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Pseudoplectania globospora (*Sarcosomataceae*, *Pezizales*), a new species from Yunnan, China

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Abstract

Pseudoplectania comprises 13 species and is characterized by dark apothecia with external hairs, hymenial hairs, spherical ascospores and the presence or absence of yellow crystals. This study introduces a new species, *P. globospora*, into this genus based on combined morphology and phylogeny. This species features dark and discoid apothecia with two types of external hairs, yellowish to brownish hymenial hairs, straight or bifurcate paraphyses, and spherical ascospores. The combined ITS and LSU analysis separated the newly-established species that was sister to *P. affinis* and differed from all other *Pseudoplectania* species.

Keywords – 1 new species – cup-fungi – morphology – phylogeny

Introduction

Pseudoplectania Fuckel is a genus of cup-fungi placed in the family *Sarcosomataceae* Kobayasi, which is typified by *Pseudoplectania nigrella* (Pers.) Fuckel (1870). Carbone et al. (2014) delimited the species concept within *Pseudoplectania* based on morphology and combined ITS and LSU phylogenetic analysis, with nine species recognized and *Pseudoplectania kumaonensis* Sanwal in doubtful status due to the absence of a herbarium specimen. Subsequently, three additional species were introduced and assigned to *Pseudoplectania* based on morphology and phylogeny (Glejdura et al. 2015, Zhang & Zhang 2020, Sochorová et al. 2022). This genus contains 13 species with nine having molecular data (Carbone et al. 2014, Zhang & Zhang 2020, Sochorová et al. 2022). The genus features discoid to cupulate, dark-coloured apothecia with or without a stalk, typically covered by brown to black hairs on the receptacle surface, *textura globulosa-angularis* cells in the ectal excipulum, hymenial hairs present in the hymenium, inamyloid asci comprise an operculate apex, typically arising from simple septa, and usually spherical and hyaline ascospores (Carbone et al. 2014, Sochorová et al. 2022).

In this study, we collected two cup fungi from Yunnan Province, China. The morphological examination showed these two samples have similar features to *Pseudoplectania* species and are distinct from other genera of Sarcosomataceae. The phylogenetic analysis based on ITS and LSU loci shows these two samples forming an independent clade within *Pseudoplectania* and distinct

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from other species. Thus, we introduce a new *Pseudoplectania* species here.

Materials & methods

Sample collection, morphological studies and deposition

Two fresh samples were collected from Yunnan Province, China, and brought to the laboratory in plastic bags containing silica gel for moisture absorption. Morphology was examined using a Nikon C-PSN stereoscope and photographed with a Nikon Eclipse Ni compound microscope equipped with a Nikon DS-Ri2 camera. Dried samples were rehydrated in distilled water and stained with Melzer's reagent and cotton blue solutions. The Tarosoft® Image Frame Work program v.0.9.7 was used for measurements. The meaning of the measured values follows Zeng et al. (2023). Samples are deposited at the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS). Facesoffungi and MycoBank numbers were obtained as in Jayasiri et al. (2015) and Robert et al. (2013).

DNA extraction, PCR amplification and sequencing

DNA was extracted from the fruiting body tissue using a Trelief^{TM} Plant Genomic DNA Extraction Kit following the manufacturer's specifications. The internal transcribed spacer (ITS) and the large subunit rRNA (LSU) were amplified by polymerase chain reaction (PCR) using the primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR5, respectively (Vilgalys & Hester 1990). PCR was performed in a 25 μL reaction mixture containing 9.5 μL sterile deionized water, 12.5 μL of 2×Power Taq PCR MasterMix, 1 μL of each primer (10 μM stock) and 1 μL DNA template. The cycling conditions of PCR amplification included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at 56 °C for 50 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The obtained PCR products were purified and sequenced by Tsingke Company, Beijing, P.R. China.

Phylogenetic analysis

Raw sequences were assembled using DNASTAR Lasergene SeqMan Pro v.7.1.0 (44.1). Relevant sequences of *Pseudoplectania* were downloaded from GenBank and listed in Table 1. Two datasets, one for each genetic marker, were built and separately aligned using MAFFT v.7.110 online (Katoh & Standley 2013). Both datasets were trimmed with TrimAl v.1.3 using the user−defined option (ITS: 0.5 value for gap threshold) and gappyout option (LSU) (Capella-Gutiérrez et al. 2009). Both datasets were assembled into a matrix using SequenceMatrix v.1.8 (Vaidya et al. 2011). AliView v.1.19−betalk was used to convert file format (Larsson 2014).

