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A new species and one new record of *Sporocadaceae* (Sordariomycetes) from Guizhou Province, China

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Abstract

Sporocadaceae species are appendaged coelomycetes. They are commonly associated with pathogens, saprobes and endophytes in a wide range of hosts. Two *Sporocadaceae* species were collected from the tobacco leaves in Guizhou Province, China. The internal transcribed spacer nrDNA (ITS) barcoding indicated that these isolations belonged to *Hymenopleella* and *Robillarda*. Morphological data in combination with molecular phylogenetic analyses based on ITS, beta-tubulin (*tub2*), and translation elongation factor 1-alpha (*tef-1α*) confirmed that our strains represented a new species, *Hymenopleella rhododendronae* sp. nov. and a new Chinese record of *Robillarda africana* associated with tobacco. These results will expand the current knowledge on fungal diversity associated with tobacco in China.

Keywords – 1 new species – *Hymenopleella* –*Robillarda* – Taxonomy

Introduction

Sporocadaceae species well-known as pestalotioid fungi, are a group of appendaged coelomycetes, including many genera treated by Nag Raj (1993). Jaklitsch et al. (2016) revised the family under Xylariales based on morphological observations and phylogenetic analyses of ITS and LSU sequence data and proposed *Sporocadaceae* as a natural group containing most asexual morph genera. In an extensive multi-gene phylogenetic study on coelomycetous taxa with appendage-bearing conidia, Wijayawardene et al. (2022) placed 35 monophyletic genera in *Sporocadaceae* including *Bartalinia* Tassi, *Clypeosphaeria* Fuckel, *Hymenopleella* Munk, *Monochaetia* (Sacc.) Allesch, *Neopestalotiopsis* Maharachch, K.D. Hyde & Crous, *Pestalotiopsis* Steyaert, *Robillarda* Sacc, *Seimatosporium* Corda, *Sporocadus* Corda, and *Strickeria* Körb.

Hymenopleella was introduced by Munk (1957) and typified with *Hymenopleella hippophaes* (Fabre) Munk. Currently, there are 10 *Hymenopleella* species listed in Species Fungorum (2024) and there are 12 species epithets are given in Index Fungorum (2024 October). These species include several important plant pathogenic taxa, such as, *H. hippophaeicola*, often causing the death of desert trees (Liu 2017). The genus was established in 1880 by Saccardo, which produces flexuous, narrow tubular, aseptate appendages and holoblastic conidiogenous cells, proliferating sympodially or percurrently near the apex (Crous et al. 2015, Wijayawardene et al. 2016). There are 42 species currently accepted in *Robillarda* (Species Fungorum 2024), and many of them are associated with living and decayed plants and also in soil (Shimoyama 2018).

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During this study, two species of *Sporocadaceae* were found in Guizhou Province, China. One collection is introduced as a novel species (*Hymenopleella rhododendronis* sp. nov.) while the other collection is reported as a new record *Robillarda africana* associated with tobacco plants from China.

Materials & Methods

Samples collection and isolation

The samples were collected in Guiyang City, Guizhou Province, China. The tobacco leaves were surface-disinfected, and pure cultures were isolated using the single-spore method (Chomnunti et al. 2014). Colonies growing from single spores were transferred to potato-dextrose agar (PDA) and incubated at room temperature (25ºC). Representative cultures of the new species described in this study are deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (GUCC).

Morphological Characterisation

Colony diameters were measured, and morphological culture characters were recorded after 1-2 weeks (Boerema et al. 2004). Microscopic slides were prepared in lactophenol. Light microscopy observations were made using BX53 compound microscopy (Olympus, Tokyo, Japan) at 1000× magnification. The morphology was observed using a compound microscope (OLYMPUS BX53), showing all necessary details of morphology and ontogeny of reproductive propagules. Measurements were made of 30 structures for conidia and conidiophores. Index Fungorum (IF) and faces of fungi (FoF) numbers were registered as mentioned in Index Fungorum (2024) and Jayasiri et al. (2015).

DNA extraction, PCR amplification, and sequencing

In this study, the strains were cultured on PDA at 25°C. Total genomic DNA was extracted from fresh mycelia grown on PDA using the Fungus Genomic DNA Extraction Kit (Biomiga #GD2416, San Diego, California, USA). The PCR amplification for ITS, *tub2*, and *tef-1α* was prepared by Bonthond et al. (2018). Three partial loci, including the 5.8S nuclear ribosomal DNA gene with the two flanking internally transcribed spacer regions (ITS), β-tubulin (*tub2*) genes, and the translation elongation factor 1-alpha (tef-1α) were amplified and sequenced using the following primer pairs: ITS4/ITS5 for ITS (White et al. 1990), EF-1/EF-2 for *tef-1α* (O'Donnell et al. 1998) and T1/Bt2b for *tub2* (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). The general PCR conditions were an initial denaturation step of 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 50 s at 52°C (ITS) or 55°C (*tub2*, *tef-1α*), and 1 min at 72°C, and a final elongation step of 7 min at 72°C. Purification and sequencing of the PCR amplicons were sequenced with both forward and reverse primers using an Applied SinoGenoMax (Beijing, China). The resulting DNA sequences were submitted to NCBI GenBank (https://ncbi.nlm.nih.gov/genbank/), and their accession numbers were provided.

