC096152	LC194446
<i>Cryptocoryneum rosae</i>	NX25-2	MZ493315	MZ493287	MZ493301	MZ508410	MZ508419
<i>Cryptocoryneum rosae</i>	NX25-1	MZ493314	MZ493286	MZ493300	MZ508409	MZ508418
<i>Cryptocoryneum</i> sp.	CBS 114518	LC194365	LC194323	LC096171	LC096153	LC194447
<i>Galeaticarpa aomoriensis</i>	KT 2563	LC194366	LC194324	LC194482	LC194393	LC194448
<i>Lophiotrema eburnoides</i>	KT 1424-1	LC001707	LC001706	LC001709	LC194403	LC194458
<i>Lophiotrema fallopiae</i>	KT 2748	LC149915	LC149911	LC149913	LC194404	LC194459
<i>Lophiotrema neohysterioides</i>	KT 713	AB619019	AB618701	LC194495	LC194408	LC194463
<i>Lophiotrema neohysterioides</i>	KT 756	AB619020	AB618702	LC194496	LC194409	LC194464
<i>Lophiotrema nucula</i>	CBS 627.86	AB619021	AB618703	LC194497	LC194410	LC194465
<i>Pseudocryptoclypeus yakushimensis</i>	KT 2186	LC194380	LC194338	LC194504	LC194417	LC194472

Results

Phylogenetic analyses

The BLASTn results and related publications indicated that our collections have affinities with Cryptocoryneaceae in Pleosporales. The combined sequence dataset of 29 taxa, including *Aquasubmersa japonica* (KT 2862) and *Aquasubmersa mircensis* (MFLUCC 11-0401) as the outgroup taxa, comprised a total of 4,785 characters (LSU: 1315 bp; SSU: 1021 bp; ITS: 519 bp; *tefl-α*: 918 bp; *rpb2*: 1012 bp). The matrix had 974 distinct alignment patterns with 5.08 % of undetermined characters or gaps. Estimated base frequencies: A = 0.249797, C = 0.251824, G = 0.267646, T = 0.230734; substitution rates: AC = 1.662220, AG = 4.397584, AT = 1.474880, CG = 1.255283, CT = 10.423588, GT = 1.000000, alpha parameter of GAMMA model: 0.119587, tree length: 0.774124. The best-scoring ML tree (final ML optimization likelihood: -18448.035943) was chosen to represent the phylogenetic relationships of the new species with other selected species in Cryptocoryneaceae and Lophiotremataceae (Dothideomycetes, Pleosporales). The evolutionary models for BI analysis with GTR+I+G for LSU and *tefl-α*, HKY+I for SSU, SYM+I+G for ITS, and GTR+G for *rpb2* were selected. The topological structure of the BI analysis is almost identical to the ML tree, and most species are highly supported statistically. Combining the phylogenetic results obtained from ML and BI analysis, the topological structure of our two isolates forms a distinct clade basal to *Cryptocoryneum* (Fig. 1).

Taxonomy

Cryptocoryneum polyramosum X.D. Liang. & Jian K. Liu, *sp. nov.* (Fig. 2)

Mycobank number MB 856774

Etymology:—Named for having numerous conidial arms.

Holotype:—HKAS 135162

Saprobic on dead twigs of *Camellia sinensis*. **Sexual morph:** Undetermined. **Asexual morph:** *Sporodochia* pulvinate, often confluent, dark brown to black. *Conidiophores* arising from stromatic cells, simple, straight, septate, hyaline to pale brown, smooth. *Conidiogenous cells* 7.0–13.0 × 4.0–8.0 μm (\bar{x} = 9 × 6.5 μm, n = 20), monoblastic, cylindrical, terminal, determinate, hyaline to pale brown. *Conidia* 33.5–58.5 × 20–51.5 μm (\bar{x} = 46.0 × 28.0 μm, n = 50), solitary, acrogenous, branched, cheiroid, with dark brown to black cap cells firmly uniting the branches together, multi-armed. *Basal cells* 3.0–7.0 × 3.0–6.5 μm (\bar{x} = 5.0 × 5.0 μm, n = 30), dark brown to black, cuneiform, smooth, thin-walled. *Arms* 17.0–44.5 × 3.5–6.0 μm (\bar{x} = 33.5 × 4.5 μm, n = 50), 7–24 armed, cylindrical, branched at base, pale brown, smooth, 3–9-septate, slightly constricted at the septa, guttulate in each cell, cells 3.0–6.0 × 3.0–4.5 μm (\bar{x} = 4.5 × 3.5 μm, n = 50).

