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Trichoderma longicollum (*Hypocreales*), a new species from Taiwan

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Coastal lagoons encompass various precious natural resources and are among the most productive ecosystems on Earth. A new *Trichoderma* species was identified during an investigation of fungi in mangrove soil in Qigu Lagoon, Taiwan. In this study, we provide the description, illustration, and phylogenetic analyses of this new species, *Trichoderma longicollum*.

Key words: coastal lagoons, mangrove soil, *Trichoderma*, phylogenetic analyses.

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Pobřežní laguny obsahují cenné přírodní zdroje a patří mezi nejproduktivnější ekosystémy na Zemi. Během výzkumu hub z mangrovové půdy v laguně Qigu na Taiwanu byl identifikován nový druh rodu *Trichoderma*. Studie přináší popis, ilustraci a fylogenetické analýzy tohoto nového druhu, *Trichoderma longicollum*.

SHORT TAXONOMIC REPORT

INTRODUCTION

The genus *Trichoderma* Persoon (1794) is a diverse group of fungi comprising mycoparasites, phytopathogenic antagonists, and plant symbionts (Nascimento Brito et al. 2023). *Trichoderma* spp. are widely distributed and can be frequently found in soil, organic matter, decomposing materials, and plant tissues as endophytes (Prameeladevi et al. 2021b, Zheng et al. 2021). Traditionally, *Trichoderma* classification was exclusively based on morphological and growth characteristics.

However, the taxonomic analysis of this genus becomes increasingly complicated with the growing number of newly discovered species due to their shared morphological characteristics (Prameeladevi et al. 2021a). DNA barcoding has significantly enhanced identification accuracy of *Trichoderma* species. Recently, the internal transcribed spacer region (ITS), translation elongation factor 1-alpha (*tef1*), and RNA polymerase subunit 2 (*rpb2*) sequences have been used for phylogenetic analyses and new *Trichoderma* species identification (Dou et al. 2020, Cai et Druzhinina 2021, Zhao et al. 2023). Morphological features combined with molecular studies have allowed for the description of more than 500 valid *Trichoderma* species to date (Sousa et al. 2023).

In this study, we describe a new *Trichoderma* species, *T. longicollum*, collected from mangrove soil in Qigu Lagoon, Taiwan.

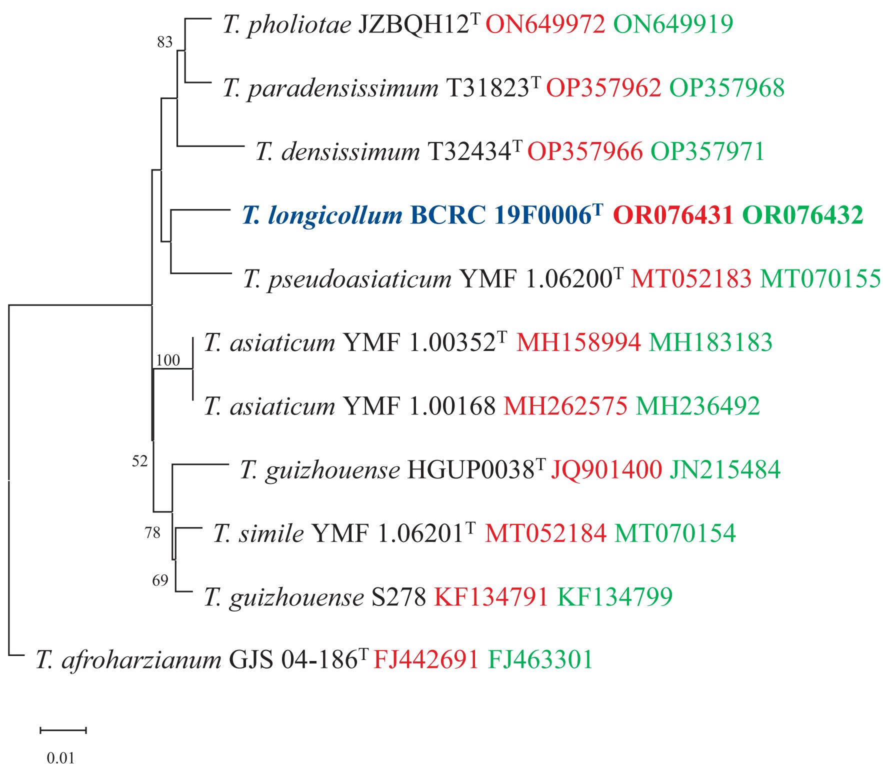

Fig. 1. Phylogenetic tree of *Trichoderma longicollum* BCRC 19F0006 and related species, based on a maximum likelihood analysis of combined *rpb2* and *tef1* sequences. *Trichoderma afroharzianum* was used as the outgroup. The *rpb2* (red) and *tef1* (green) GenBank accession numbers are provided following the strain numbers. The bootstrap values > 50% from 1000 replicates are indicated at the nodes. The new species proposed in this study is shown in a blue and bold font.

