Phytotaxa 756 (2): 138–150
https://www.mapress.com/pt/
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Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.756.2.3

Morphology and multi-locus phylogeny reveal a new *Rhamphoriopsis* species from Guizhou Province, China

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Abstract

During a mycological survey conducted in Zhenyuan County, Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province, China, a *Phaeoisaria*-like fungus was collected from decayed wood in a terrestrial habitat. Multi-locus phylogenetic analyses of ITS, LSU, SSU, *rpb*2, and *tef*1-α sequence data revealed that the taxon forms an independent lineage within *Rhamphoriopsis,* sister to *R. yunnanensis.* Based on morphology and multi-gene phylogeny, the fungus is described herein as a new species*, Rhamphoriopsis guizhouensis*. A comprehensive description, illustration, and phylogenetic analysis results showing the placement of the new species are provided. This study contributes to a better understanding of the genus *Rhamphoriopsis* and expands the knowledge of fungal diversity in Guizhou Province, China.

Key words: 1 new species, microfungi, *Phaeoisaria*-like fungi, Rhamphoriaceae, taxonomy

Introduction

Rhamphoriales was established based on Rhamphoriaceae Réblová within Sordariomycetes O.E. Erikss. & Winka, supported by phylogenetic, molecular dating, and morphological evidence (Hyde *et al*. 2021). Members of Rhamphoriales are predominantly saprobic, typically occurring on plant debris and decomposing organic matter in terrestrial and freshwater habitats, though some taxa exhibit endophytic lifestyles (Hyde *et al*. 2021, Pitakpattanakul *et al*. 2025). Morphologically, they are characterized by ascomycetous sexual morphs and hyphomycetous asexual morphs (Hyde *et al*. 2021, 2024). Their ecological presence across diverse decaying substrates underscores their role in organic matter decomposition and nutrient cycling (Réblová & Štěpánek 2018, Hyde *et al*. 2021, 2024).

Rhamphoriaceae was established by Réblová & Štěpánek (2018) to accommodate *Rhamphoria* Niessl as the type genus, with three other genera, *viz. Rhamphoriopsis* Réblová & Gardiennet, *Rhodoveronaea* Arzanlou, W. Gams & Crous, *Xylolentia* Réblová. The dematiaceous hyphomycete *Linkosia multiseptum* W.P. Wu (HKUCC 10825) was also included based on molecular evidence (Réblová & Štěpánek 2018). However, the placement of this strain remains uncertain due to the lack of morphological data and the unavailability of both morphological and sequence data for the holotype specimen (WUWP WU 1374b) (Hyde *et al*. 2021). Therefore, this species is not accepted as a member of Rhamphoriaceae (Hyde *et al*. 2021). Divergence time estimates indicate that the stem age of Rhamphoriaceae is approximately 133 million years ago (MYA), which falls within the temporal range of orders (Hyde *et al*. 2017, 2021, 2024). Members of Rhamphoriaceae are characterized by perithecial, immersed to superficial ascomata lacking stromatic tissues or a clypeus, papillate to elongate necks, a two-layered peridium, unitunicate asci with a non-amyloid apical annulus, and hyaline to brown, transversely septate to dictyoseptate ascospores that may produce ascoconidia (Müller & Samuels 1982, Réblová & Štěpánek 2018, Hyde *et al*. 2020b). Asexual morphs are hyphomycetous, with mononematous or loosely fasciculate, macronematous conidiophores or reduced conidiogenous cells, and hyaline

or brown, aseptate to septate conidia (Réblová & Štěpánek 2018, Hyde *et al*. 2020a, b). Species of Rhamphoriaceae are predominantly saprobic on decaying woody substrates in terrestrial habitats, rarely occurring in freshwater environments, and have been reported mainly from Europe, with additional records from Argentina, Australia, China, South Africa, and the USA (Hyde *et al*. 2020b, 2024, Yang *et al.* 2023a, b, Pitakpattanakul *et al*. 2025, Xiao *et al.* 2025). Despite their ecological relevance in organic matter decomposition, the taxonomic diversity and phylogenetic relationships within the family remain insufficiently explored, indicating that further integrative studies are likely to reveal additional taxa (Hyde *et al*. 2024, Pitakpattanakul *et al*. 2025).

