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Two new species in *Neomyrmecridium* and two new records in *Myrmecridium* (*Myrmecridiaceae*, *Myrmecridiales*) from the Tibetan Plateau, China

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Abstract

This study aims to investigate the species diversity of lignicolous freshwater fungi in the Tibetan Plateau, China. Four hyphomycetous taxa were identified and subjected to molecular analysis utilizing combined ITS and LSU sequence data. The phylogenetic analysis identified two novel species in *Neomyrmecridium*, namely *N. gaoligongense* and *N. luguense*, as well as two new records in *Myrmecridium*, namely *M. iridis* and *M. schulzeri*. These species are characterized by possessing cylindrical, septate, unbranched conidiophores, integrated, terminal, polyblastic conidiogenous cells, and subhyaline, obovoid conidia. The four taxa are comprehensively described with colour photographs and phylogenetic analyses.

Key words – 2 new species – Freshwater fungi – Hyphomycetes – Phylogeny, *Sordariomycetes* – Taxonomy

Introduction

Myrmecridiales was introduced by Crous et al. (2015a) with a monophyletic family *Myrmecridiaceae* which comprises two genera including *Myrmecridium* and *Neomyrmecridium* (Arzanlou et al. 2007, Crous et al. 2018a). *Myrmecridium* was introduced by Arzanlou et al. (2007), with *M. schulzeri* as its type species. It is characterized by its flat colonies, immersed mycelium that grows vertically and consists of unbranched, straight or flexuose, septate conidiophores. Conidiogenous cells are polyblastic, integrated, cylindrical and solitary with obovoidal or fusiform, smooth or finely verrucose-walled conidia (Arzanlou et al. 2007). Members of *Myrmecridium* are widely distributed on decaying plant branches in freshwater and soil habitats (Arzanlou et al. 2007, Jie et al. 2013, Peintner et al. 2016, Réblová et al. 2016, Tibpromma et al. 2017, Crous et al. 2018a, 2018b, 2020, 2021, 2022, Serrano et al. 2020). So far, 24 species are accepted in *Myrmecridium* (http://www.indexfungorum.org/Names/Names.asp accessed on 18 September 2023). Crous et al.

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(2018a, 2021) have transferred *Myrmecridium aquaticum* and *M. sorbicola* to *Neomyrmecridium* based on morphological and phylogenetic studies.

Neomyrmecridium was introduced by Crous et al. (2018a) with *N. septatum* as the type species. The genus is characterized by solitary, unbranched conidiophores, polyblastic conidiogenous cells bearing several denticles at the apex, and fusoid-ellipsoid, septate conidia with upper two-thirds encased in a mucoid sheath. Based on morphological and phylogenetic analyses, seven species are currently recognized in *Neomyrmecridium* (Crous et al. 2021, Species Fungorum 2023).

The Tibetan Plateau is the largest and most unique geographical region on earth, which encompasses remarkable endemic diversity (Wang et al. 2016, Guo et al. 2020, Xu et al. 2021). In recent years, global research on the taxonomy and phylogeny of saprobic fungi have increased significantly, with diversity of freshwater fungi in China being well-studied (Hyde et al., 2016, Luo et al., 2018, 2019, Dong et al. 2020, 2021, Hongsanan et al. 2020, Hyde et al. 2020, Bao et al. 2021, Shen et al., 2022). However, there is a lack of study in freshwater fungi from the Tibetan Plateau. In this study, we introduce the two new species in *Neomyrmecridium* as well as two new records in *Myrmecridium* from freshwater habitats in the Tibetan Plateau environments.

Materials & Methods

Specimens and morphological studies and isolation

During investigations of fungal diversity in the Tibetan Plateau, China, decaying wood submerged in freshwater habitats was collected following the methods described in Luo et al. (2018) and Senanayake et al. (2020). Samples were placed in a zip lock bag and were taken back to the laboratory for observation. Samples were incubated for a week in a sterile plastic box containing sterilized wet tissues. Samples were observed and examined following the instruction outlined in Luo et al. (2018). Macroscopic and microscopic morphology of filamentous fungi (e.g., colonies, conidiomata, conidiophores or conidia) were examined using a stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy GmBH, Germany) and microphotographs were taken using a compound microscope (Nikon ECLIPSE 80i, Nikon, Japan) fitted with a NikonDS-Ri2 digital camera (Nikon, Japan). Measurements were made with the Tarosoft (R) Image Frame Work program and photographic plates used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems, USA). Single spore isolation was carried out following the method described in Senanayake et al. (2020). All specimens were deposited in the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), after natural air-drying. The living culture was deposited in the Kunming Institute of Botany Culture Collection (KUNCC), Kunming, China. Index Fungorum and Facesoffungi numbers were registered as mentioned in Index Fungorum (2022) and Jayasiri et al. (2015).