PHYLOGENETIC ANALYSIS

We extracted the DNA of this new species and sequenced the ITS as well as the *rpb2* and *tef1* genes. Next, we applied the maximum likelihood algorithm to determine the phylogenetic relatedness of our new species by combining the concatenated partial *rpb2* and *tef1* sequences via the MEGA 11 software package (Tamura et al. 2021). The reference sequences of related species were retrieved from the NCBI database based on previous publications (Zheng et al. 2021, Cao et al. 2022, Zhao et al. 2023).

Phylogenetically, *T. longicollum* resides in the *Harzianum* clade, closely related to *Trichoderma pseudoasiaticum* Z.F. Yu et Y.F. Lv (Fig. 1). According to the BLASTn analyses, *T. longicollum* differs from *T. pseudoasiaticum* both in the *rpb2* (97.34% similarity) and *tef1* (97.48% similarity) sequences.

TAXONOMY

Trichoderma longicollum Y.-H. Wei, S.-S. Tzean et G.-Y. Liou, **sp. nov.** Fig. 2

MycoBank: MB849086

Gene sequences: OR075258 (ITS); OR076431 (*rpb2*); OR076432 (*tef1*)

Etymology. The Latin specific epithet "*longicollum*" refers to the slender phialide neck of this species.

Diagnosis. *Trichoderma longicollum* is distinct by the conspicuous diffusing yellow pigment on PDA. Furthermore, it is easily distinguished from *T. pseudoasiaticum* and other related *Trichoderma* species by its longer phialides of (6.1)6.6–12.4(15.7) in length, higher phialide l/w ratio and a phialide number of 3–5(7) per whorl.

Holotype. Taiwan, Tainan City, Qigu Lagoon, isolated from soil under grey mangrove (*Avicennia marina*), 13 Jan. 2018, leg. C.-C. Chen et G.-Y. Liou. Type BCRC 19F0006, stored in metabolically inactive state (deep-frozen) in the Bioresource Collection and Research Center of the Food Industry Research & Development Institute, Hsinchu, Taiwan.

Description. Colonies on PDA (potato dextrose agar) after 72 h: colony radius 45–49 mm at 25 °C, 46–52 mm at 30 °C, and 36–40 mm at 35 °C; mycelium white, abundant, dense, floccose; pustules forming a distinct zone around the inoculation plug, white at first, then gradually turning green; yellow pigment conspicuously displayed. No distinct odour.

Colonies on SNA (synthetic nutrient-poor agar) after 72 h: colony radius 66–69 mm at 25 °C, covering the 90-mm plate at 30 °C, and 58–64 mm at 35 °C; colonies hyaline, mycelium sparse, with dark green to dull green pustules, irregular in shape, forming 2 concentric rings; no diffusing pigment; no distinct odour.

Conidiophores straight or curved, comprised of a distinct main axis and often terminating in a whorl of 3–5(7) phialides, occasionally in a solitary phialide, side branches paired or unilateral, sometimes substituted by a solitary phialide or verticillated phialides. Side branches terminating in a whorl of 2–5 phialides,