Maximum likelihood (ML) analysis was performed using the GTR+GAMMA substitution model with 1,000 rapid bootstrap replicates in RAxMLGUI v.1.3 (Silvestro & Michalak 2011). Bayesian inference (BI) analysis was carried out using Mrbayes v.3.2.6 (Ronquist et al. 2012). The model of evolution was estimated using MrModeltest v.2.3 (Nylander et al. 2004) based on the Akaike information criterion (Posada & Buckley 2004). Markov Chain Monte Carlo Sampling (MCMC) was used to calculate posterior probabilities (PP) (Rannala & Yang 1996, Huelsenbeck & Ronquist 2001). Two independent runs comprising six simultaneous Markov Chains each were run for 75,000 generations and trees were sampled every 100 generations (Cai et al. 2005). 25% of the trees were discarded as burn-in and analysis was stopped when the standard deviation of split frequencies reached 0.01. Phylogenetic trees were viewed in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and beautified using Adobe Illustrator CS5 (Adobe Systems, USA).

Results

Phylogenetic analysis

Phylogenetic trees were inferred using 23 *Pseudoplectania* taxa as ingroup taxa and two

Sarcosoma taxa as outgroup taxa based on combined ITS and LSU sequence data (Fig. 1). The alignment comprised 1,597 total characters (ITS: 1−575 bp; LSU: 576−1,597 bp). The best sorting RaxML tree had a final likelihood value of − 6888.456207. In addition to the two unidentified *Pseudoplectania* taxa, the rest of the taxa were separated into nine monophyletic clades, each of which represents a species, with moderate or high supports. Our new species is represented by two taxa, which group together with maximum support (100BS/1.00PP) and are further sister to *Pseudoplectania affinis* also with maximum support (100BS/1.00PP).

Taxonomy

Pseudoplectania globospora M. Zeng & Q. Zhao, sp. nov. Fig. 2

Diagnosis: This species is characterized by black apothecia covering two types of brown to dark brown hairs, straight, curved, or branched paraphyses with or without notches close to the apex, spherical and smooth ascospores with one to multiple guttules, and additional granular contents.

MycoBank number: MB852406; Facesoffungi number: FoF15216

Etymology – The epithet refers to the globose ascospores.

Holotype – HKAS 127988

Saprobic on soil associated with moss. Teleomorph: *Apothecia* up to 1.5 cm broad, up to 1 cm high, scattered to gregarious, discoid to shallowly cupulate, sessile, with basal tomentum. *Receptacle* shallow cupulate, receptacle surface black, pubescent, margin conspicuous, entire, slightly involute. *Disc* discoid to shallowly cupulate, concolorous with the receptacle surface. *Ectal excipulum* 90−340 µm thick, composed of sub-hyaline to brown or dark brown *textura globulosa-angularis*, cells 17−22 × 14−17 µm, with abundant hairs. *Hairs* of two types: 1) Hyphoid hairs: 5−9 µm broad, long, brown to dark brown, friable, septate, straight to slightly wavy, smooth, tapering towards the tip with a rounded end, abundant close to the base; 2) Clavate hairs: 6.5−11 µm broad, short, brown to dark brown, tight, septate, smooth or faintly rough, with a rounded end, sometimes with an enlarged cell at the end. *Medullary excipulum* 90−200 µm thick, composed of sub-hyaline to brownish or brown *textura intricata*, hyphae 3−5.5 µm wide. *Hymenium* ca. 260 µm thick, brown to dark brown, existing hymenial hairs. *Hymenial hairs* 3−4 µm broad, yellowish to brownish, filiform, straight, smooth, non-septate, tapering towards the tip. *Paraphyses* 1.5−2.5 µm wide in the middle part, hyaline or sub-hyaline, filiform, septate, straight or normally with bifurcate, sometimes curved or with notches close to the apex. *Asci* 230−264 × 11−14 µm, 8-spored, terminal operculate, apices obtuse, cylindrical, becoming narrow towards the base, J-. *Ascospores* [20/1/1, in H_2O] (10–)10.5–12.5(–13.0) × (11.5–)9.8–13.5(–14) µm (Q = 0.93–1.09, **Q** = 0.99 ± 0.05), spherical, uniseriate, hyaline, with one to multiple big guttules, and additional granular contents, smooth-walled. Anamorph: not seen.