The genus *Rhamphoriopsis* was introduced by Réblová & Štěpánek (2018) to accommodate the type species *R. muriformis* Réblová & Gardiennet, originally described from decaying wood of *Buxus sempervirens* (Buxaceae) in France. Later, additional species of *Rhamphoriopsis* have been discovered and described, based on morphological observations and multi-gene phylogenetic analyses (Hyde *et al*. 2020, Crous *et al*. 2023, Lin *et al*. 2023, 2025, Yang *et al*. 2023a, Pitakpattanakul *et al*. 2025). The genus is characterized by globose to subglobose ascomata with cylindrical to occasionally flattened necks, unitunicate, hyaline, 8-spored asci, and cylindrical to fusiform, transversely septate to muriform ascospores that become light to dark brown at maturity. The asexual morph is *Phaeoisaria*-like, characterized by mononematous conidiophores (solitary or in loose fascicles), polyblastic denticulate conidiogenous cells, and ellipsoidal, hyaline, aseptate conidia (Réblová & Štěpánek 2018, Pitakpattanakul *et al*. 2025). Members of *Rhamphoriopsis* are saprobic, occurring on decomposing woody substrates in both aquatic and terrestrial habitats, and frequently form coelomycetous conidiomata or hyphomycetous conidiophores on the host surface and tissue (Réblová & Štěpánek 2018, Pitakpattanakul *et al*. 2025). Currently, 13 species are accepted in *Rhamphoriopsis*, which are reported from China, France, South Africa, and the USA (Réblová & Štěpánek 2018, Hyde *et al*. 2020 a, b, Crous *et al*. 2023, Lin *et al*. 2023, 2025, Yang *et al*. 2023a, Pitakpattanakul *et al*. 2025, Réblová *et al*. 2025).

During our survey of fungal diversity in Qiandongnan Miao and Dong Autonomous Prefecture**,** Guizhou Province, a new hyphomycetous species, *Rhamphoriopsis guizhouensis*, was isolated from the surface of decayed wood in a terrestrial habitat. Initial morphological observations revealed that our collection exhibits characteristics typical of a *Phaeoisaria*-like species. The species was identified based on morphological characteristics and phylogenetic analyses of combined sequence datasets of ITS, LSU, SSU, *rpb*2, and *tef*1-α. Full descriptions, photoplates illustrating macro- and micromorphological characteristics, and phylogenetic trees showing the placement of the new taxon are provided in this study.

Materials and Methods

Specimen collection, isolation, and examination of morphological characteristics

Specimens were collected from a terrestrial habitat in Zhenyuan County, Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province, China. Samples were placed in sterile, moistened plastic bags containing sample information, such as collector, date, location, and other relevant details (Rathnayaka *et al*. 2024), and transported to the mycology laboratory at Southwest Forestry University, Kunming. Fresh specimens were dissected and observed for morphological characteristics, including the structures of conidiophores, conidiogenous cells, and conidia, using a stereo microscope (SMZ 745 and SMZ 800N, Nikon, Tokyo, Japan) and an ECLIPSE Ni compound microscope (Nikon, Tokyo, Japan). Measurements were obtained with the Tarosoft (R) Image Framework software, and photoplates were assembled using Adobe Photoshop CS6 software (Adobe Systems, USA).

Single-spore isolation was performed according to the methodology described by Senanayake *et al*. (2020). Pure cultures were maintained at 28 °C under continuous illumination, and colony characteristics were examined and documented in detail. Dried specimens were deposited in the herbarium of the Guizhou Academy of Agriculture Sciences (GZAAS), Guiyang, China, while living cultures were preserved in the Guizhou Culture Collection (GZCC), Guiyang, China. Index Fungorum (http://www.indexfungorum.org; 20 March 2026) and Faces of Fungi (Jayasiri *et al*. 2015) numbers were obtained following standard protocols. The new species was described in accordance with the guidelines provided by Chethana *et al*. (2021).