Table 1 Taxa are used for molecular phylogenetic analyses and their GenBank accession numbers.

Species name	Strain number	country	host	GenBank Accession numbers		
				ITS	*Tub2*	*tef1-α*
Bartalinia bella	CBS 464.61T	Brazil	Air	MH554051	MH554727	MH554486
B. pini	CBS 143891	Uganda	*Pinus patula*	MH554125	MH554797	MH554559
B. pondoensis	CBS 125525	Netherlands	–	MH863602	MH554663	MH554421
B. robillardoides	CBS 122705	Italy	*Leptoglossus occidentalis*	LT853104	LT853252	LT853202
Hymenopleella austroafricana	CBS 143886T	South Africa	*Gleditsia triacanthos*	MH554115	MH554788	MH554549

Table 1 Continued

Species name	Strain number	country	host	GenBank Accession numbers		
				ITS	*Tub2*	*tef1-α*
H. austroafricana	CBS 144026	South Africa	*Bridelia mollis*	MH554117	MH554790	MH554551
H. austroafricana	CBS 144027	Zambia	*Combretum hereroense*	MH554119	MH554792	MH554553
H. endophytica	EML-AS5-1T	Korea	*Abies firma*	KX216520	–	–
H. hippophaeicola	CBS 113687	Sweden	*Hippopha€e rhamnoides*	MH553969	MH554628	MH554387
H. hippophaeicola	CBS 140410T	Austria	*Hippopha€e rhamnoides*	KT949901	MH554678	MH554436
H. hippophaes	CBS 320.71	Netherlands	–	MH860144	–	–
H. polyseptata	CBS 143887T	South Africa	*Combretum* sp.	MH554116	MH554789	MH554550
H. rhododendronis	**GUCC 21237.7**	**China**	***Rhododendron simsii***	**OR438795**	**OR453871**	**OR453918**
H. schefflerae	COAD 2371	Brazil	–	MH128360	MH231215	MH231216
H. subcylindrica	CBS 164.77	India	*Cocos nucifera*	MH554009	MH554685	MH554443
H. subcylindrica	CBS 647.74T	India	*Gypsophilla seeds*	MH554062	MH554739	MH554498
Robillarda africana	CBS 122.75T	South Africa	–	KR873253	MH554656	MH554414
	GUCC 2178.5	**China**	***Nicotiana tabacum***	**OR438796**	**OR453872**	**OR453919**
	GUCC 2178.11	**China**	***Nicotiana tabacum***	**OR438797**	**OR438793**	**OR453920**
R. australiana	CBS 143882T	Australia	–	MH554091	MH554764	MH554525
R. mangiferae	KUMCC 18-0180T	Thailand	*Mangifera indica*	MK353084	–	–
R. roystoneae	CBS 115445T	Hong Kong	*Roystonea regia*	KR873254	KR873317	KR873310
R. sessilis	CBS 114312T	Germany	Dust	KR873256	KR873319	KR873312
R. terrae	CBS 587.71T	India	Soil	MH860276	MH554734	MH554493
Robillarda sp. GoF-062015	CPC 25020	Netherlands	–	KR873259	KR873322	KR873315
Strickeria kochii	CBS 14041 1T	Austria	*Robinia pseudoacacia*	NR_15442 3	MH554679	MH554437

(T) = ex- type strain. CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; COAD; Coleção Octávio de Almeida Drummond (UFV); CPC: Culture collection of Pedro Crous, housed at the Westerdijk Institute; EML: Environmental Microbiology Laboratory Fungal Herbarium, Chonnam National University, Gwangju, Korea; GUCC: Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University

Results

Phylogenetic Analysis of Hymenopleella

Our final concatenated alignment of *Hymenopleella* species and its closely related taxa included 1722 characters, (ITS: 1–487; *tef-1α*: 488–1058; *tub2*: 1059–1722). *Bartalinia pini* (CBS 143891) and *B. pondoensis* (CBS 125525) were used as the outgroup taxa. The MP analysis included 358 parsimony-informative characters. The MP inference resulted in two equally parsimonious trees (TL = 856, CI = 0.81, RI = 0.81, HI= 0.19, RC = 0.66) and one of them was selected to show the

topology (FIG. 1). Eleven *Hymenopleella* strains clustered together as a clade, which received strong support from both ML and BI analyses. *Hymenopleella rhododendronis* sp. nov. (GUCC 2137.7) was retrieved as a sister taxon of *H. hippophaeicola* (CBS 140410) with 100% ML, 93% MP bootstrap and 1.00 BYPP support.