Material examined – China, Yunnan, Kunming, on soil associated with moss, 23 August 2021, Hongli Su, ZM381 (HKAS 127988, holotype); *ibid.*, 17 September 2021, Ming Zeng, ZM391 (HKAS 127989, paratype).

Notes – This species is similar to other *Pseudoplectania* species in that it has black apothecia with abundant external hairs, existing hymenial hairs, and spherical ascospores (Carbone et al. 2014, Sochorová et al. 2022). The most distinguishing taxonomic feature is that this new species contains two types of external hairs; hyphoid and clavate hairs. In the phylogenetic analysis, the new species has a distinct placement as sister to *Pseudoplectania affinis* with maximum support (100BS/1.00PP) (Fig. 1). However, *Pseudoplectania affinis* differs from the newly established species in that it has one type of external hairs, and slightly narrower hymenial hairs (2.5–3 µm) (Carbone et al. 2014). The ITS region of *Pseudoplectania globospora* (HKAS 127988) differed from that of *Pseudoplectania affinis* (PDD 81842) by 6.5% over a 464 bp fragment (including four gaps), which further supports the establishment of our new species.

Table 1 Sequences used to infer phylogeny in this study.

Species name	Location	Voucher/ Strain number	GenBank number		Sources
			ITS	LSU	
Pseudoplectania affinis	**New Zealand, Auckland**	**PDD 81842 (Holotype)**	**JX669826**	**JX669865**	**Carbone et al. (2014)**
Pseudoplectania africana	**South Africa**	**PRM 954013 (Holotype)**	**MT496892**	**MT496884**	Sochorová et al. (2022)
Pseudoplectania episphagnum	Finland, Varsinais-Suomi	TUR 064171	KF305712	–	Carbone et al. (2014)
Pseudoplectania episphagnum	Finland, Perä-Pohjanmaa	TUR 064173	KF305711	KF305724	Carbone et al. (2014)
Pseudoplectania ericae	Italy, Liguria	MCVE 27581	KF305721	KF305731	Carbone et al. (2014)
Pseudoplectania ericae	Spain, Girona	TUR-A 195789	JX669822	JX669862	Carbone et al. (2013)
Pseudoplectania ericae	Spain	TUR-A 195790	JX669823	JX669863	Carbone et al. (2013)
Pseudoplectania lignicola	**Czech Republic**	**HR 89756 (Paratype)**	**MT496886**	**MT496882**	Sochorová et al. (2022)
Pseudoplectania lignicola	Slovaki	SAV 105/17	MT496881	MT496883	Sochorová et al. (2022)
Pseudoplectania melaena	Italy, Veneto	MCVE 27433	JX669806	JX669842	Carbone et al. (2013)
Pseudoplectania melaena	France, Doubs	MCVE 27579	KF305717	KF305728	Carbone et al. (2014)
Pseudoplectania melaena	USA, Washington	TUR-A 198588	KF305719	KF305729	Carbone et al. (2014)
Pseudoplectania nigrella	**Austria, Carinzia**	**KL BK-4914 (Neotype)**	**JX669807**	**JX669843**	**Carbone et al. (2013)**
Pseudoplectania nigrella	Italy, Lombardia	MCVE 27396	KF305715	KF305725	Carbone et al. (2014)
Pseudoplectania nigrella	Canada, British Columbia	MCVE 27580	KF305713	KF305726	Carbone et al. (2014)
Pseudoplectania nigrella	Finland, Koski	TUR 169888	JX669821	JX669859	Carbone et al. (2013)
Pseudoplectania sp.	China, Guangxi	3-1-7-2-4-1	KX065280	–	Li et al. (2016)
Pseudoplectania sinica	**China**	**CGMCC3.19892 (Holotype)**	**MN831477**	**MN396768**	Zhang & Zhang (2020)
Pseudoplectania sp.	China, Zhejiang	DO87	KP050642	–	https://www.ncbi.nlm.nih.gov/nuccore/KP050642
Pseudoplectania tasmanica	Australia, Tasmania	MCVE 27584	KF305723	KF305733	Carbone et al. (2014)
Pseudoplectania tasmanica	**Australia, Tasmania**	**MCVE 27583 (Holotype)**	**KF305722**	**KF305732**	**Carbone et al. (2014)**
Pseudoplectania globospora	**China, Yunnan**	**HKAS 127988 (Holotype)**	**PP357153**	**OR879981**	**This study**
Pseudoplectania globospora	China, Yunnan	HKAS 127989	PP357154	OR879982	This study
Sarcosoma globosum	Sweden	KH.07.04 (S)	FJ499393	–	Hansen et al. (2008)
Sarcosoma globosum	Russia, Leningrad	LE-BIN 3794	KY344789	–	https://www.ncbi.nlm.nih.gov/nuccore/KY344789