DNA extraction, PCR amplification, and sequencing

Actively growing mycelia were collected from PDA cultures with sterile toothpicks and transferred into 1.5 mL microcentrifuge tubes. Total genomic DNA was isolated using the Ezup Column Fungi Genomic DNA Purification Kit in accordance with the manufacturer's protocol. Five genetic markers, including the internal transcribed spacer (ITS)

with the primer pair of ITS4 and ITS5 (White *et al*. 1990), large subunit rDNA (LSU) with the primer pair of LR0R and LR5 (Vilgalys & Hester 1990), small subunit ribosomal RNA (SSU) with the primer pair of NS1 and NS4 (White *et al*. 1990), the RNA polymerase II second largest subunit (*rpb*2) with the primer pair of RPB2–5f and RPB2–7cR (Liu *et al*. 1999), and translation elongation factor 1-alpha (*tef*1-α) with the primer pair of EF1–983F and EF1–2218R (Carbone & Kohn 1999).

PCR amplifications were carried out in 25 μL reaction mixtures containing 21 μL of 1.1× T3 Super PCR Mix (Tsingke Biotechnology Co., Ltd., Chengdu, China), 1 μL of each forward and reverse primer, and 2 μL of genomic DNA. Amplifications were performed on a JS-G9612 thermal cycler (Shanghai Peiqing Technology Co., Ltd., Shanghai, China). Cycling conditions for ITS, SSU, LSU, *rpb*2, and *tef*1-α followed the protocols described by Ma *et al*. (2023). PCR products were examined by electrophoresis on 1% agarose gels, purified, and subsequently sequenced by Tsingke Biotechnology Co., Ltd. (Chengdu, China). All newly generated sequences were deposited in GenBank (https://ncbi.nlm.nih.gov/WebSub/).

Phylogenetic analyses

A combined gene dataset of ITS, LSU, SSU, *rpb*2 and *tef*1-α was used for phylogenetic analyses. Newly generated sequence data were subjected to the BLASTn search in the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) to identify the taxa most closely related to our strains. Reference sequences used in this study were obtained from previous publications (Pitakpattanakul *et al*. 2025, Lin *et al*. 2025) and downloaded from GenBank via the One-click Fungal Phylogenetic Tool (OFPT) (Zeng *et al*. 2023). Raw sequences were initially checked using BioEdit V.7.0.5.2 (Hall 1999). Forward and reverse sequences were assembled into consensus sequences using SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) and Geneious Pro.v4.8.5 (https://www.geneious.com/). Each gene region was aligned separately using MAFFT V. 7.215 (https://mafft.cbrc.jp/alignment/server/; Katoh & Standley 2016) and trimmed with TrimA1.v1.2rev59 (Capella-Gutiérrez *et al*. 2009), and manually edited in BioEdit V.7.0.5.2 (Hall 1999). The multi-gene alignments were concatenated using SequenceMatrix 1.7.8 (Vaidya *et al*. 2011) and further inspected in AliView (Larsson 2014) or BioEdit V.7.0.5.2 (Hall 1999). The final alignment in FASTA format was converted to PHYLIP and NEXUS formats using the Alignment Transformation Environment (ALTER) (http://sing.ei.uvigo.es/ ALTER/) (Glez-Peña *et al*. 2010) for Maximum Likelihood analysis (ML) and Bayesian inference (BI) analyses (Glez-Peña *et al*. 2010), respectively.

Phylogenetic analyses were conducted on the CIPRES Science Gateway platform, including Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian inference (BI) analyses (Miller *et al*. 2010). The RAxML was performed using RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis *et al*. 2008; Stamatakis 2014) under the GTRGAMMA nucleotide substitution model with 1,000 bootstrap replicates. The best-fit model for each gene was determined using MrModeltest 2.2 for Bayesian inference (BI) (Nylander 2004). The best models for ITS were TIM2e+R4 and TNe+I+G4 for LSU; K2P+G4 for SSU; TN+F+I+G4 for *rpb*2; and TIM2+F+R5 for *tef*1-α. Bayesian inference analyses were conducted using MrBayes on XSEDE (3.2.7a) (Ronquist *et al*. 2012) by the Markov Chain Monte Carlo (MCMC) method to evaluate posterior probabilities (BYPP) (Richard & Lippmann 1991; Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002). Six simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled at every 100th generation. The phylogenetic tree was visualized with FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Microsoft PowerPoint 2021. The final figure design and layout were completed with Adobe Photoshop CS6 software (Adobe Systems, California, USA). All newly generated sequences in this study were deposited in GenBank, and the accession numbers are listed in Table 1.