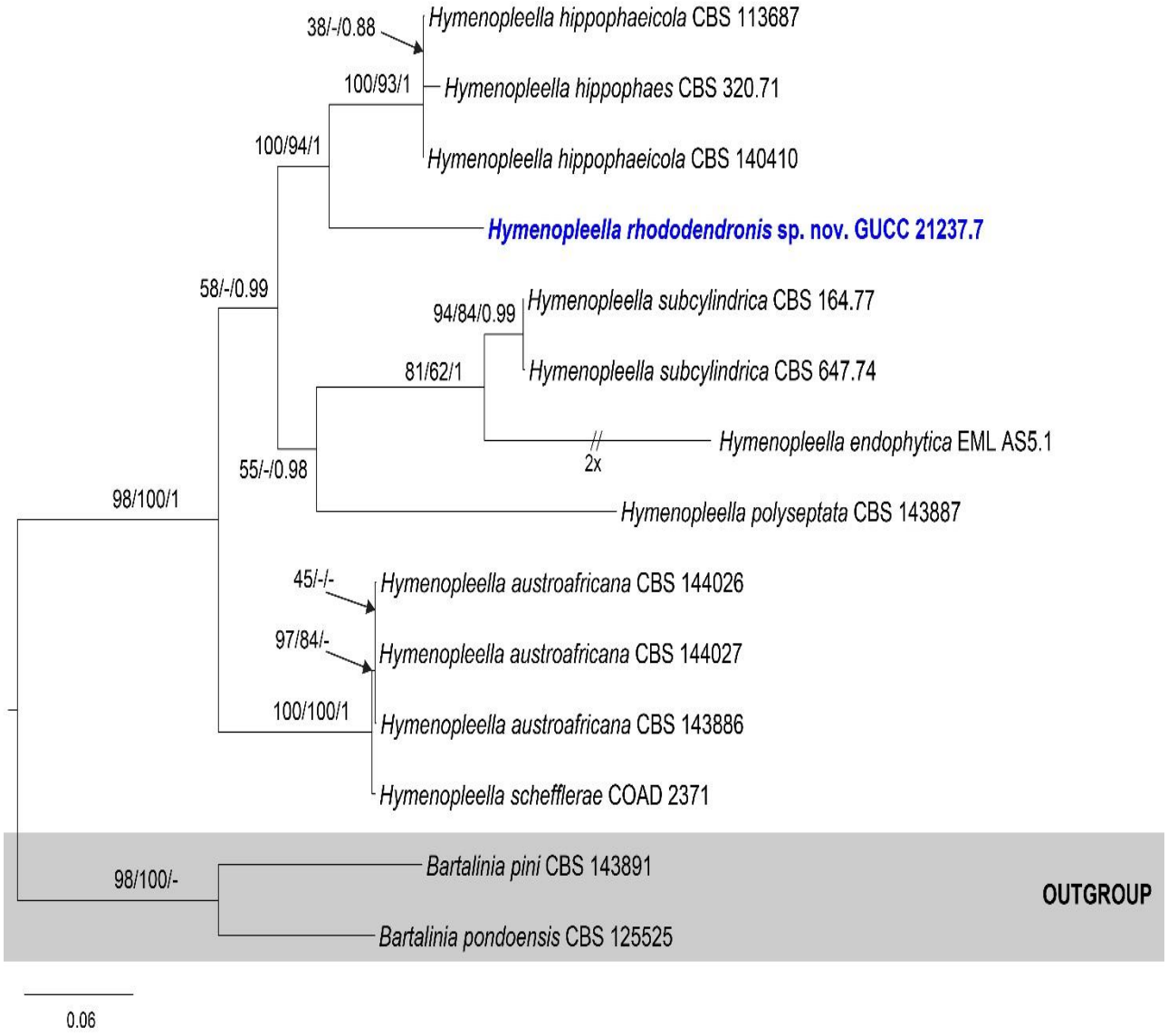


Fig. 1 – Phylogenetic tree inferred from a Maximum Parsimonious analysis based on a concatenated alignment of ITS, *tub2*, and *tef-1α* sequences. ML bootstrap support values (MLBS) ≥ 50, BI posterior probabilities (BIPP) ≥ 0.90, and MP bootstrap support values (MPBS) ≥ 50 are given at the nodes. The tree was rooted to *Bartalinia pini* (CBS 143891) and *B. pondoensis* (CBS 125525). Newly generated isolates are in bold. Ex-type strains are marked by an asterisk (T).