Culture characteristics:—Conidia germinating on PDA within 24 h and germ tubes arising from cells of the arms. Colonies growing on PDA reaching a diam. of 3 cm after 4 weeks at 25 °C. Velvety, grey olivaceous to olivaceous black, reverse almost black with white edges, without sporulation.

Material examined:—CHINA. Sichuan Province: Le Shan City, Mabian Yi Autonomous County, Fu Lai Village, 28°55'52"N, 103°33'15"E, 1100 m, on dead twigs of *Camellia sinensis*, 22 October 2023, X.D. Liang, M5 (HKAS 135162, holotype; ex-type living culture, CGMCC 3.27592); *ibid.*, M5-2 (HUEST 24.0151, isotype; ex-isotype living culture, UESTCC 24.0134).

Notes:—In the multi-gene phylogenetic tree (Fig. 1), two isolates of *Cryptocoryneum polyramosum* form a distinct clade basal to other species within the genus *Cryptocoryneum* (Cryptocoryneaceae). Morphologically, *C. polyramosum* resembles *Cryptocoryneum* species by appearing as pulvinate sporodochia, with cheiroid conidia, and occasionally branched conidial arms that irregularly developed downward from the cap cells. However, asexual morph of adjacent family Lophiotremataceae species, which are characterized by pycnidial colonies, globose to subglobose, scattered, semi-immersed ostiolates, differ distinctly from *C. polyramosum* (Hashimoto *et al.* 2017). Comparison with the morphological key to *Cryptocoryneum* (Hashimoto *et al.* 2016) and ten other species lacking molecular data reveals that *C. polyramosum* shares similarities with *C. rilstonei* in conidial size (Hashimoto *et al.* 2016). However, *C. polyramosum* has slightly longer and wider conidia than *C. rilstonei* (33.5–58.5 × 20.0–51.5 μm vs. 21–40 × 17–32.5 μm) and more arms (7–24, \bar{x} = 13 arms vs. 5–9, \bar{x} = 7 arms). Further morphological comparison with nine species for which molecular data are available indicates that *C. polyramosum* closely resembles *C. rosae* (Boonmee *et al.* 2021)

in conidial morphology. Nevertheless, *C. polyramosum* has slightly longer and wider conidia ($33.5\text{--}58.5 \times 20.0\text{--}51.5 \mu\text{m}$ vs. $30\text{--}50 \times 20\text{--}30 \mu\text{m}$), fewer septa (3–9, $\bar{x} = 6$ septa vs. 8–12, $\bar{x} = 9$ septa) and more arms (7–24, $\bar{x} = 13$ arms vs. 4–12, $\bar{x} = 9$ arms). This combination of unique morphological features and robust phylogenetic evidence supports the recognition of *C. polyramosum* as a novel species. The morphological distinctions and basal phylogenetic position underscore its evolutionary significance and contribute to a more comprehensive understanding of species diversity within the Cryptocoryneaceae.

