Lambertella (Rutstroemiaceae, Helotiales) from Northern Thailand

Anis Sri Lestari (anislestari1@gmail.com)
Mae Fah Luang University School of Science https://orcid.org/0000-0002-1606-9884
Thilini Chethana Kandawatte Wedaralalage
Mae Fah Luang University School of Science https://orcid.org/0000-0002-5816-9269

Research Article

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Abstract

During our excursions for discomycetes from 2019–2021 in forests and plantations in northern Thailand, several Lambertella-like specimens were found. Morphological observation and BLAST sequence data search confirmed that six newly collected specimens belong to *Lambertella*. Further phylogenetic analysis using maximum likelihood and Bayesian inference analyses based on combined ITS and LSU sequence data and morphological examination coupled with chemical reactions, confirmed six *Lambertell*a species. *Lambertella aurantiaca* was established as a new geographical record, and the other five specimens, *L. fusoidea, L. phanensis, L. sessilis, L. takensis*, and *L. tectonae* were introduced as novel species. The highlight of the current study is to contribute a complete morphological description of *Lambertella aurantiaca* since its introduction in 1964, emend the morphological criteria for *Lambertella sensu stricto*, and provide sequence data for all the *Lambertella* species described in the current study, including the extant species, *L. aurantiaca* for the first time, which are essential for future studies.

Introduction

Rutstromiaceae was established based on nuclear rDNA phylogeny comprising *Lambertella, Lanzia, Poculum*, and *Verpatinia*, and typified with *Rutstroemia* (Kohn & Grenville, 1989; Holst-Jensen et al., 1997). The family was characterized by brownish and greenish, stipitate to substipitate apothecia or sometimes cleistothecia with inoperculate, cylindric-clavate asci, and hyaline to brown, fusiform to ellipsoid ascospores. It mainly includes saprobes and a few parasites (Holst-Jensen et al., 1997; Galán et al., 2015; Wiseman et al., 2015; Zhao et al., 2016; Baral, 2017; Johnston et al., 2019). With several taxonomic revisions based on morpho-phylogeny and novel species discoveries, *Rutstroemiaceae* has been expanded from its initial members to include six genera, *Bicornispora*, *Bryorutstroemia, Dencoeliopsis, Lambertella, Lanzia, Pseudolanzia, Rutstroemia* and *Torrendiella* (Johnston & Park 2013; Johnston et al., 2014; Galán et al., 2015; Pärtel et al., 2017; Baral, 2017, Wijayawardene et al., 2022; Baral et al., 2023).

Lambertella was established by Höhnel (1918), with *Lambertella corni-maris* as the type species in *Sclerotiniaceae* (Seaver, 1951). Whetzel (1943) published the first monograph for *Lambertella*, contributing eight novel species, *Lambertella cephalanthi, L. colombiana, L. hicoriae, L. jasmine, L. pruni, L. tropicalis, L. viburni*, and *L. corni-maris.* Subsequently, the genus was re-evaluated and placed in *Rutstroemiaceae* with *Lanzia, Poculum, Rutstroemia* and *Verpatinia* based on rDNA phylogeny and substratal stroma (Kohn & Grenville, 1989; Holst-Jensen et al., 1997). Several *Lambertella* species were reported from China (Korf & Zhuang, 1985), Europe (Dumont, 1971; Schumacher & Holøs, 1989), India (Tewari & Pant, 1967; Elliot & Sharma, 1976; Gautam et al., 1982; Sharma, 1985), Japan (Korf &Zhuang, 1985; Hosoya & Otani, 1997), Malaysia (Dumont, 1974), North America (Cash, 1958; Tewari, 1963), South America (Dumont, 1974), the Philippines (Dumont, 1971) and UK (Abdullah & Webster, 1981). Additionally, some species in the genera *Helotium* (Dumont, 1974), *Humaria* (Dumont, 1971), *Moellerodiscus* (Hosoya & Otani, 1997), *Mollisia* (Dumont, 1971), *Phaeociboria* (Dumont, 1971), *Rutstroemia* (Dumont, 1974), *Phialea* (Korf, 1982) and *Velutaria* (Dumont, 1971) were synonymized under *Lambertella*. Currently, six species of *Lambertella* are accepted in Wijayawardene et al. (2022), while 69 *Lambertella* species are listed in the Index Fungorum (2023) and Species Fungorum (2023).