Phylogenetic Analysis of Robillarda

Our final concatenated alignment for *Robillarda* included 1733 characters including gaps) (ITS: 1–522, *tef-1α*: 523–1105; *tub2*: 1106–1773). *Strickeria kochii* (CBS 14041) was selected as the outgroup taxa. The MP analysis included 415 parsimony-informative characters. The MP inference resulted in two equally parsimonious trees (TL = 1026, CI = 0.83, RI = 0.81, HI= 0.17, RC = 0.68) and one of them was selected to show the topology (FIG. 2). Strains GUCC 2178.5 and 2178.11 grouped with *R. africana* (CBS 122.75T) (Crous et al. 2015) and *R. australiana* (CBS 143882) with 92% ML, 87% MP bootstrap values and 1.00 BYPP support showing a closer relationship to *Robillarda africana*.

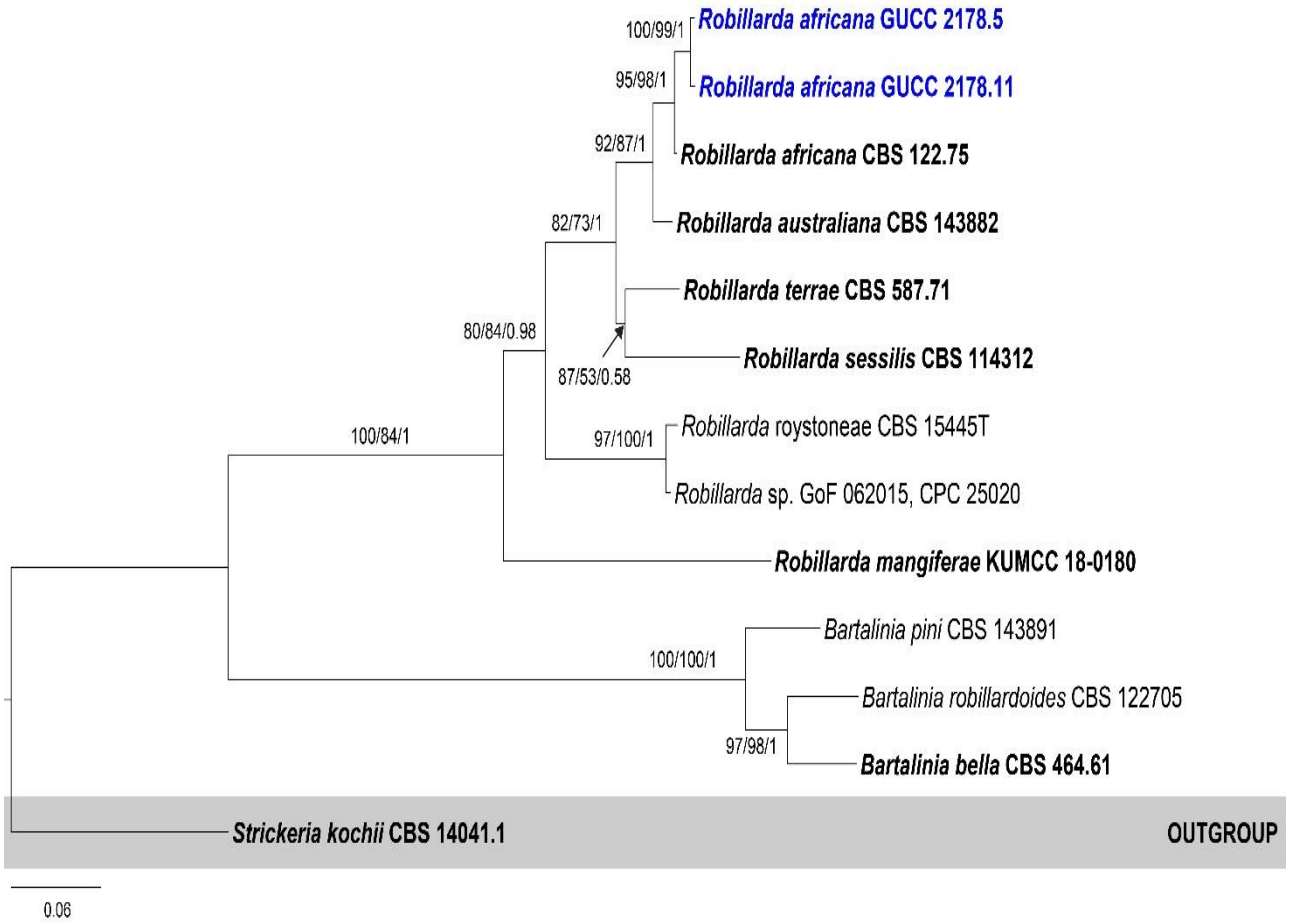


Fig. 2 – Phylogenetic tree inferred from a Maximum Parsimonious analysis based on a concatenated alignment of ITS, *tub2,* and *tef-1α* sequences. ML bootstrap support values (MLBS) ≥ 50, BI posterior probabilities (BIPP) ≥ 0.90, and MP bootstrap support values (MPBS) ≥ 50 are given at the nodes. The tree was rooted in *Strickeria kochii* (CBS 14041). Newly generated isolates are in bold. Ex-type strains are marked by an asterisk (T).

Taxonomy

Hymenopleella Munk, Dansk bot. Ark. 15(no. 2): 89 (1953)

This genus can possess a coelomycetous morph (3–7-septate conidia with apical and basal appendages) mainly distributed in Africa, China and India (Jeewon et al. 2003, Liu et al. 2019). Based on multi-locus phylogenetic analyses, in combination with morphological data, this genus was placed in *Sporocadaceae* (Liu et al. 2019). There are currently 12 epithets of *Hymenopleella* in the database of Index Fungorum.