DNA extraction, PCR amplification and sequencing

Fresh mycelium scraped from colonies grown on PDA plates was used for DNA extraction using a DNA extraction kit according the manufacturer's instructions (TOLOBIO Plant Genomic DNA Extraction Kit, Shanghai Co. Ltd. P.R. China). PCR amplification was performed using primers ITS4/ITS5 for the internal transcribed spacer (ITS1-5.8S-ITS2, ITS), LR0R/LR5 for large subunit ribosomal RNA (LSU rRNA) (White et al. 1990, Vilgalys and Hester 1990). PCR was carried out in 25 µl reaction volume containing 21 µl of 1 × Power Taq PCR Master Mix, 1 µl of each primer (10 µl stock) and 2 µl of genomic DNA template. Amplifications were carried out using the BioTeke GT9612 thermocycler (Beijing City, China). The PCR amplification conditions for ITS and LSU consisted of initial denaturation at 98 ℃ for 3 minutes, followed by 35 cycles of denaturation at 98 ℃ for 20 seconds, annealing at 53 ℃ for 10 seconds, extension at 72 ℃ for 20 seconds, final extension at 72 ℃ for 5 minutes. PCR products were visualized using 1% agarose gel electrophoresis. The PCR products were sequenced by Tsingke Company, Beijing, P.R. China.

Phylogenetic study

The qualities of generated sequences were checked with BioEdit v7.0.9 (Hall 1999) and each sequence was subjected to a BlastN search in NCBI's GenBank to reveal the closet taxa and exclude possible contamination. The reference sequences were selected based on the blast results of ITS and LSU as well as the recent literatures listed in (Table 1). Each matrix was aligned using MAFFTv6.8 (Katoh et al. 2005) and manually improved employing BioEdit v7.0.9. The trimmed sequence alignments were subjected to Maximum likelihood (ML) and Bayesian inference (BI) analyses. Maximum likelihood (ML) analysis was carried out using the RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2006, Stamatakis et al. 2008) of CIPRES Science Gateway website (Miller et al. 2010: http://www.phylo.org/portal2) and the estimated proportion of invariant sites is (GTRGAMMA+I) model. Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012) and the best-fit models of ITS and LSU gene regions were independently determined by MrModeltest 2.2 (Guindon and Gascuel 2003, Nylander 2004, Darriba et al. 2012). The Markov Chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (Rannala and Yang 1996). Bayesian analyses of six simultaneous Markov chains were run for 10,000,000 generations with trees sampled every 1000 generations. Bayesian posterior probabilities (BYPP) values were calculated from the 50% majority-rule consensus tree, and branches with BYPP equal to or greater than 0.95 were considered as significantly supported. The best trees were printed with FigTree v1.4.0 (Rambaut 2012) and the layout was done with Adobe Illustrator CS v. 6. *Lanspora cylindrospora* (NFCCI 4427, NFCCI 4391) was chosen as the outgroup taxon following (Hyde et al. 2020).

Table 1 Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers. The newly generated sequences are indicated in red and bold font. Ex-type strains are bold, while unavailable sequences are indicated by a symbol "–"

Species	Specimen. Voucher	GenBank accession numbers		References
		ITS	LSU	
Lanspora cylindrospora	**NFCCI 4391**	**MN168889**	**MN168891**	Hyde et al. 2020
L. cylindrospora	NFCCI 4427	MN168890	MN168892	Hyde et al. 2020
Myrmecridium banksiae	**CBS 132537**	**JX069871**	**JX069855**	Crous et al. 2012
M. dactylidis	**CBS 148281**	**OK664729**	**OK663768**	Crous et al. 2021
M. flexuosum	**CBS 398 76**	**EU041768**	**EU041825**	Arzanlou et al. 2007
M. fluviae	**CNUFC YR61 1**	**KX839678**	**KX839677**	Tibpromma et al. 2017
M. fluviae	CNUFC YR61 2	KX839679	KX839676	Tibpromma et al. 2017
M. hiemale	**CBS 141017**	**KP714695**	**KU302612**	Peintner et al. 2016
M. hiemale	JMRC SF 12083	KT380622	–	Peintner et al. 2016
M. iridis	**CBS 139917**	**KR476744**	**KR476777**	Crous et al. 2015a
M. iridis	KUNCC 10792	OP326189	OP326201	This study
M. iridis	KUNCC 10793	OP326190	OP326202	This study
M. junci	**CBS 148274**	**OK664725**	**OK663764**	Crous et al. 2021
M. juncicola	**CBS 148316**	**OK664731**	**OK663770**	Crous et al. 2021
M. juncicola	CBS 148267	OK664733	OK663772	Crous et al. 2021
M. juncigenum	**CBS 148268**	**OK664735**	**OK663774**	Crous et al. 2021
M. montsegurinum	**PRM 934684**	**KT991674**	**KT991664**	Réblová et al. 2016
M. obovoideum	**HGUP 0314**	**KC136140**	**KC136139**	Jie et al. 2013
M. phragmiticola	**CBS 146628**	**MT373366**	**MT373349**	Crous et al. 2020
M. phragmitis	**CBS 131311**	**JQ044425**	**JQ044444**	Crous et al. 2011
M. pulvericola	**DAOM 250405**	**KU309312**	**KU309313**	Crous et al. 2016
M. sambuci	**CBS 148444**	**OK664707**	**OK663746**	Crous et al. 2021
M. schulzeri	CBS 325 74	EU041775	EU041832	Arzanlou et al. 2007
M. schulzeri	CBS 134 68	EU041770	EU041827	Arzanlou et al. 2007
M. schulzeri	KUNCC 10798	OP326191	–	This study
M. spartii	**CBS 140006**	**KR611884**	**KR611902**	Crous et al. 2015b
M. splendidum	**GZCC 19-0549**	**MW133875**	**OP377931**	Yang et al. 2023
M. thailandicum	**CBS 136551**	**KF777169**	**KF777222**	Crous et al. 2013
Neomyrmecridium aquaticum	**MFLUCC 15 0366**	**MK828657**	**MK849804**	Luo et al. 2019
N. aquaticum	MFLUCC 18 1489	MK828656	MK849803	Luo et al. 2019