The majority of *Lambertella* species were found as saprobes on lignocellulose substrates, such as petioles, twigs, and fallen fruits in freshwater or terrestrial habitats (Dumont, 1971; Abdullah & Webster, 1981; Zhao et al., 2013), while some were found on dung (Dumont, 1976) or associated with other fungi (Dumont, 1971; Korf & Zhuang, 1985). Following its introduction, the vital chemical properties of *Lambertella*, which may potentially suppress other fungal diseases, have also been studied. For example, a recent study by Vasić et al. (2022) documented the antagonistic properties of the apple post-harvest pathogen, *Lambertella corni-marris*, against the brown fruit rot pathogen, *Monilia polystroma*. Further studies extracted the metabolites with antagonistic properties against bacteria and fungi from *Lambertella hicoriae* and *L. corni-maris* (Sproston, 1963; Murakami et al., 2008) and patented later as bactericides and fungicides (Dumont, 1971). *Lambertella* species also exhibited mycoremediation properties that reduce toxicity on intermediate and old landfill leachates (Siracusa et al., 2020).

Lambertella is characterized by sub- to stipitate apothecia, *textura prismatica* ectal excipulum, substratal stroma formation from vegetative hyphae, and brown-pigmented ascospores (Dumont, 1971; Korf & Zhuang, 1985; Holst-Jensen et al., 1997). According to Baral (1992), *Lambertella*'s brown ascospores can be observed in mature apothecia and are often overlooked in immature herbarium specimens. However, brown pigmentation in the ascospores of helotian discomycetes is also observed in *Hymenoscyphus, Lanzia, Pseudolanzia*, and *Rutstroemia* (Zhao & Hosoya, 2015; Zhao et al., 2016; Baral, 2017) and also supported by LSU and *RPB2* phylogeny. Zhao et al. (2016) defined *Lambertella sensu stricto* clade to accommodate those *Lambertella*-like specimens characterized by brown ascospores in the asci. Holst-Jensen et al. (1997) demonstrated polyphyletic relationships of the *Lambertella* clade, which was further confirmed by Zhao et al. (2016) with the arrangement of *Lambertella sensu stricto* and suggested that species reevaluation in *Lambertella sensu lato* requires careful observation and may be assigned to new genera in the future.

In Thailand, helotian discomycetes were recorded (Phanichapol, 1986; Ekanayaka et al., 2019; Phutthacharoen et al., 2022), however, there are no records of *Rutstroemiaceae* members, especially *Lambertella*. Following the suggestions of Chethana et al. (2021), the current paper employed polyphyletic approaches for identifying *Lambertella* species, which resulted in five new species and a geographical record from Thailand.

Materials and Methods

Sample collection and morphological studies

Lambertella-like fungi were collected in some forests and plantations in Chiang Mai and Chiang Rai Provinces, Thailand, during 2019–2021. Fungal specimens enclosed in paper boxes were brought to the laboratory. Macromorphology of apothecia was observed, dissected on a Leica EZ4 stereomicroscope (Leica Microsystems Company, Germany), and photographed by Olympus SC 180 digital camera connected to the microscope. Apothecial micromorphology consists of examining excipular cells, paraphyses, asci, and ascospores on a compound microscope Nikon Eclipse N*i*-U connected to the Nikon DS-R*i*2 digital

camera for photographs while Tarosoft (R) Image Frame Work software was used for the measurement (Senanayake et al. 2020). Chemical reactions during micromorphology observation were observed by mounting the apothecial section in distilled water or 2–5% KOH, stained in Congo red and Melzer reagent (MLZ) for the amyloid test. The photo plates were prepared with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA). The dried herbarium specimens were deposited at the Mae Fah Luang University Herbarium (MFLU), Thailand. Faces of fungi and Index Fungorum numbers were obtained as in Jayasiri et al. (2015) and Index Fungorum (2023), respectively. Ascospore germination was observed by mounting the macerated apothecial section on water agar and incubating for 24 to 48 hours at 23–25 ^{o}C.