Hymenopleella rhododendronis W.D. Mo & Yong Wang bis, sp. nov. Fig. 3

Index Fungorum: IF900994; Facesoffungi number: FoF 16682

Etymology: Latin, *rhododendronis*, refers to the host plant (*Rhododendron simsii* Planch.) from which the fungus was isolated.

Sexual morph: undetermined. Asexual morph: coelomycetous, *Conidiomata* 240–860 µm (av. 400 µm) in diam., pycnidial, globose, solitary, black, semi-immersed on PDA, septate, branched, hyaline, thin-walled. *Conidiogenous cells* discrete to lageniform, obclavate, hyaline, or rarely light brown, smooth-walled. *Conidia* (19–)24 × 6(–7) µm ($\bar{x}$ = 21.4 × 6.5 µm, n = 30), fusiform to clavate, straight to slightly curved, 3–5-septate, mostly 4-septate; basal cell cylindrical to obconic, hyaline, thin-walled, smooth, 2–4.5 µm ($\bar{x}$ = 3.2 µm, n = 30); the three median cells 13–16.5 µm ($\bar{x}$ = 14.5 µm, n = 30), concolorous, dark brown with septa darker than the rest of the cells, the second cell from

base 3.5–5.5 µm ($\bar{x}$ = 4.4 µm, n = 30); the third cell 3–5.5 µm ($\bar{x}$ = 4.3 µm, n = 30); the fourth cell 4.5–6.5 µm ($\bar{x}$ = 5.3 µm, n = 30); apical cell 2.5–5 µm ($\bar{x}$ = 3.6 µm, n = 30), cylindrical, hyaline; 1 tubular apical appendage, arising from the apex of the apical cell each at different points, flexuous, 3–7.5 µm ($\bar{x}$ = 5.1 µm, n = 30); basal appendage present, single, tubular, unbranched, 1.5–4.5 µm ($\bar{x}$ = 2.7 µm, n = 30).