Table 1 Continued

Species	Specimen. Voucher	GenBank accession numbers		References
		ITS	LSU	
N. asiaticum	**CBS 145080**	**MK047444**	**MK047494**	Crous et al. 2018a
N. asymmetricum	**CCMCIBE H304**	**MN014057**	**MN014055**	Serrano et al. 2020
N. asymmetricum	CCMCIBE H304 A	MN014058	MN014056	Serrano et al. 2020
N. guizhouense	**GZCC 20 0008**	**MT002305**	**MT002307**	Hyde et al. 2020
N. gaoligongense	**KUNCC 10794**	**OP326185**	**OP326197**	This study
N. gaoligongense	KUNCC 10795	OP326186	OP326198	This study
N. luguense	**KUNCC 10796**	**OP326187**	**OP326199**	This study
N. luguense	KUNCC 10797	OP326188	OP326200	This study
N. naviculare	**GZCC 20-0484**	**OP377827**	**OP377927**	Yang et al. 2023
N. naviculare	MFLUCC 19-0303	OP377828	OP377928	Yang et al. 2023
N. septatum	**CBS 145073**	**MK047442**	**MK047492**	Crous et al. 2018a
N. sorbicola	**CBS 143433**	**MH107901**	**MH107948**	Crous et al. 2018a

Results

Phylogenetic analyses

The concatenated sequence dataset of ITS and LSU gene regions comprised 39 strains. The dataset contained 1224 characters including gaps (ITS = 454 bp, LSU = 770 bp). The aligned sequence matrix comprises 1401 distinct alignment patterns with 5.42% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.239397, C = 0.251091, G = 0.287768, T = 0.221745, with substitution rates AC = 2.502621, AG = 3.221060, AT = 2.368767, CG = 0.716144, CT = 10.894950, GT = 1.000000; gamma distribution shape parameter α = 0.188231. The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of -6163.440829. The tree topologies obtained from ML and BI analyses were largely consistent and shown in the Fig. 1. The phylogenetic analysis showed that the two collections (KUNCC 10792 and KUNCC 10793) clustered with the type strain *Myrmecridium iridis* (CBS 139917) with 83% MLBS and 0.99 BYPP support. The isolate KUNCC 10798 clusters with the two isolates of *M. schulzeri* (CBS 325.74, CBS 134.68) with 98% MLBS and 1.00 BYPP support. Four isolates including KUNCC 10796, KUNCC 10797, KUNCC 10796 and KUNCC 10797 form a monophyletic clade with *Neomyrmecridium* taxa (Fig. 1).

Taxonomy

Neomyrmecridium gaoligongense R.J. Xu, Q. Zhao & Boonmee, sp. nov. Fig. 2

MycoBank number: MB 850054; Facesoffungi number: FoF 14345

Etymology – the epithet "*gaoligongense*" is named after the collection sites, the Gaoligong Mountains, China.

Saprobic on decaying wood. Sexual morph: undetermined. Asexual morph: hyphomycetous. *Colonies* on natural substrate superficial, effuse, pale brown, velvety. *Mycelium* immersed, composed of brown, branched, septate hyphae. *Conidiophores* 138–226 × 4–7 µm ($\bar{x}$ =176 × 6 µm, n = 25), macronematous, mononematous, solitary, erect, unbranched, single or in groups of two or three, straight or slightly flexuous, subcylindrical, tapering towards the apex, 4–7-septate, slightly constricted at septa, brow to dark brown at the base and pale brown towards the apex. *Conidiogenous cells* 34–68 × 3–6 µm ($\bar{x}$ = 47–5 µm, n = 20), subcylindrical, polyblastic, terminal, integrated, determinate, subhyaline, with several denticles at the apex. *Conidia* 16–24 × 5–7 µm ($\bar{x}$ =19 × 6 µm, n = 25), solitary, clavate-cymbiform, truncate at the base, subhyaline to pale brown, aseptate when immature, 0–3-septate when mature, guttulate, smooth-walled.

Culture characteristics – Conidia germinated on PDA within 24 hours and germ tube arising from terminal conidium. *Colonies* circular, edge entire, mycelia superficial and dense at center, becoming sparse towards the circumference, yellowish white to yellow from upper and lower view.

Material examined – China, Yunnan Province, Lushui City, Pian Ma, Gaoligong Mountains, on submerged decaying wood in freshwater habitats, 3050 msl, 25°58′9″N, 98°41′1″E, 29 Apr 2021, R.J. Xu, GLG-07, (HKAS 124621, **holotype**), ex-type living culture, KUNCC 10794; *ibid*., Gaoligong Mountains, saprobic on submerged decaying wood in freshwater habitats, 2600 msl, 25°30′28″N, 97°55′3″E, 30 Apr 2021, R.J. Xu, GLG-14, (HKAS 124622, paratype), living culture, KUNCC 10795.