TABLE 1. Names, strain numbers, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses.

Taxa	Strains	GenBank accession numbers				
		LSU	ITS	SSU	*tef*1-α	*rpb2*
Myrmecridium schulzeri	CBS 100.54	EU041826	EU041769	–	–	–
Myrmecridium sorbicola	CBS 143433 T	MH107948	MH107901	–	–	–
Rhamphoria delicatula	CBS 132724	FJ617561	MG600391	–	–	–

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TABLE 1. (Continued)

Taxa	Strains	GenBank accession numbers				
		LSU	**ITS**	**SSU**	***tef*1-α**	***rpb2***
Rhamphoria delicatula	PRA-13612	AF261068	MG600390	–	–	–
Rhamphoria pyriformis	CBS 139024 T	MG600397	MG600392	MG600405	–	MG600401
Rhamphoria pyriformis	CBS 139033	KT991665	KT991677	MG600406	–	KT991656
Rhamphoriopsis aquimicrospora	GZCC 20-0515 T	OP377911	OP377812	OP377996	OP472992	OP473085
Rhamphoriopsis brevis	GZCC 18-0072 T	PQ898783	PQ898747	PQ898817	PV040799	–
Rhamphoriopsis cuprea	CBS 147991 T	PV455956	PV455942	PV455969	PV483440	PV483456
Rhamphoriopsis denticulata	CBS 147996 T	PV455957	PV455943	PV455970	PV483441	PV483457
Rhamphoriopsis denticulata	CBS 147997	PV455958	PV455944	PV455971	PV483442	PV483458
Rhamphoriopsis glauca	CBS 480.75	MH872702	–	–	–	–
Rhamphoriopsis globularis	GMBC5313 T	PV933641	PV933623	PV933658	PX392348	PX373386
Rhamphoriopsis globularis	GMBC5314	PV933642	PV933624	PV933659	PX392349	PX373387
Rhamphoriopsis guizhouensis	GZCC 25-0756 T	PX853881	PX853883	**PX853885**	**PZ238795**	**PZ238784**
Rhamphoriopsis guizhouensis	GZCC 25-0757	PX853882	PX853884	**PX853886**	**PZ238796**	**PZ238785**
Rhamphoriopsis hyalospora	GZCC 18-0056 T	MN846343	–	–	–	–
Rhamphoriopsis hyalospora	GZCC 18-0066	MN846342	MN846344	–	–	–
Rhamphoriopsis muriformis	CBS 127683	MG600395	MG600389	MG600403	–	MG600400
Rhamphoriopsis muriformis	CBS 131269 T	MG600396	–	MG600404	–	MG600399
Rhamphoriopsis sympodialis	DS 2-38	MT079191	MT079187	–	–	–
Rhamphoriopsis sympodialis	HKAS 105172 T	NG_079672	NR_174906	–	–	–
Rhamphoriopsis synnematosa	CBS 150066	–	OR680774	–	–	–
Rhamphoriopsis synnematosa	CPC 45231 T	OR717029	OR680773	–	–	OR683730
Rhamphoriopsis uniseptata	GMBCC1144 T	PV933767	PV910738	PV933826	PV944196	PV944194
Rhamphoriopsis uniseptata	GMBCC1146	PV939605	PV933765	PV933827	PV944197	PV944195
Rhamphoriopsis yunnanensis	GMBC6917 T	PV939432	PV939404	PV939456	PX392366	PX373388
Rhamphoriopsis yunnanensis	GMBC6919	PV939433	PV939405	PV939457	PX392367	PX373389

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TABLE 1. (Continued)