DNA extraction, amplification, and sequencing

DNA extraction using the E.Z.N.A Fungal DNA Mini Kit D3390-02 (Omega Bio-Tek, USA) was conducted from the mycelia and/or apothecia (Dissanayake et al., 2020). Apothecia were collected into 1.5 ml microtubes and ground using pestles with liquid nitrogen. DNA extractions followed the manufacturer's protocol with slight modifications on the additional incubation and reduced elution volume of the extracted DNA. Extracted DNA was then stored at -20°C until further processing. The internal transcribed spacer (ITS) and large sub-unit of 28S rRNA (LSU) gene regions were amplified using primers ITS5/ITS4 (White et al., 1990) and LROR/LR5 (Vilgalys & Hester, 1990), respectively. The PCR reactions were carried out in 50 µl volumes, containing 12.5 µl of 2 × Go Taq@ Green Master Mix (Promega. com, USA), one µl of each primer (20 µM), two µl genomic DNA and 8.5 µl of de-ionized water. PCR amplification conditions for ITS and LSU include initial denaturation at 94 ^{o}C for 3 minutes, followed by 35 cycles of denaturation at 94 ^{o}C for 40 seconds, annealing at 55 ^{o}C for 50 seconds, extension at 72 ^{o}C for 90 seconds, and a final extension at 72 ^{o}C for 10 minutes. The PCR products were stained with DL5000-DNA Fluorescent loading dye (Smobio Technology Inc, Taiwan), visualized on 1.5% agarose gels, and sequenced at Solgent Co. Ltd., Korea. The newly produced sequences were deposited in the GenBank (Table 1).

Table 1
Accession numbers and origin of fungal taxa used in the phylogenetic analysis.