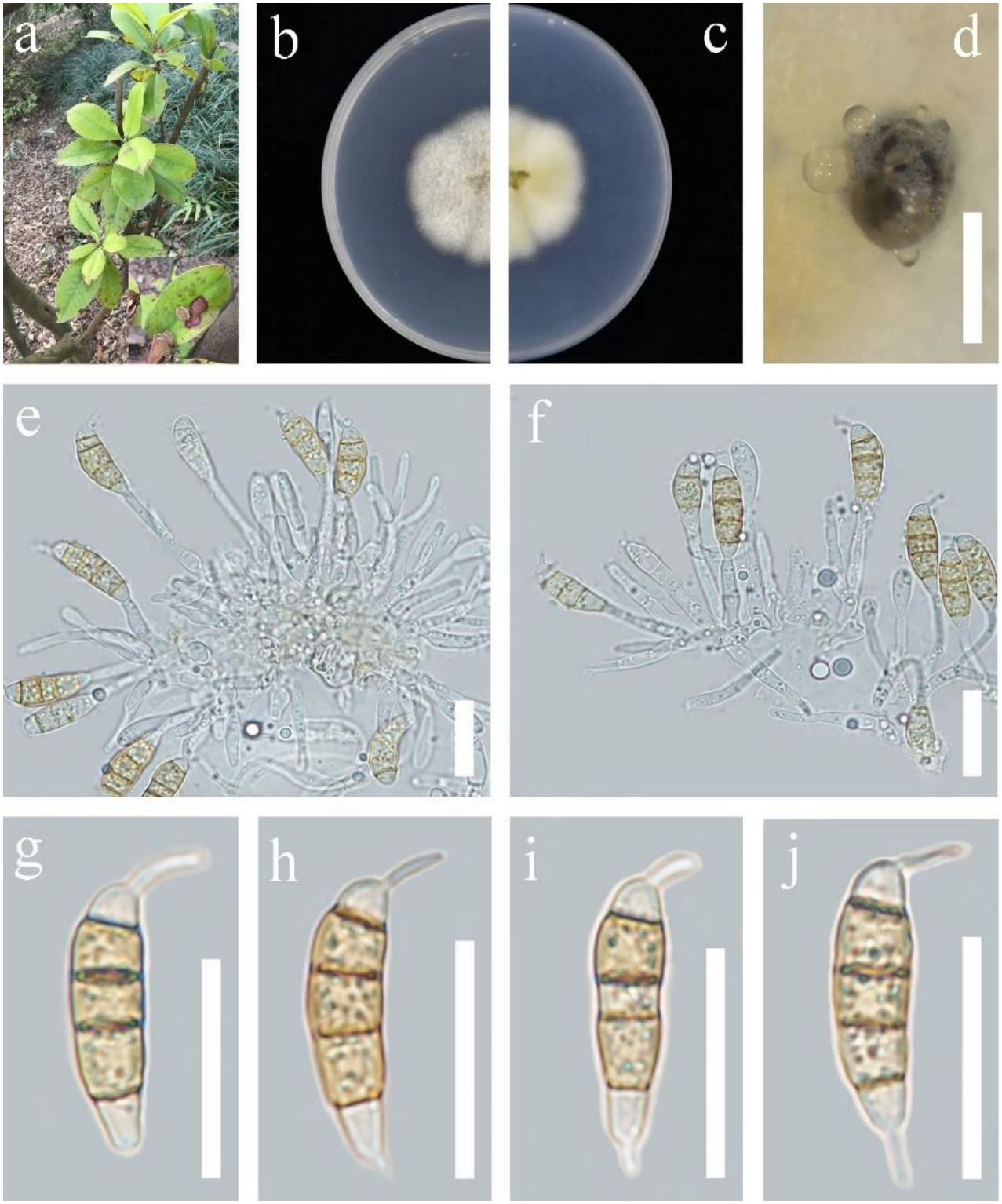


Fig. 3 – ***Hymenopleella rhododendronis*** (GUCC 2137.7; **holotype**). a Leaf spots of *Hymenopleella rhododendronis*. b, c Culture on PDA (b-above, c-reverse). d Colony sporulating on PDA. e–f Conidiophores. g–j Conidia. Scale bars: d = 1000 µm, e–j = 20 µm.

Culture characteristics: *Colonies* on PDA reaching 7–8 cm in diam., After 7 d at room temperature (25℃), under light, 12 hr/dark. *Colonies* filamentous to circular, whitish, with clustered black fruiting bodies and filiform and fluffy margins, white from above and white from the reverse. Material examined: China, Guizhou Province, Guiyang City, from leaves of *Rhododendron simsii* Planch., 11 May 2020, Q. Zhang, (HGUP 137, holotype); ex-type culture GUCC 2137.7.

Note – Based on ITS, *tub2*, and *tef-1α* gene loci, phylogenetic analyses showed that GUCC 2137.7 was closer to *H. hippophaeicola*. Morphologically, *H. rhododendronis* can produce black conidiomata 240–860 µm in diam, whereas those of *H. hippophaeicola* 140–300 µm in diam, and the colony was a 15–50 µm thick wall of reddish-brown hyphae (Jaklitsch et al. 2016). The cells of *H. rhododendronis* are discrete to lageniform, obclavate, hyaline or rarely light brown, smooth-walled, but conidiophores of *H. hippophaeicola* are hyaline, simple, with fine annellations (Jaklitsch et al. 2016). Furthermore, the basal appendage of *H. rhododendronis* is single, tubular, unbranched, 1.5–4.5 µm, while the basal appendage of *H. hippophaeicola* with an acuminate 3–6 µm long appendage sometimes separated by an additional septum. Meanwhile, we also compared the micro-morphology of our new taxon with the other known *Hymenopleella* species. Conidia of *H. polyseptata* is longer than those of *H. rhododendronis* (Liu et al. 2019). Conidia of *H. subcylindrica* have fewer septa than those of *H. rhododendronis* (Liu et al. 2019). Our present taxon has basal appendage. However, *H. schefflerae* is aseptate appendage and basal cell without a basal appendage (Samarakoon et al. 2020). The basal cells of *H. austroafricana* and *H. endophytica* are conical or with a truncate base (Liu et al. 2019), while *H. rhododendronis* is cylindrical to obconical. In summary, comparisons of DNA base pair differences in the three loci and morphological differences confirmed that GUCC 2137.7 represented a new species. A comparison of DNA bases (Table 2) demonstrated that between *H. rhododendronis* (GUCC 2137.7) and *H. hippophaeicola* (CBS 140410), there were nine bp differences in the ITS region, 56 in the *tub2* and 81 in the *tef-1α.* While comparing our strain (GUCC 2178.5) with *R. africana*, there were 8, 9, and 5 differences in the ITS, *tub2, and tef-1α* regions.