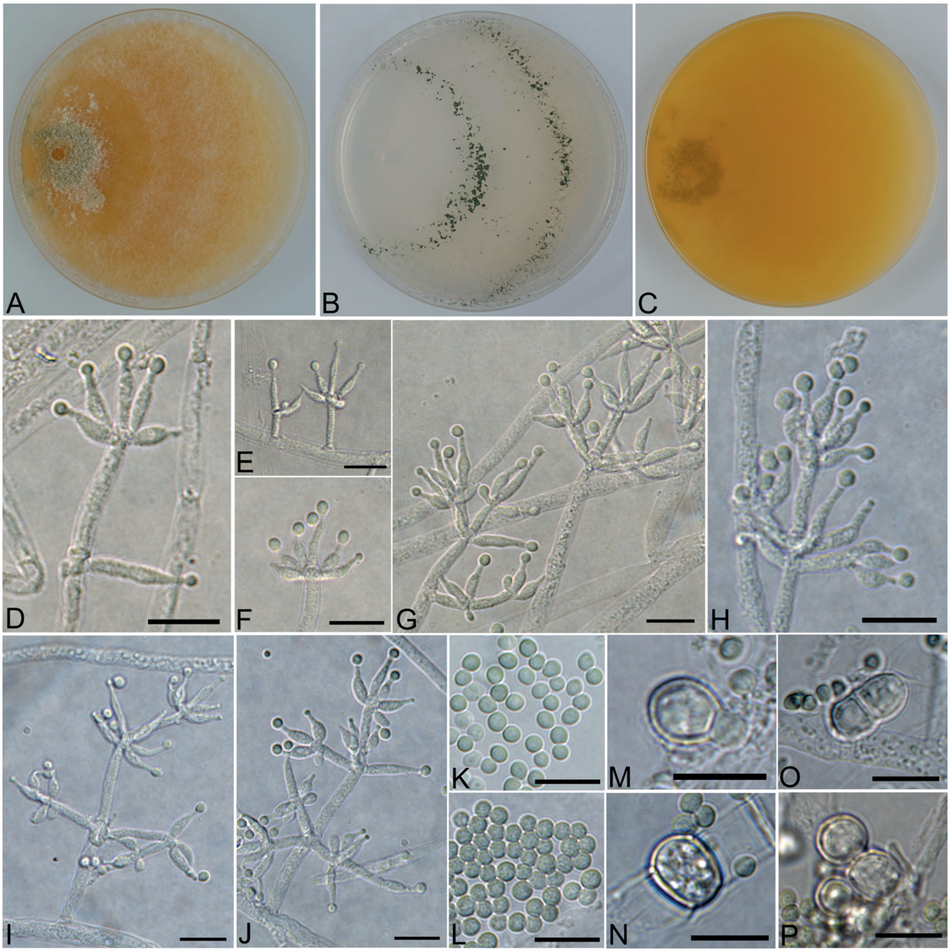

Fig. 2. *Trichoderma longicollum* BCRC 19F0006. **A–C** – colony after 7 days at 25 °C: on PDA (A), on SNA (B), reverse on PDA (C); **D–J** – conidiophores, phialides, and conidia formed on SNA; **K–L** – conidia; **M–P** – chlamydospores. Scale bars: 10 µm. Photographs by Y.-H. Wei.

rarely 6–7 phialides. Phialides lageniform to lanceolate, straight or slightly curved, often with slender, sometimes slightly bent neck, (6.1)6.6–12.4(15.7) × (1.5)2.1–3.2(3.6) µm, l/w ratio 2.0–4.2(7.9), 1.6–2.4 µm wide at the base. Conidia green in mass, smooth to slightly rough, globose to subglobose, occasionally broadly ellipsoidal to ellipsoidal, 2.4–3.7(4) × 2.4–3.7 µm, l/w ratio 1.0–1.1(1.4). Chlamydospores smooth, subglobose (5.7–9.2 × 5.4–8.7 µm) or irregular, terminal or intercalary.

Habitat. Soil in mangrove stand (*Avicennia marina*) of Qigu Lagoon, the largest lagoon in Taiwan. Soil characteristics: texture silty sand; silt-clay ratios = 6.25 ± 0.84; organic content (%) = 3.75 ± 1.43; TOC/TN (C/N) = 9.85 ± 8.10 (Chiu et al. 2011).

Note. Phylogenetically, *T. longicollum* is closest to *Trichoderma pseudoasiaticum*. However, the features of *T. longicollum* were different from *T. pseudoasiaticum* when cultured on two different media, and these species also differ in their phialides. *Trichoderma longicollum* produced a conspicuous yellow pigment on potato dextrose agar (PDA) and grew well at 35 °C on synthetic nutrient-poor agar (SNA; radius 58–64 mm), whereas *T. pseudoasiaticum* displayed no diffusing pigments on PDA and its growth was restricted on SNA (radius 2 mm) (Zheng et al. 2021). Differences in morphological characters of *T. longicollum* and species related to it (length of their phialides, phialide l/w ratios, and phialide numbers per whorl) are provided in Tab. 1.

Tab. 1. Comparison of morphological characteristics for *Trichoderma longicollum* and closely related species.