Note: Names in red indicate new species in this study. Names in bold indicate type species.
Abbreviations: CGMCC: China General Microbiological Culture Collection Center, Beijing, China; HKAS: Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences, China; HR: Muzeum

Východních Cech, Austria; KH: K. Hirayama; KL: Herbarium of the Landesmuseum Kärnten, Austria; MCVE: Herbarium del Museo Civico di Storia Naturale, Venezia, Italy; PDD: New Zealand Fungarium, Auckland, New Zealand; PRM: Herbarium of the Prague National. Museum, Czech Republic; TUR: Herbarium, Centre for Biodiversity, University of Turku, Finland.

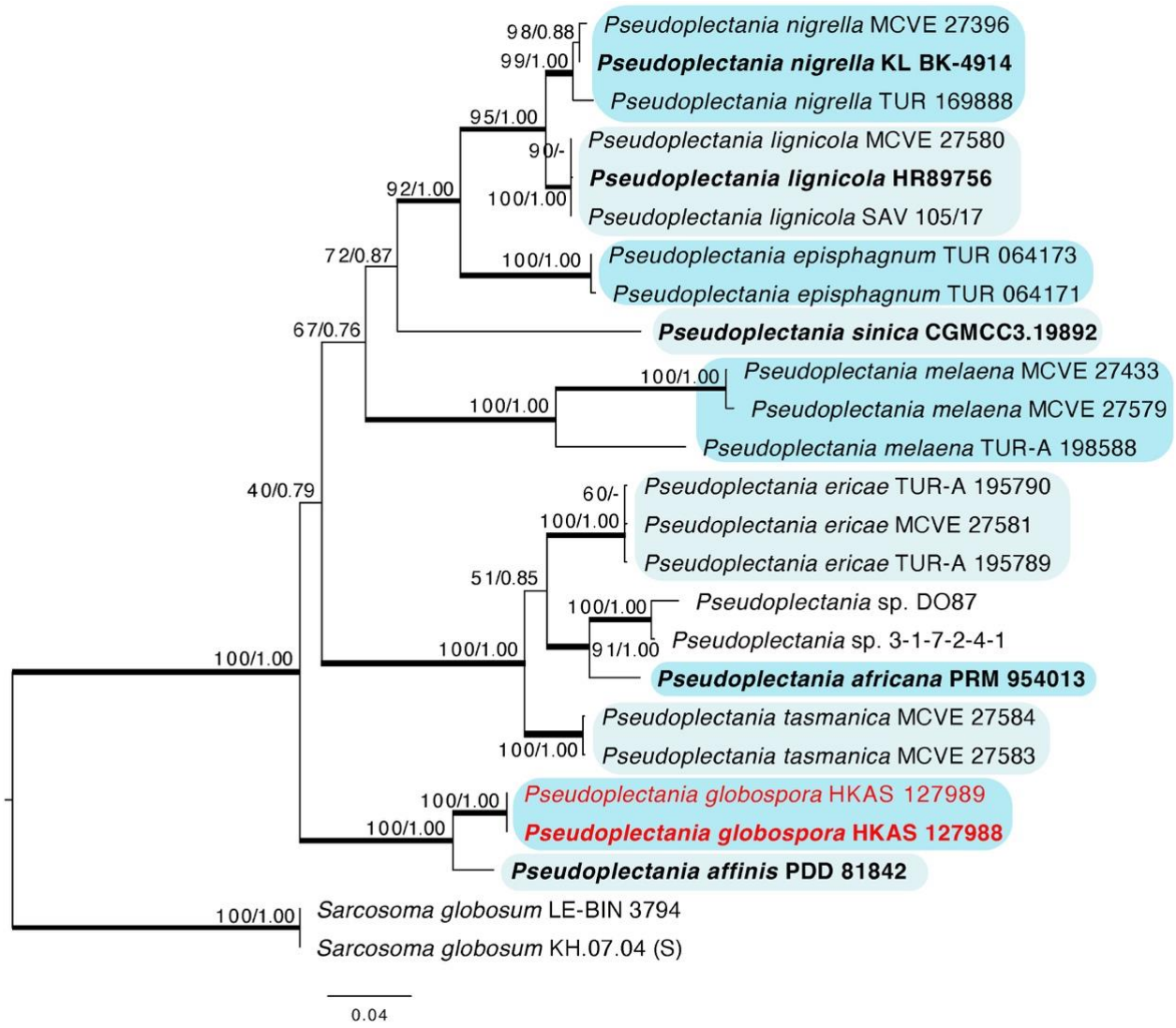

Fig. 1 – Phylogenetic tree of combined ITS and LSU sequence data inferred from 23 *Pseudoplectania* taxa and two *Sarcosoma* taxa. Values at the nodes denote maximum likelihood bootstrap support and posterior probabilities in this order. Values greater than 75% (maximum likelihood) and 0.90 (Bayesian inference) are indicated with thick branches. Names in red indicate new species and names in bold indicate type species.

Discussion

Pseudoplectania species are normally found in dead wood, soil, or *Sphagnum* (Sochorová et al. 2022). Aside from the one doubtful species *P. kumaonensis*, the rest of *Pseudoplectania* species have been characterized by combined ecological, morphological, and phylogenetic information (Carbone et al. 2014, Sochorová et al. 2022). The presence of yellow crystals mainly in the hymenium and ectal excipulum is an important feature in species determination and is found in *P. africana*, *P. ericae*, and *P. tasmanica* (Carbone et al. 2014, Sochorová et al. 2022). These three species share the trait of having sheath-surrounded ascospores with *P. nigrella*, *P. melaena*, *P. lignicola*, and *P. stygia* (Carbone & Agnello 2012, Carbone 2013, Van Vooren et al. 2013, Carbone et al. 2014, Glejdura et al. 2015, Sochorová et al. 2022).