Table 2 The DNA base differences between our strains and related taxa in the three gene regions.

Species	**Strain number**	**ITS (1– 554 bp)**	***tub2*** (555– 1320 bp)	***tef-1α*** (1321–1768 bp)
Hymenopleella rhododendronis* sp. nov.	**GUCC 2137.7**	–	–	–
H. hippophaeicola	CBS 113687	8	54	81
	CBS 140410	9	56	81
H. hippophaes	CBS 320.71	16	–	–
Species	Strain number	ITS (1– 585 bp)	*tub2* (586–1122 bp)	*tef-1α* (1123–1860 bp)
Robillarda africana*	**GUCC 2178.5**	–	–	–
	CBS 122.75T	8	5	9
R. australiana	CBS 143882T	4	13	34

Asterisks (*) denote our material.

Robillarda Sacc., Michelia 2 (no. 6): 8 (1880)

This genus with a coelomycetous morph was established by Saccardo in 1880. It produces holoblastic conidiogenous cells, conidia flexuous, narrow tubular with aseptate appendages, proliferating sympodially or percurrently near the apex (Crous et al. 2015, Wijayawardene et al. 2016). This fungal group was distributed widely as saprobes (Wijayawardene et al. 2017, Farr & Rossman 2019), and according to Liu et al. (2019), it was accommodated to *Sporocadaceae*.

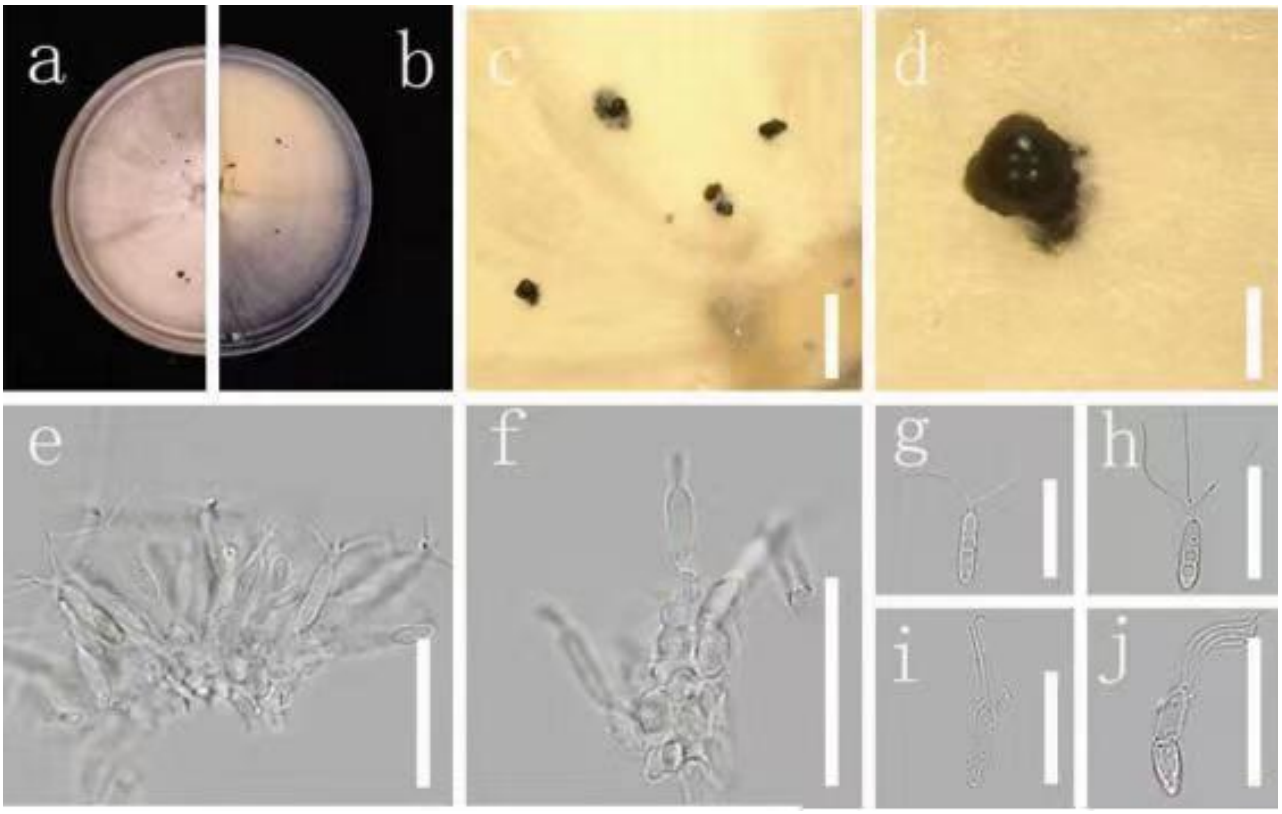
Robillarda africana Crous & Giraldo, IMA Fungus 6 (1): 184 (2015)