Taxa	Strains	GenBank accession numbers				
		LSU	**ITS**	**SSU**	***tef*1-α**	***rpb2***
Rhamphoriopsis zhaotongensis	GMBC6918 T	PV939434	PV939406	PV939458	PX392368	PX373390
Rhamphoriopsis zhaotongensis	GMBC6920	PV939435	PV939407	PV939459	PX392369	PX373391
Rhodoveronaea aquatica	GZCC 20-0447	OP377947	OP377862	OP378027	OP473041	OP473107
Rhodoveronaea aquatica	MFLUCC 18-1339 T	MK849785	MK828641	MK828310	MN194046	–
Rhodoveronaea everniae	CBS 148309 T	OK663776	OK664737	–	–	OK651172
Rhodoveronaea hainanensis	GZAAS 22-2020T	OP748932	OP748935	–	–	–
Rhodoveronaea hyalina	GZCC 23-0622 T	PP102207	PP102206	PP102214	PP259403	PP259399
Rhodoveronaea hyalina	GZCC 23-0623	PP102208	PP102211	PP102215	PP259404	PP259400
Rhodoveronaea lignicola	GZCC 23-0624 T	PP102209	PP102212	PP102216	PP259405	PP259401
Rhodoveronaea lignicola	GZCC 23-0625	PP102210	PP102213	PP102217	PP259406	PP259402
Rhodoveronaea nieuwwulvenica	CBS 149447 T	OQ629048	OQ628466	–	OQ627955	OQ627935
Rhodoveronaea varioseptata	CBS 123472	FJ617559	MG600393	MG600408	–	JX066701
Rhodoveronaea varioseptata	CBS 123473	FJ617560	KT991676	JX066710	–	JX066700
Rhodoveronaea varioseptata	CBS 431.88 T	EU041870	EU041813	–	–	–
Xylolentia aseptata	GZCC 20-0424 T	OP377944	OP377859	OP378024	OP473038	OP473104
Xylolentia aseptata	GZCC 20-0426	OP377945	OP377860	OP378025	OP473039	OP473105
Xylolentia bambusae	ZHKUCC 24-1142	PQ376584	PQ376583	PQ380133	PQ383292	PQ383291
Xylolentia brunneola	PRA-13611 T	MG600398	MG600394	MG600407	–	MG600402
Xylolentia matsushimae	NN043170	–	OL627569	–	–	–
Xylolentia palmicola	NN055349	–	OL627827	–	–	–
Xylolentia reniformis	CC.ZGS48	PP407643	PP407785	PP415854	–	PP558325
Xylolentia reniformis	GZCC 18-0048 T	MK547648	MK547646	–	–	–
Xylolentia simplex	BCRC FU31758	OQ146974	OQ146961	–	–	LC745948
Xylolentia simplex	BCRC FU31869 T	–	OQ146962	–	–	–

Note: "T" represents the type strain. "–" indicates data unavailability. Newly generated sequences are represented in bold.

Results

Phylogenetic analyses

The phylogenetic trees obtained from ML and BI analyses were essentially similar. The phylogeny of Rhamphoriaceae was based on combined ITS, LSU, SSU, *rpb*2, and *tef*1-α sequence data. The aligned dataset encompassed 52 strains, representing 32 taxa, including *Rhamphoria* (four strains representing two taxa), *Rhamphoriopsis* (24 strains representing 14 taxa), *Rhodoveronaea* (12 strains representing seven taxa), *Xylolentia* (10 strains representing seven taxa), and the outgroup taxa of *Myrmecridium schulzeri* (Sacc.) Arzanlou, W. Gams & Crous (CBS 100.54) and *M. sorbicola* Crous & R.K. Schumach. (CBS 143433). The RAxML analysis of the combined dataset yielded a best-scoring tree (Fig. 1) with a final ML optimisation likelihood value of -21155.209579. The alignment contained 42,63 bp (ITS 1–511 bp, LSU 512–1,356 bp, SSU 1,357–2,365 bp, *rpb*2 2,366–3,359 bp, and *tef*1-α 3,360–4,263 bp). The matrix contained 1,572 distinct alignment patterns, with 26.84% of characters undetermined or missing. Estimated base frequencies; A = 0.246438, C = 0.262156, G = 0.278715, T = 0.212691; substitution rates AC = 0.212691, AG = 3.074099, AT = 1.140286, CG = 1.221733, CT = 7.890758, GT = 1.000000; proportion of invariable sites I = 0.564899; gamma distribution shape parameter α = 0.515848.

Four genera (*Rhamphoria*, *Rhamphoriopsis*, *Rhodoveronaea*, and *Xylolentia*) of Rhamphoriaceae are represented in the phylogenetic tree (Fig. 1). The topology of the ML and BI analyses results is consistent with the phylogenetic results of Pitakpattanakul *et al*. (2025). Multi-gene phylogenetic analyses show that our strains belong to *Rhamphoriopsis* and form a distinct clade (Fig. 1). *Rhamphoriopsis guizhouensis sp. nov.* forms a distinct clade sister to *R. yunnanensis,* W.M. Li, K. Habib & Q.R. Li (GMBC6917 and GMBC6919), with 95% ML bootstrap support and 1.00 Bayesian posterior probability.