Notes – The new taxon, *Neomyrmecridium gaoligongense* shares similar characteristics with *N. sorbicola* and *N. asymmetricum* in having unbranched, subcylindrical conidiophores, terminal, integrated conidiogenous cells and solitary conidia. However, *N. gaoligongense* differs from *N. sorbicola* in its clavate-cymbiform, larger conidia without mucoid sheath, whereas *N. sorbicola* has obovoid, smaller conidia with mucoid sheath (see Table 2). In addition, *N. gaoligongense* differs from *N. asymmetricum* in number of conidial septa (0–3-septate vs 0–1-sepate) and conidial size (5–7 µm vs 2–3 µm) (Crous et al. 2018a, Serrano et al. 2020).

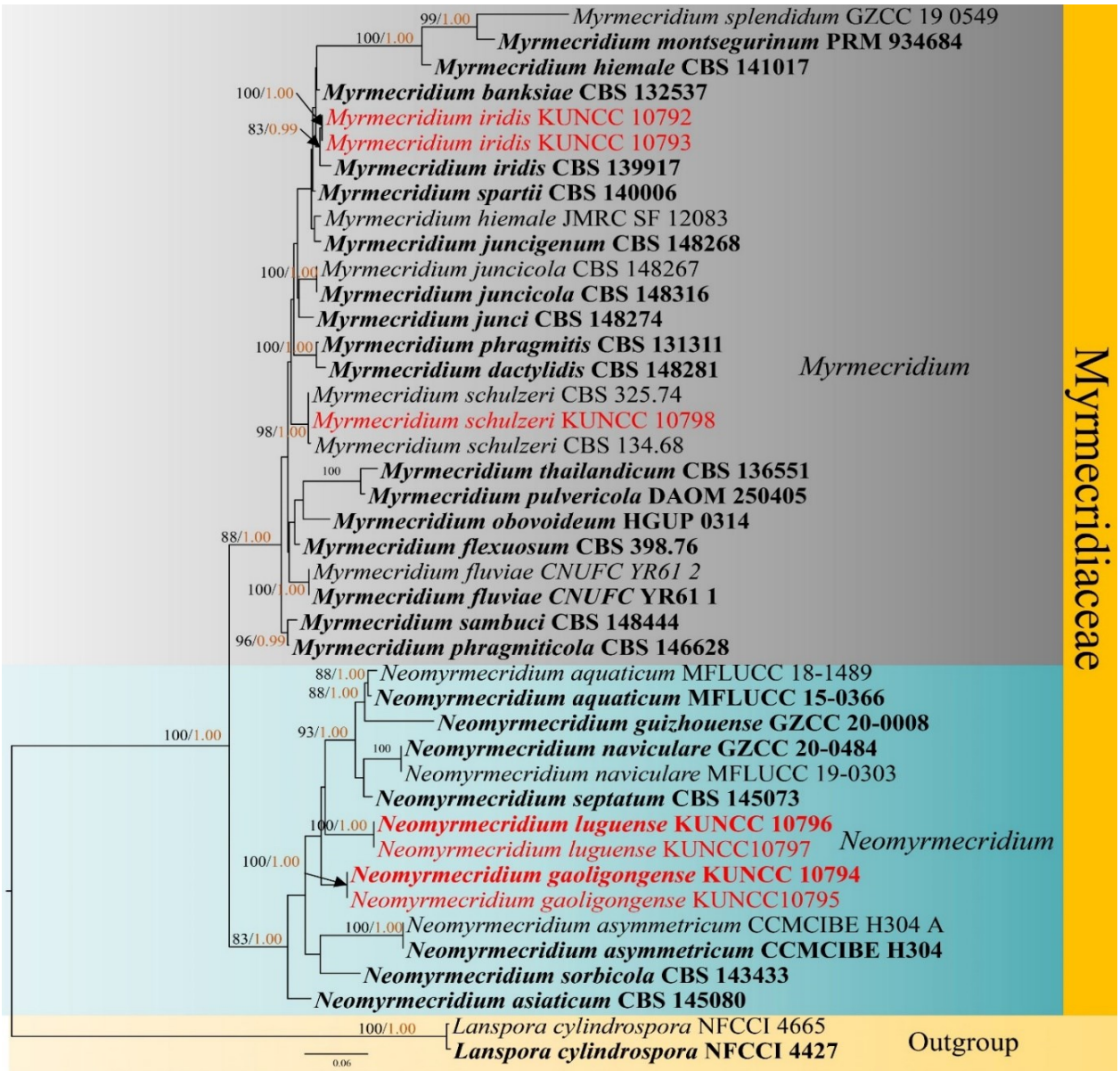

Fig. 1 – Phylogenetic tree generated from RAxML analysis of a combined ITS and LSU sequence dataset. Bootstrap support values (MLBS) equal to or greater than 75% and posterior probability (BYPP) equal to or greater than 0.95 are provided above the nodes. The new isolates in this study are in red bold. *Lanspora cylindrospora* (NFCCI 4427, NFCCI 4391) is selected as the outgroup taxon (Hyde et al. 2020).