Index Fungorum: IF812797; Facesoffungi number: FoF 10700

Sexual morph: unknown. Asexual morph: *Conidiophores* reduced to very short conidiogenous cells, smooth, colourless. *Conidiogenous cells* discrete, thin-walled, guttulate, lageniform, ampulliform or irregular, 7–9 × 2–3 μm ($\bar{x}$ = 7.7 × 2.5 μm), colourless, smooth. Each conidiogenous cell produces 2–4 small but distinct protuberances at the apex. *Conidia* comprise a 1-septate conidium body and a septate apical cell modified into a branched appendage. Conidium body cylindrical, straight, 1-septate, smooth, hyaline to pale brown, guttulate, not constricted at the median septum, 9.5–13.5 × 2–3 μm ($\bar{x}$ = 11.3 × 2.4 μm), lower cell and upper cell in ± equal length; apical cell cylindrical for 1–2.5 μm (($\bar{x}$ = 1.7 μm) long, then divide into 2-4 divergent branches; apical appendages unbranched, attenuated, 11.5–17 μm ($\bar{x}$ = 14.3 μm) long; basal appendages absent or not.

Culture characteristics: Colonies on PDA reaching 7.5–8 cm diam after 7 d at room temperature (25°C), under light 12 hr/dark. The hyphae change from white to light pink, colonies circular, slightly undulated at the edge, whitish, with black fruiting bodies clustered, have filiform and fluffy margins, light pink from above, and light yellow from the reverse.

Materials examined: China, Guizhou Province, Guiyang City, from the leaves of *Nicotiana tabacum* L., 14 November 2019, Y. Wang, HGUP 178, living culture GUCC 2178.5 and GUCC 2178.11.



Figs 4 – *Robillarda africana* (GUCC 2178.5). a, b Culture on PDA (a-above, b-reverse). c, d Colony sporulating on PDA. e, f Conidiogenous cells give rise to conidia. g, j Conidia. Scale bars: c = 500 μm， d = 1000 μm, g-j = 20 μm.

Discussion

In this study, *Hymenopleella rhododendronis* (GUCC 2137.7) and *Robillarda africana* (GUCC 2178.5 and 2178.11) belonging to *Sporocadaceae* were collected from Guizhou Province in China. For strains GUCC 2178.5 and GUCC 2178.11, the molecular phylogenetic data (FIG. 4) showed that they were very close to *R. africana* and *R. australiana*. The *tub2* sequences of strain GUCC 2178.5 and *R. africana* (CBS 122.75) are almost identical but different from *R. australiana* (CBS 143882).

The morphological comparison shows that the conidia of our material (GUCC 2178.5) consistent with the description of *R. africana*; although they are a little shorter (*R. africana*: 9.5–13.5 × 2–3 μm) (Crous et al. 2015). According to previous reports, tobacco leaves were able to accommodate a large fungal community reaching to1,528,500 colony-forming units (CFUs) per gram of tobacco (Welty 1972, El-Ansary et al. 2013). A new disease gray spots surrounded by a yellowish ring was first discovered in Guizhou Province, which was caused by *Nigrospora aurantiaca* (Huang et al. 2021). Gang et al. (2024) also reported *Pseudopithomyces palmicola* causing a new leaf spot disease on tobacco in Guizhou, China. However, this is the first report about *Robillarda* species isolated from tobacco leaves. As a kind of worldwide saprobic fungi, we believe in the future more members of this genus can be discovered in the different tissues of this important economic crop.

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