Species	Phialide length (µm)	l/w ratio	Phialide number / whorl	Reference
T. longicollum	(6.1)6.6–12.4(15.7)	2.0–4.2(7.9)	3–5(7)	This study
T. pseudoasiaticum	(5.2)6.1–9.0(9.7)	(1.1)1.5–3.6(5.2)	2–4	Zheng et al. 2021
T. densissimum	(3.4)5.7–8.0(10.1)	(1.4)2.1–3.1(3.9)	3–4	Zhao et al. 2023
T. paradensissimum	(5.4)7.4–11.0(15.0)	(1.4)2.1–3.1(3.9)	3	Zhao et al. 2023
T. pholiotae	(4.1)4.9–10.9(11.6)	1.4–3.4(3.9)	3–4	Cao et al. 2022

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REFERENCES

CAI F., DRUZHININA I.S. (2021): In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. – Fungal Divers. 107: 1–69. DOI: https://doi.org/10.1007/s13225-020-00464-4

CAO Z.-J., QIN W.-T., ZHAO J., LIU Y., WANG S.-X., ZHENG S.-Y. (2022): Three new *Trichoderma* species in *Harzianum* clade associated with the contaminated substrates of edible fungi. – J. Fungi 8: 1154. DOI: https://doi.org/10.3390/jof8111154

CHIU Y.-J., SHEN T.-Y., HWANG C.-C., WANG Y.-K., HSEU J.-R., YEH S.-L. (2011): Abundance and species diversity of benthic polychaetes in relation to sediment environmental variables in the intertidal

zone of Cigu Lagoon, Taiwan. – TW J. of Biodivers. 13: 135–151.
DOI: https://doi.org/10.7064/TJB.201104.0135 [in Chinese]

Dou K., Lu Z., Wu Q., Ni M., Yu C., Wang M., Li Y., Wang X., Xie H., Chen J., Zhang C. (2020): MIST: a multilocus identification system for *Trichoderma*. – Appl. Environ. Microbiol. 86: e01532-20. DOI: https://doi.org/10.1128/AEM.01532-20

Nascimento Brito V., Lana Alves J., Sírio Araújo K., de Souza Leite T., Borges de Queiroz C., Liparini Pereira O., de Queiroz M.V. (2023): Endophytic *Trichoderma* species from rubber trees native to the Brazilian Amazon, including four new species. – Front. Microbiol. 14: 1095199. DOI: https://doi.org/10.3389/fmicb.2023.1095199

Persoon C.H. (1794): Neuer Versuch einer systematischen Eintheilung der Schwämme. – In: Römer J.J., ed., Neues Magazin für die Botanik in ihrem ganzen Umfange, pp. 63–128, Zürich.
DOI: https://www.e-rara.ch/zut/doi/10.3931/e-rara-37279

Prameeladevi T., Kamil D., Sudeep Toppo R., Choudhary S.P. (2021a): *Trichoderma dumbbelliforme* sp. nov. an undescribed fungus of order *Hypocreales* from India. – Phytotaxa 520: 285–295. DOI: https://doi.org/10.11646/phytotaxa.520.3.8

Prameeladevi T., Narayanasamy P., Kamil D., Choudhary S. (2021b): Polyphasic taxonomy of Indian *Trichoderma* species. – Phytotaxa 502: 1–27. DOI: https://doi.org/10.11646/phytotaxa.502.1

Sousa T.F., Vieira Reça B.N.P., Castro G.S., da Silva I.J.S., Caniato F.F., de Araújo Júnior M.B., Yamagishi M.E.B., Koolen H.H.F., Bataglion G.A., Hanada R.E., da Silva G.F. (2023): *Trichoderma agriamazonicum* sp. nov. (*Hypocreaceae*), a new ally in the control of phytopathogens. – Microbiol. Res. 275: 127469. DOI: https://doi.org/10.1016/j.micres.2023.127469

Tamura K., Stecher G., Kumar S. (2021): MEGA11: Molecular evolutionary genetics analysis version 11. – Mol. Biol. Evol. 38: 3022–3027. DOI: https://doi.org/10.1093/molbev/msab120

Zhao R., Mao L.J., Zhang C.L. (2023): Three new species of *Trichoderma* (*Hypocreales*, *Hypocreaceae*) from soils in China. – MycoKeys 97: 21–40. DOI: https://doi.org/10.3897/mycokeys.97.101635

Zheng H., Qiao M., Lv Y., Du X., Zhang K.-Q., Yu Z. (2021): New species of *Trichoderma* isolated as endophytes and saprobes from southwest China. – J. Fungi 7: 467.
DOI: https://doi.org/10.3390/jof7060467