Fig. 2 – *Neomyrmecridium gaoligongense* (HKAS 124621, **holotype**). a Colonies on natural substrate. b–e Conidiophores with conidiogenous cells and conidia. f–h Conidiogenous cells with immature conidia. i–k Conidia. l, m Culture on PDA at 30 days old. Scale bars: b–e = 50 µm, f–k = 10 µm.

Neomyrmecridium luguense R.J. Xu, Q. Zhao & Boonmee, sp. nov. Fig. 3

MycoBank number: MB 850055; Facesoffungi number: FoF 14346

Etymology – Name refers to Lugu Lake, the place where this fungus was collected.

Saprobic on decaying wood. Sexual morph: undetermined. Asexual morph: hyphomycetous. *Colonies* on natural substrate effuse, greyish white, velvety. *Mycelium* immersed, composed of brown, branched, septate hyphae. *Conidiophores* 152–298 × 4–6 μm ($\bar{x}$ = 230 × 5 μm, n = 25), macronematous, mononematous, solitary, single or in groups of 2 or 3, erect, straight or slightly flexuous, subcylindrical, medium brown, paler towards apex, unbranched, 5–10-septate. *Conidiogenous cells* 39–68 × 3–5 μm ($\bar{x}$ = 58–4 μm), polyblastic, terminal, integrated, intercalary, subcylindrical, subhyaline, with several denticles at apex. *Conidia* 12–15 × 4–7 μm ($\bar{x}$ = 14 × 6 μm, n = 30), solitary, obovoid, tapering at the base, subhyaline to pale brown, (0–)2–3-septate, sometimes surrounded by a mucilaginous sheath, guttulate, smooth-walled.

Culture characteristics – Conidium germinated on PDA within 24 hours and germ tube arising from apical conidium. Mycelia superficial, circular, with entire edge, mycelia dense at centre, sparse towards circumference, yellowish to white mycelium growing towards the edge from above, yellowish at center, bright orange on the edge from below.

Material examined – China, Yunnan Province, Ninglang County, Lugu Lake, saprobic on submerged decaying wood in freshwater habitats, 2672 msl, 27°40′4″N, 100°47′5″E, 05 March 2021, H.W. Shen, L1127, (HKAS 124601, **holotype**), ex-type living culture, KUNCC 10796; China, Sichuan Province, Yanyuan County, Lugu Lake, saprobic on submerged decaying wood in freshwater habitats, 2731 msl, 27°44′40″N, 100°48′40″E, 23 October 2021, H.W. Shen, MD-GLG1, (HKAS 124613, paratype), living culture, KUNCC 10797.

Notes – The new species, *Neomyrmecridium luguense* shares similar characters with *N. aquaticum* in having unbranched, subcylindrical conidiophores, terminal, integrated, intercalary conidiogenous cells, and solitary, obovoid conidia (Luo et al. 2019). However, *N. luguense* can be distinguished from *N. aquaticum* by the presence of mucilaginous sheath conidia. In the phylogenetic tree, *N. luguense* (KUNCC 10796 and KUNCC 10797) isolates formed a distinct lineage indicating they represent a new species (Fig. 1).

Myrmecridium iridis Crous, in Crous et al., Persoonia 34: 219 (2015) Fig. 4

MycoBank number: MB 812462

Saprobic on decaying wood. Sexual morph: undetermined. Asexual morph: hyphomycetous. *Colonies* on natural substrate effuse, greyish white, velvety. *Mycelium* immersed, composed of brown, branched, septate hyphae. *Conidiophores* 75–140 × 2–4.5 μm ($\bar{x}$ = 98 × 3 μm, n = 15), macronematous, mononematous, solitary, groups of 2 or 3, erect, straight or slightly flexuous, subcylindrical, unbranched, 2–5-septate, medium brown, paler towards apex, smooth, thin-walled. *Conidiogenous cells* 2–4 μm wide, polyblastic, terminal, integrated, subcylindrical, subhyaline, bearing several denticles. *Conidia* 9–13 × 3–5 μm ($\bar{x}$ = 10 × 4 μm, n = 30), solitary, fusoid, truncate at the base, hyaline to pale brown, 1-septate, smooth-walled.

Known distribution – the Netherlands, China.

Culture characteristics – *Conidium* germinated on PDA within 24 hours and germ tube arising from both ends of conidium. *Mycelia* superficial, spreading, with even, sparse to moderate aerial mycelium, mycelia dense at centre, radially striate, with fimbriate margins, sparse towards circumference, pale to apricot from above and below.

Material examined – China, Xinjiang Uygur Autonomous Region, Bayingolin Mongolian Autonomous Prefecture, Qiemo County, on submerged decaying wood in freshwater habitats, 1537 msl, 38°7′32.5″N, 85°34′22.5″E, 24 July 2021, R.J. Xu, MD-309, (HKAS 124614), living culture, KUNCC 10792; China, Xinjiang Uygur Autonomous Region, Bayingolin Mongolian Autonomous Prefecture, Qiemo County, on submerged decaying wood in freshwater habitats, 1581 msl, 36°35′55.44″N, 88°7′29.9″E, 25 July 2021, R.J. Xu, MD-309-1, (HKAS 124615, living culture, KUNCC 10793.