Taxonomy

Rhamphoriopsis guizhouensis X.F. Liu & S. Shen *sp. nov.* Fig. 2

Fungal Names number: IF 904834; Facesoffungi Number: FoF 19504.

Etymology: Refers to the type locality, Guizhou Province.

Holotype: GZAAS 25-07826.

Saprobic on decayed wood in terrestrial habitat. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Colonies* on natural substrate effuse, gregarious, dark brown to black, bearing abundant conidia. *Mycelium* partly superficial, composed of branched, septate, and hyaline. *Conidiophores* 58–180 × 1–3 µm ($\overline{x}$ = 111 × 2 µm, n = 30), synnematous, polyblastic, integrated, terminal becoming intercalary, cylindrical, tapering apically, sympodial, with numerous indistinct denticles, hyaline towards the apex, pale brown near the base, smooth-walled. *Conidiogenous cells* 27–41 × 1–2 µm ($\overline{x}$ = 32 × 1.5 µm, n = 20), polyblastic, integrated, terminal, cylindrical, tapering apically, hyaline towards the apex, pale brown near the base, smooth. *Conidia* 2.2–3.2 × 1.2–1.8 µm ($\overline{x}$ = 2.6 × 1.5 µm, n = 30), ellipsoidal to obovoid, straight, rounded at the apex, hyaline, aseptate, smooth-walled.

Culture characteristics: Conidia germinated on PDA within 12 hours. Colonies growing on PDA reached 18 mm diam. after 30 days at 25 °C, circular, entire margin, surface beige, and pale brown from below, not producing pigmentation.

Material examined: CHINA, Guizhou Province, **Qiandongnan Miao and Dong Autonomous Prefecture, Zhenyuan County,** 27°14′40" N, 108°18′30" E, elevation 650 m, on decayed wood surface in a terrestrial habitat, 5 May 2025, Xingjuan Xiao & Xiangfu Liu, GF5-A (GZAAS 25-07826, holotype), ex-type GZCC 25-0756; *ibid*., GF5-B (GZAAS 25-07827, isotype), ex-isotype GZCC 25-0757.

Notes: Phylogenetic results showed that the new isolates (GZCC 25-0756 (ex-type) and GZCC 25-0757) formed a distinct clade and sister to *R. yunnanensis* (GMBC6917 (ex-type) and GMBC6919) with 95%/1.00 ML/PP statistical support (Fig. 1). The pairwise nucleotide comparisons between *R. guizhouensis* (GZCC 25-0756, ex-type) and *R. yunnanensis* (GMBC6917, ex-type) showed that there are 4% (14/505 bp, 4 gaps) base pair differences in ITS, 1% (1/819 bp, without gap) base pair differences in LSU, 7% (69/998 bp, without gap) base pair differences in *rpb*2, 1% (2/995 bp, without gap) base pair differences in SSU, and 3% (31/908 bp, without gap) base pair differences in *tef*1-α. Morphologically, *R. guizhouensis* differs from *R. yunnanensis* in that the latter possesses longer conidiophores (58–180 µm vs. 100–255 µm) and was isolated from an aquatic habitat (Liu *et al*. 2025). Based on the phylogenetic analyses and morphological characteristics, *R. guizhouensis* is described as a new species.