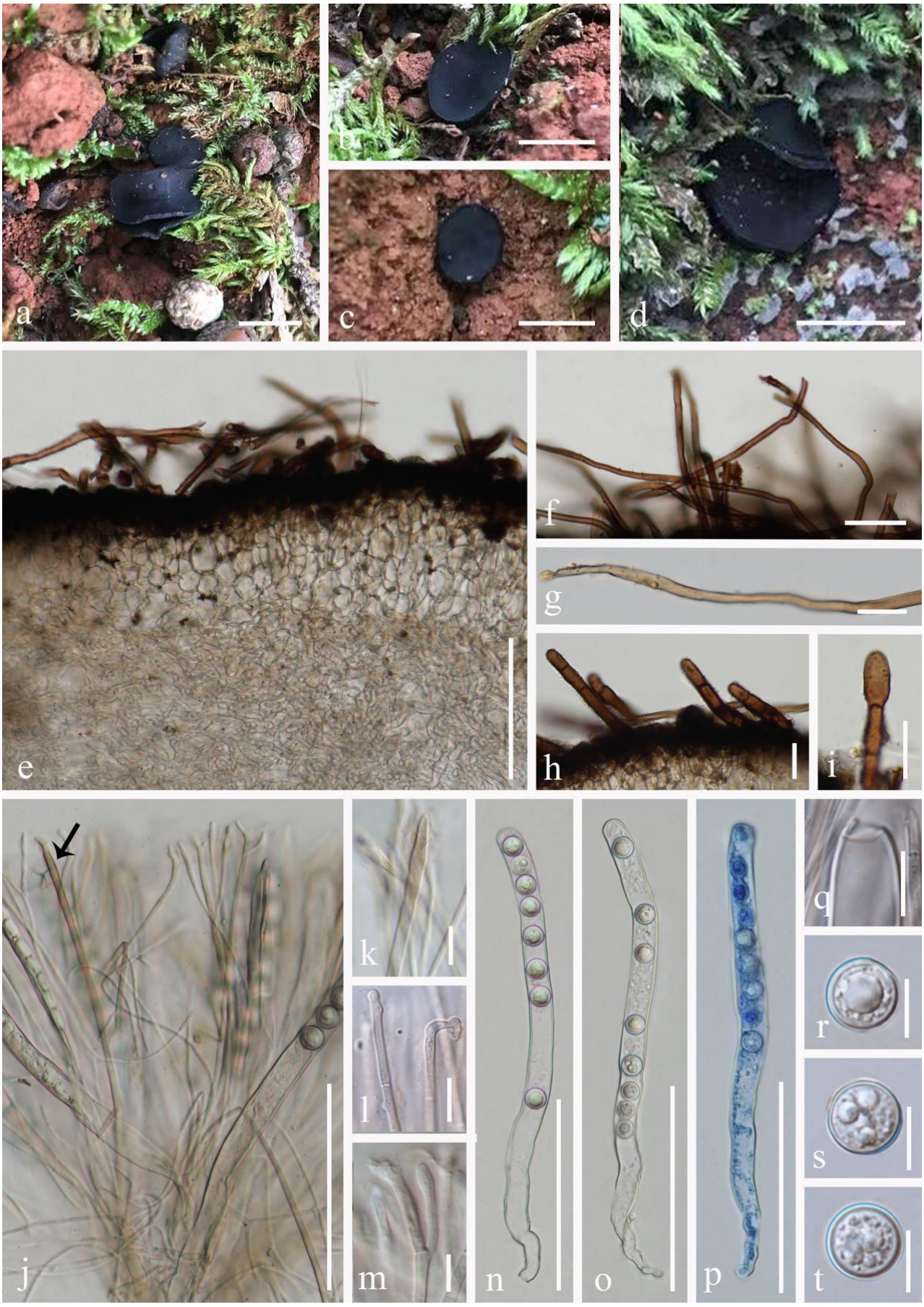

Fig. 2 – *Pseudoplectania globospora* (HKAS 127988, holotype). a–d Fresh apothecia. e Vertical section of receptacle ectal excipulum and part of medullary excipulum. f, g Hyphoid hairs.

h, i Clavate hairs. j Paraphyses and hymenial hairs (arrow). k Apex of hymenial hair. l, m Apexes of paraphyses. n–p Asci (p Ascus in Cotton blue). q Ascus apex. r–t Ascospores. Scale bars: a–d = 1 cm, e, j, n–p = 100 μm, f = 50 μm, g–i = 20 μm, k–m, q–t = 10 μm.

In contrast, we did not observe the yellow crystals and sheath-surrounded ascospores in our new species or the sister species, *P. affinis* (Carbone et al. 2014, Sochorová et al. 2022). *Pseudoplectania episphagnum* is phylogenetically distant from our new species (Fig. 1), and there are also significant differences in morphology. It has coiled external hairs and an apothecium that is usually smaller than 1 cm. Meanwhile, it is often found on *Sphagnum* in bogs (Sochorová et al. 2022). With regard to the new species comparison to those lacking molecular data, *P. ryvardenii* differs in its ornamented ascospores and missing hymenial hairs (Iturriaga et al. 2012), while *P. carranzae* has shorter asci (170–200 × 10–14 μm) (Calonge & Mata 2002). Aside from our new species, there are two additional species reported from China, *P. nigrella* (Zhuang 2004) and *P. sinica* (Zhang & Zhang 2020). This study adds to the diversity of *Pseudoplectania*, hence enriching our knowledge on this genus specifically and *Sarcosomataceae* more broadly.

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