Notes – *Myrmecridium iridis* was described by Crous et al. (2015a) from symptomatic leaves of *Iris* sp. (Iridaceae) in the Netherlands. In this study, our two isolates KUNCC 10792 and KUNCC 10793 were found on decaying wood submerged in freshwater habitats in Xinjiang, China, and phylogenetic analysis showed these two strains cluster with the ex-type strain *M. iridis* (CBS 139917) with 78% MLBS and 0.99 BYPP support (Fig. 1). KUNCC 10792 and KUNCC 10793 strains share similar characteristics with *M. iridis* (CBS 139917) in having terminal, denticles conidiogenous cells and solitary, fusoid conidia (Crous et al. 2015a). Therefore, we identified the two isolates as a new record for *Myrmecridium iridis* from freshwater habitat in China.

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, Studies in Mycology 58: 84 (2007) Fig. 5

MycoBank number: MB 504560; Facesoffungi number: FoF 14347

Saprobic on decaying wood. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* on natural substrate effuse, greyish white, velvety. *Mycelium* immersed, composed of brown, branched, septate hyphae. *Conidiophores* 88–115 × 2–4 μm ($\bar{x}$ = 106 × 3 μm, n = 20), macronematous, mononematous, solitary, erect, straight or slightly flexuous, subcylindrical, unbranched, 3–5-septate, paler towards apex, smooth, thick-walled. *Conidiogenous cells* 38–42 × 3–4 μm ($\bar{x}$ = 40 × 3 μm, n = 20), polyblastic, terminal, integrated, subcylindrical, subhyaline, verrucose, with several denticles at the apex. *Conidia* 6–8 × 3–4 μm ($\bar{x}$ = 7 × 3 μm, n = 25), solitary, ellipsoid, obovoid or fusiform, subhyaline, aseptate, smooth or finely verrucose-walled.

Known distribution – Australia, China, France, Germany, Ibid, Ireland, Korea, Netherlands, Spain, Thailand, Ukraine and Uruguay

Culture characteristics – *Conidia* germinating on PDA within 24 hours. Germ tubes produced from one or both ends. *Mycelia* superficial, circular, flat, rather compact, with entire margin, mycelia dense at centre, sparse towards circumference, pale orange to orange.

Material examined – China, Yunnan Province, Ninglang County, Lugu Lake, saprobic on submerged decaying wood in freshwater habitats, 2672 msl, 27°40′4″N, 100°47′5″E, 05 March 2021, H.W. Shen, L145, (HKAS 124585), living culture, KUNCC 10798.

Notes – Arzanlou et al. (2007) introduced the genus *Myrmecridium* with the type species *M. schulzeri* (Sacc.) Arzanlou, W. Gams & Crous (≡ *Psilobotrys schulzeri* Sacc.) based on morphological and phylogenetic analyses, and treated this genus in *Sordariomycetes incertae sedis*. *Myrmecridium schulzeri* can be found as plant pathogens or saprobes in soil (Arzanlou et al. 2007, Rezakhani et al. 2019). Whereas, our collection was found as a saprobe on decaying wood in freshwater habitat. Phylogenetic analysis showed that our isolate KUNCC 10798 clustered with *M. schulzeri* with 99% MLBS and 1.00 BYPP support (Fig. 1). The shape and size of conidiophores, conidiogenous cells and conidia of our strains are identical to those of the type strain of *M. schulzeri* (CBS 325.74) (Arzanlou et al. 2007). We therefore identified a new isolate of KUNCC 10798 as new freshwater habitats record of *Myrmecridium schulzeri* from Yunnan, China.

Discussion

Myrmecridiales comprises a single family *Myrmecridiaceae* which has two genera, *Myrmecridium* and *Neomyrmecridium.* The main features of *Myrmecridiales* are the solitary, unbranched, straight or flexuose, septate conidiophores, polyblastic, integrated, denticulate, cylindrical conidiogenous cells and fusoid-ellipsoid, navicular or fusiform, smooth or sheath conidia (Arzanlou et al. 2007, Crous et al. 2018a, Yang et al. 2023). These characteristics are heterogeneous and polyphyletic in the phylogenetic tree of *Myrmecridiaceae* based on combined of ITS and LSU sequence data, suggesting that morphologic traits have low resolution in generic demarcation. For example, *Myrmecridium sorbicola* and *M. aquaticum* has the typical characteristics of *Myrmecridium*, while phylogenetic analysis showed they clustered with species of *Neomyrmecridium*, thus they were transferred to the latter genus (Crous et al. 2018a). It is worth to mentioning that conidial morphology (e.g., septa, shape and sheath) were usually used for interspecific identification. In this study, our new species *Neomyrmecridium luguense* phylogenetically is closely related to *N. aquaticum*,

Fig. 3 – *Neomyrmecridium luguense*. (HKAS 124601, **holotype**). a Colonies on natural substrate. b–e Conidiophores with conidiogenous cells and conidia. f Conidiogenous cells. g–l Conidia. m Germinated conidium. n Culture on PDA at 30 days old. – Scale bars: b–e = 50 µm, f= 20 µm, g–l = 5 µm.