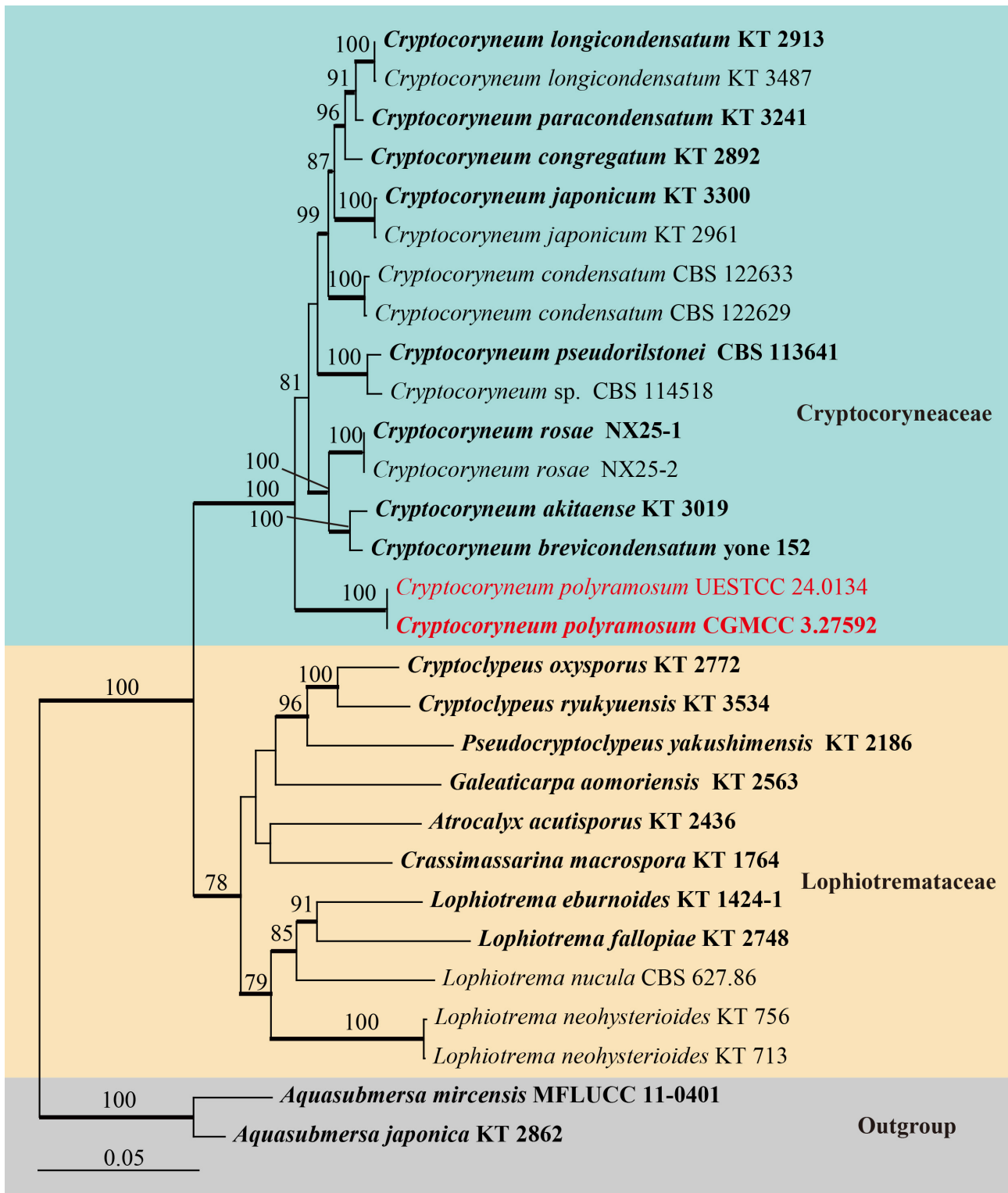


FIGURE 1. Phylogenetic tree generated from RAxML analysis of combined LSU, SSU, ITS, *tef1-a* and *rpb2* dataset. ML bootstrap values equal to or greater than 75% are shown above the nodes. Branches in bold indicate BI posterior probabilities (PP) equal to or greater than 0.95. Newly generated isolates are indicated in red and ex-type strains are in bold. The tree is rooted with *Aquasubmersa japonica* (KT 2862) and *A. mircensis* (MFLUCC 11-0401).