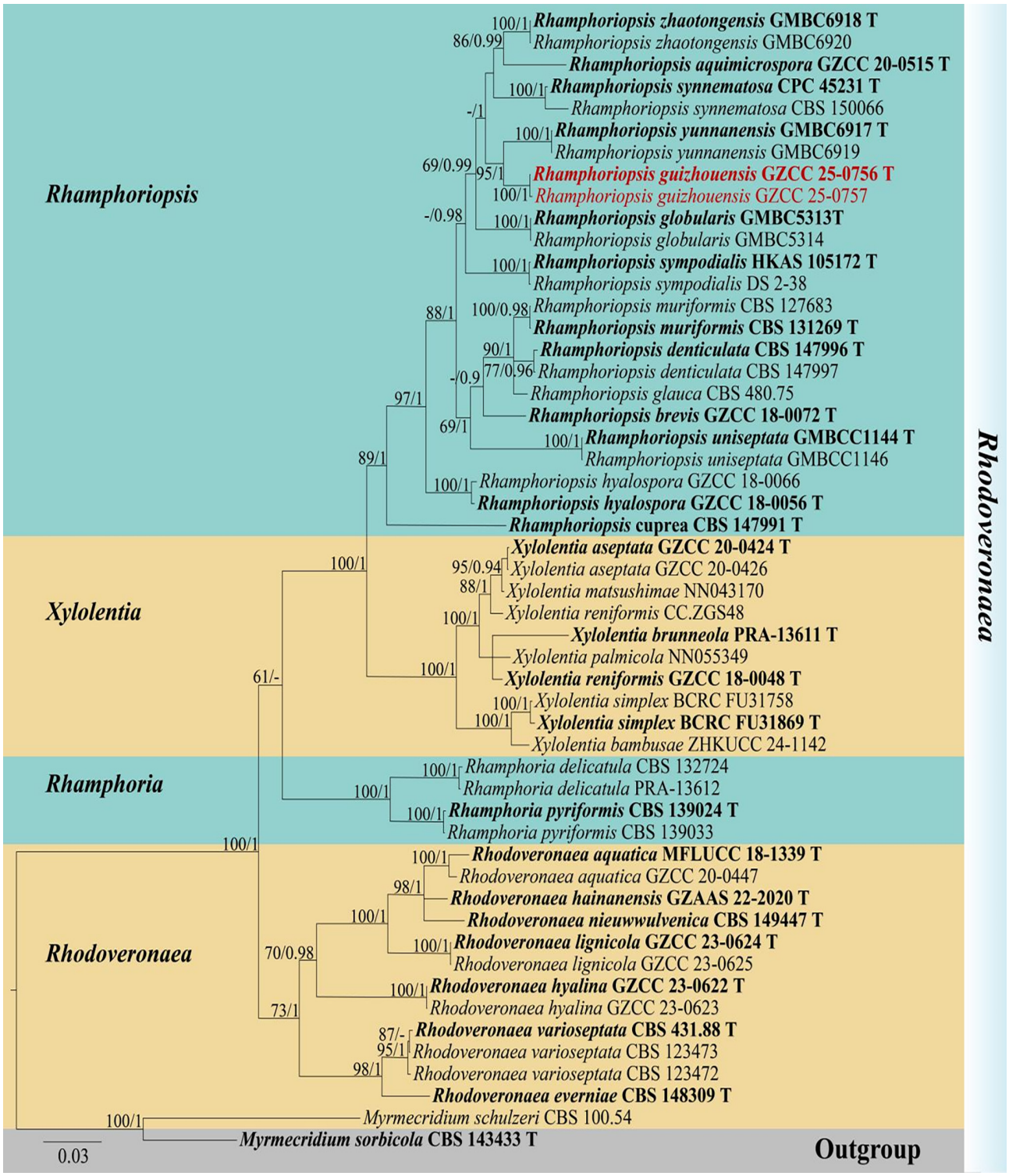

FIGURE 1. Phylogenetic tree from maximum likelihood analysis based on combined ITS, LSU, SSU, *rpb*2, and *tef*1-α sequenced data for the family Rhamphoriaceae. ML bootstrap support values (MLB ≥ 60 %) and Bayesian posterior probabilities (BYPP ≥ 0.90) are indicated below or above the nodes. Ex-type strains are marked with T and in bold, and the newly generated sequences are in red.

FIGURE 2. *Rhamphoriopsis guizhouensis* (GZAAS 25-07826, holotype). **a, b** Colonies on the natural substrate. **c–e**. Conidiophores, conidiogenous cells, and conidia. **f** Conidiogenous cells and conidia. **g–i** Conidia. **j** Germinated conidia. **k, l** Colonies on PDA medium (k from above, l from below). Scale bars: c–e = 50 μm; f, j = 10 μm; g–i = 5 μm.