Fig. 4 – *Myrmecridium iridis* (HKAS 124614). a Colonies on natural substrate. b–e Conidiophores with conidiogenous cells and conidia. f–l Conidia. j, k Culture on PDA at 30 days old. Scale bars: b–e = 50 µm, f–k = 5 µm.

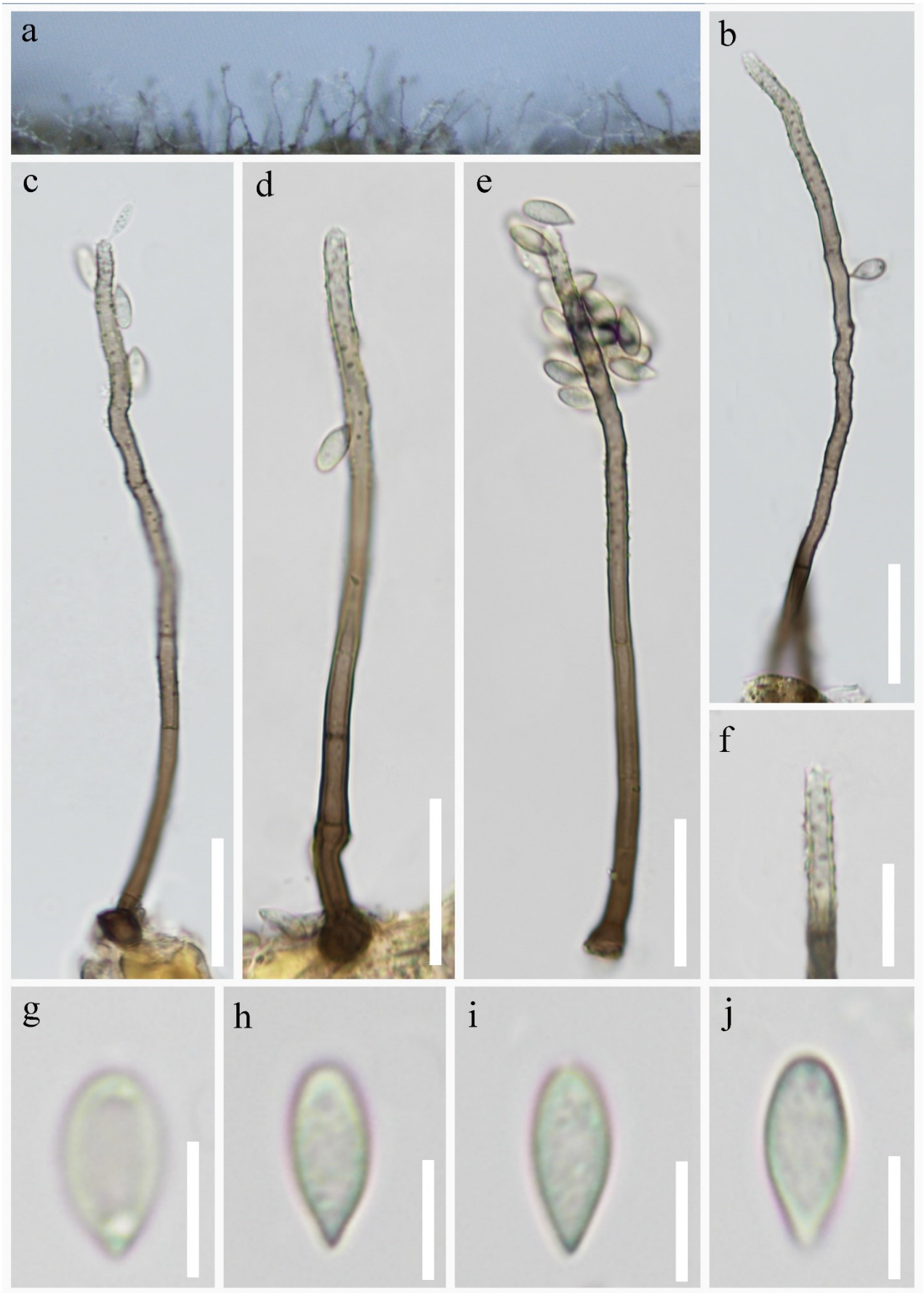

Fig. 5 – *Myrmecridium schulzeri* (HKAS 124585). a Colonies on natural substrate. b–e Conidiophores with conidiogenous cells and conidia. f Conidiogenous cells. g–j Conidia. Scale bars: b–e = 20 µm, f =10 µm, g–j = 5 µm.