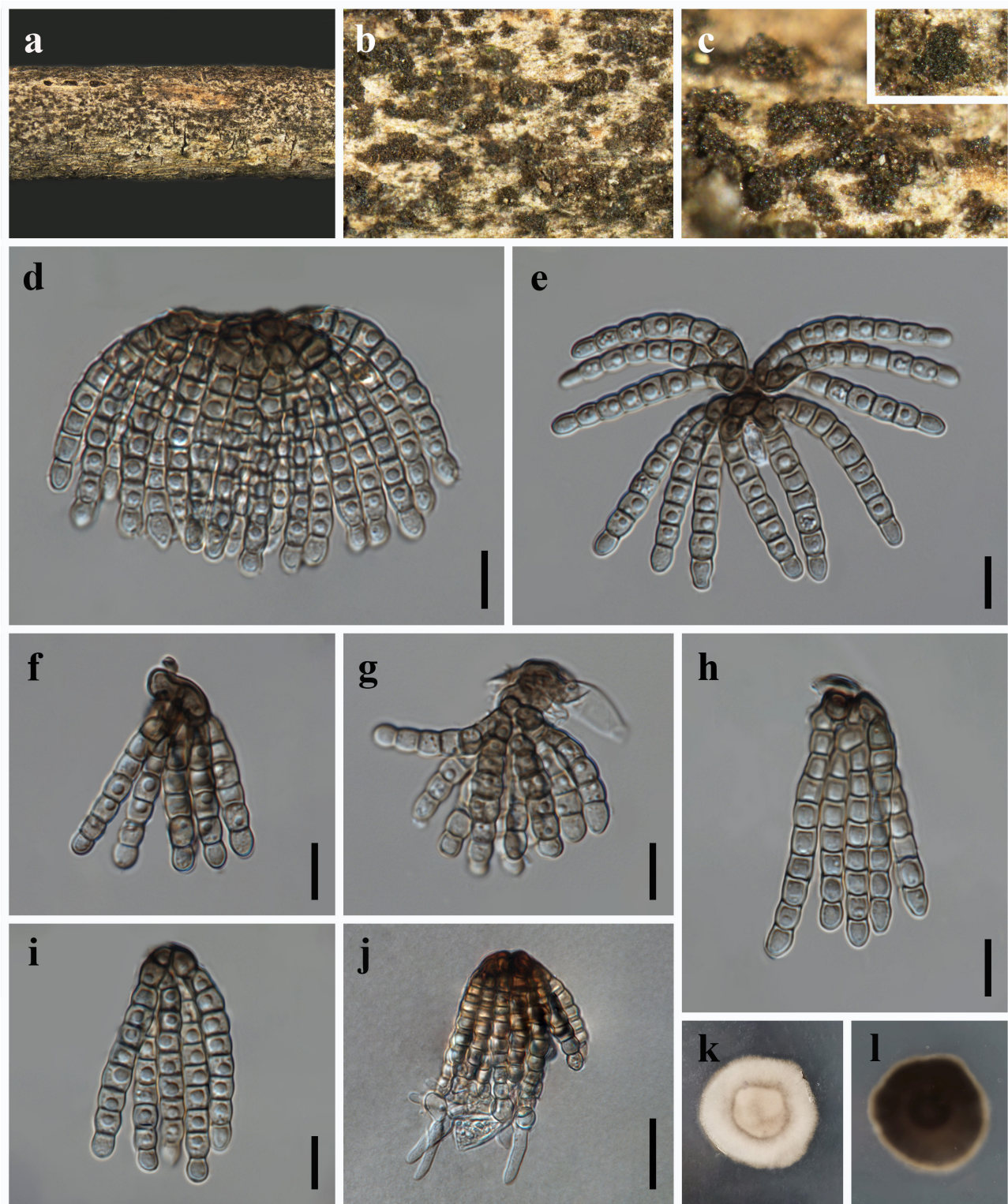


FIGURE 2. *Cryptocoryneum polyramosum* (HKAS 135162 holotype). a–c. Appearance of sporodochia on host surface. d, e. Cheiroid conidia. f, g. Conidia with exfoliated cap cells. h, i. Conidia with brown basal cells. j. Germinating conidium. k, l. Culture on PDA from above and below. Scale bars: d–i = 10 μ m, j = 20 μ m.

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