Discussion

Phaeoisaria-like asexual morphs are characterized by macronematous, mononematous to loosely fasciculate conidiophores, polyblastic conidiogenous cells bearing distinct denticles, and solitary, aseptate, hyaline to pale brown conidia (Réblová & Štěpánek 2018, Hyde *et al*. 2020a, Lin *et al*. 2023, Yang *et al*. 2023b). Molecular phylogenetic studies have consistently demonstrated that these morphologies are polyphyletic within Sordariomycetes, reflecting extensive morphological convergence rather than monophyly (Höhnel 1909, Hughes 1958, de Hoog & Papendorf 1976, Shenoy *et al.* 2006, Hyde *et al*. 2020a, 2021, Yang *et al*. 2023b). Ecologically, taxa exhibiting *Phaeoisaria*-like morphs are predominantly saprobic on decaying woody substrates in terrestrial or freshwater environments (Réblová & Štěpánek 2018, Hyde *et al*. 2020a). Although these asexual characters alone are insufficient for resolving higher-level systematic relationships (Seifert & Gams 2011, Hyde *et al*. 2020a), they retain taxonomic value at the species and genus levels when interpreted in conjunction with sexual morphology and multilocus phylogenetic data (Réblová & Štěpánek 2018, Hyde *et al*. 2021). This integrative approach is well illustrated in Rhamphoriaceae, particularly in *Rhamphoriopsis*, where *Phaeoisari*a-like asexual morphs provide important phenotypic support within a robust, multi-evidence taxonomic framework (Réblová & Štěpánek 2018, Hyde *et al*. 2020b, Pitakpattanakul *et al*. 2025).

Rhamphoriopsis represents a well-supported genus that clearly demonstrates both the utility and limitations of *Phaeoisaria*-like asexual morphs in fungal taxonomy. Species of *Rhamphoriopsis* consistently produce hyphomycetous asexual morphs with macronematous conidiophores, sympodially proliferating polyblastic conidiogenous cells, and denticulate conidiogenous loci, closely conforming to the classical concept of *Phaeoisaria* (Réblová & Štěpánek 2018; Hyde *et al*. 2020b). However, multigene phylogenetic analyses unequivocally place *Rhamphoriopsis* in a distinct, well-resolved clade separated from *Phaeoisaria*, thereby demonstrating that asexual morphology alone is inadequate for generic delimitation (Réblová & Štěpánek 2018, Hyde *et al*. 2020, Crous *et al*. 2023, Lin *et al*. 2023, 2025, Yang *et al*. 2023a, b, Pitakpattanakul *et al*. 2025, Réblová *et al*. 2025). Ecologically, members of *Rhamphoriopsis* are saprobic on decaying wood in terrestrial habitats, a lifestyle shared with other rhamphoriaceous taxa and indicative of ecological adaptation rather than close morphological affinity (Réblová & Štěpánek 2018, Hyde *et al*. 2020a). Recent studies further show that subtle yet consistent differences in conidiophore architecture, conidiogenous cell morphology, and conidial dimensions, when integrated with multilocus phylogenetic evidence, allow reliable species delimitation within the genus (Hyde *et al*. 2020b, Pitakpattanakul *et al.* 2025). Collectively, *Rhamphoriopsis* underscores the need for integrative taxonomy that combines morphology and molecular phylogeny to achieve a natural classification within morphologically convergent groups of hyphomycetes.

Globally, 14 species (including the species described in this study) of *Rhamphoriopsis* have been formally described to date, with records from China, France, the Czech Republic, South Africa, and the USA (Réblová & Štěpánek 2018, Hyde *et al*. 2020, Crous *et al*. 2023, Lin *et al*. 2023, 2025, Yang *et al*. 2023a, Liu *et al*. 2025, Pitakpattanakul *et al*. 2025, Réblová *et al*. 2025). Notably, 71.43% of these species (ten in total) were originally described from China, highlighting that the genus remains insufficiently explored worldwide and suggesting a high potential for the discovery of additional species through future surveys.

Acknowledgements

The research was supported by the Science Research Funding of Yunnan Provincial Department of Education (Project No. 2026J0726), the Forestry and Grass Science and Technology Innovation Joint Project of Yunnan Province (Project No. 202404CB090008), Modern Industry School of Edible-fungi, Southwest Forestry University (SYJ25).

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