Table 2 Synopsis of known species in *Neomyrmecridium.*

Species	Conidiophores	Conidiogenous cells	Conidia	Host	Distribution	References
Neomyrmecridium aquaticum	Macronematous, mononematous, erect, unbranched, multi-septate, 211–308 × 5–7 µm.	Holoblastic, polyblastic, integrated, terminal.	Obovoid, 3-septate, 14–16 × 4–6 µm.	On decaying wood in freshwater habitat.	China	Luo et al. 2019
N. asiaticum	Arising from hyphal coils on creeping hyphae, mostly unbranched, 2–5-septate, 50–100 × 3–5 µm.	Polyblastic, integrated, terminal, denticles, 5–35 × 4–7 µm.	Ellipsoid to obovoid, 2–3-septate, 13–17 × 4–5 µm, wing-like gelatinous sheath.	On leaves of unidentified vine.	Thailand	Crous et al. 2018a
N. asymmetricum	Macronematous, mononematous, erect, unbranched, 3–15-septate, 40–210 × 3.5–9 µm.	Polyblastic, terminal, integrated, denticulate, 8–35 × 3.5–5 µm.	Narrow clavate or subclavate, 1-euseptate, 12–15 × 2–3 µm.	On decaying leaves of *Theobroma cacao.*	Ecuador	Serrano et al. 2020
N. gaoligongense	Macronematous, mononematous, solitary, erect, unbranched, 4–7-septate, 138–226×4–7 µm.	Polyblastic, terminal, integrated, denticles, 34–68 × 3–6 µm.	Clavate-cymbiform, 0–3 septate, 16–24×5–7 µm.	On decaying wood in freshwater habitat.	China	This study
N. luguense	Macronematous, mononematous, solitary, erect, unbranched, 5–10-septate, 75–140×2–4.5 µm.	Polyblastic, terminal, integrated, intercalary, denticles, 39–68 × 3–5 µm.	Obovoid, (0–)2–3-septate, 12–15 × 4–7 µm.	On decaying wood in freshwater habitat.	China	This study
N. naviculare	macronematous, mononematous, erect, unbranched, 100–200×4–5.6 µm.	Polyblastic, integrated, terminal, sympodial, denticulate.	Navicular to fusiform, tapering to a hilum towards the base, (1–)3–septate, 16–24×5.5–7.5 µm, with a thin mucilaginous sheath	On decaying submerged wood in freshwater habitats	China	Yang et al. 2023
N. septatum	Solitary, erect, straight, unbranched, 1–4-septate, 40–70 × 4–5 µm.	Polyblastic, terminal, integrated, denticles, 30–40 × 4–5 µm.	Fusoid-ellipsoid, 1–3-septate, 12–20 × 3.5–5 µm, mucoid sheath	On leaves of unidentified vine.	Thailand	Crous et al. 2018a
N. sorbicola	Solitary, erect, unbranched, 1–18-septate, 50–200 × 4–7 µm.	Polyblastic, integrated, terminal and intercalary, denticles, 20–65 × 3–4 µm.	Obovoid, 0–3-septate, 7–15 × 4–5 µm, mucoid sheath.	On branch of *Sorbus aucuparia*	Germany	Crous et al. 2018a, 2018b

however, the former can be distinguished from the latter by the presence of mucilaginous sheath on the conidia. On the other hand, species with similar conidial morphology could be phylogenetically distantly related, for example, *Neomyrmecridium asiaticum* resembles *N. septatum* based on its conidial morphology, but these two species are phylogenetically distinct. Therefore, utilization of morphological and phylogenetic data is needed to resolve a natural classification of *Myrmecridiales* species.

Most species of *Myrmecridiaceae* present their asexual morph on the natural substrates and artificial medium (Arzanlou et al. 2007, Jie et al. 2013, Peintner et al. 2016, Tibpromma et al. 2017, Luo et al. 2019, Hyde et al. 2020, Serrano et al. 2020, Crous et al. 2020, 2021, 2022). *Myrmecridium montsegurinum* is the only one that is known from its sexual morph (Réblová et al. 2016). This species is characterized by solitary or gregarious ascomata with subglobose to conical venter and a papilla or short central necks, cylindrical periphysate ostiole, slender stipe, non-amyloid with apical annulus asci and ellipsoidal, slightly inequilateral, without sheath or appendages ascospores (Réblová et al. 2016).

Members of *Myrmecridiaceae* are usually saprobes on dead plant branches, such as *Myrmecridium junci*, *M. juncicola* and *M. juncigenum* are saprobes on culms of *Juncus effusus* (Juncaceae) (Crous et al. 2021). *Myrmecridium phragmiticola*, *M. phragmitigenum* and *M. phragmitis* occurred on dead culms of *Phragmites australis* (Poaceae) (Crous et al. 2020, 2021). Some species such as *M. hiemale*, *M. obovoideum*, *M. flexuosum* and *M. schulzeri* were isolated from soil (Arzanlou et al. 2007, Jie et al. 2013, Peintner et al. 2016). In addition, *M. montsegurinum*, *M. fluviae*, *Neomyrmecridium aquaticum*, *N. gaoligongense*, *N. luguense* and *N. navicularеare* were collected from freshwater habitats (Réblová et al. 2016, Tibpromma et al. 2017, Luo et al. 2019, Yang et al. 2023).

The Tibetan Plateau is renowned for its exceptional biological diversity and extensive range of aquatic habitats, encompassing lakes, rivers, and wetlands that harbor diverse fungal communities (Yao et al. 2019). Although freshwater fungi play a crucial role in the ecosystem (Calabon et al. 2023), their study has been neglected in this region due to limited research. Our investigation of freshwater fungi diversity on the Tibetan Plateau has led to two new species and two new records in *Myrmecridiaceae*, based on phylogenetic analysis and morphologic observation. The discovery of these novel species contributes to our comprehension of the fungal diversity in the Tibetan Plateau and will allow comparison along a north/south gradient in Asia (Hyde et al. 2016) and underscores the significance of sustained exploration and investigation into fungal species.

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