Taxon name	Strain codes[a]	GenBank accession no		Origin	References
		ITS	LSU		
Bicornispora seditiosa (T)	CBS 135998	NR170723	-	Spain	Galán et al. (2015)
Bicornispora seditiosa	AH 44702	KF499362	-	Spain	Galán et al. (2015)
Bicornispora seditiosa	WU 32446	KF499360	-	Spain	Galán et al. (2015)
Bicornispora seditiosa	AH 44701	KF499361	-	Spain	Galán et al. (2015)
Bicornispora seditiosa	WU 32445	KF499359	-	Spain	Galán et al. (2015)
Bicornispora exophiala	AH 15779	KF499363	-	Spain	Galán et al. (2015)
Bryorutstroemia fulva	Z.S.7	OP035830	OP035830	Czech Republic	Baral et al. (2023)
Bryorutstroemia fulva	Z.S.9	OP035829	OP035829	Czech Republic	Baral et al. (2023)
Bryorutstroemia fulva	Z.S.19	OP035828	OP035828	Czech Republic	Baral et al. (2023)
Dencoeliopsis johnstonii	C-F32113	LT158456	-	Denmark	Pärtel et al. (2017)
Dencoeliopsis johnstonii	C-F90563	LT158455	-	Denmark	Pärtel et al. (2017)
Dencoeliopsis johnstonii	C-F28009	LT158454	-	Denmark	Pärtel et al. (2017)
Erioscyphella abnormis	MFLU 18-1826	MK584950	MK591977	China	Ekanayaka et al. (2019)
Erioscyphella aseptata	MFLU 16–0590	NR163780	NG066456	Thailand	Ekanayaka et al. (2019)
Lachnum virgineum	OSC 100002	DQ491485	AY544646	N/A	Spatafora et al. (2006)
Lambertella aurantiaca	**MFLU 23–0090**	**OP967479**	**OP965344**	**This study**	**This study**
Lambertella corni-maris	CBS 197.47	MH856215	MH867745	Switzerland	Vu et al. (2019)
Lambertella corni-maris	TNSF 40083	AB926069	AB926139	Japan	Zhao et al. (2016)
Lambertella fusoidea	**MFLU 23–0086**	**OP967481**	**OP965340**	**This study**	**This study**
Lambertella hicoriae	CBS 294.54	MH856216	MH867746	USA	Vu et al. (2019)
Lambertella himalayensis	CBS 230.77	MH861053	MH872822	Myanmar	Vu et al. (2019)
Lambertella langei	2852	Z81435	Z814ll	Norway	Holst-Jensen et al. (1997)
Lambertella palmeri	AHsn	KF499365	-	Spain	Galán et al. (2015)
Lambertella palmeri	AH 7576	KF499364	-	Spain	Galán et al. (2015)
Lambertella phanensis	**MFLU 23–0091**	**OP967478**	**OP965343**	**This study**	**This study**
Lambertella pruni	CBS 199.47	MH856217	MH867747	USA	Vu et al. (2019)
Lambertella pruni	WMA1-4	DQ335471	-	USA	Marek et al. (2008)
Lambertella pyrolae (T)	TNSF 40132	AB926081	AB926164	Japan	Zhao et al. 2016
Lambertella sessilis	**MFLU 23–0092**	**OQ650294**	**OQ650293**	**This study**	**This study**
Lambertella subrenispora (T)	CBS 811.85	MH861915	MH873604	Japan	Vu et al. (2019)
Lambertella subrenispora	1879	KC533549	-	Japan	Aynardi et al. (2016)
Lambertella takensis	**MFLU 23–0089**	**OP967480**	**OP965339**	**This study**	**This study**
Lambertella tectonae	**MFLU 23–0087**	**OP967476**	**OP965342**	**This study**	**This study**
Lambertella tectonae	**MFLU 23–0088**	**OP967477**	**OP965341**	**This study**	**This study**
Lambertella tetrica	F142281	KJ941068	-	Spain	Galán et al. (2015)
Lambertella tubulosa	CBS 202.79	MH861201	MH872970	Netherlands	Vu et al. (2019)
Lambertella tubulosa	CBS 281.51	MH856859	MH868377	Sweden	Vu et al. (2019)
Lambertella tubulosa	CBS 125202	MH863518	MH875004	Austria	Vu et al. (2019)
Lambertella viburni	CBS 200.47	AB926098	AB926153	Japan	Zhao et al. (2016)
Lanzia allantospora	PDD 60137	AY755334	-	New Zealand	Johnston and Park (2005)

Taxon name	Strain codes[a]	GenBank accession no		Origin	References
		ITS	LSU		
Lanzia allantospora	CBS 1243.34	AB926099	-	Japan	Zhao et al. (2016)
Lanzia berggrenii	ICMP 19614	KC164645	KC164640	Australia	Johnston and Park (2013)
Lanzia berggrenii	ICMP 19615	KC164647	-	Australia	Johnston and Park (2013)
Lanzia echinophila	F132998	KF588371	KJ941053	Spain	Perić & Baral (2017)
Lanzia echinophila	CBS 111547	KF545332	-	Netherlands	Perić & Baral (2017)
Lanzia griseliniae	PDD 64240	AY755333	-	New Zealand	Johnston and Park (2005)
Lanzia ovispora	PDD 70881	MH578500	MH587172	New Zealand	Johnston and Park (2013)
Lanzia ovispora	PDD 104600	MH578498	-	New Zealand	Johnston and Park (2013)
Lanzia ovispora	PDD 103388	MH578497	-	New Zealand	Johnston and Park (2013)
Pseudolanzia piceetorum (T)	HOB 1013	MK679683	-	Germany	Baral (2017)
Rutstroemia alnobetulae	G00273761	MW677580	-	Switzerland	Senn-Irlett et al. (2021)
Rutstroemia bolaris	TU 104236	LT158432	-	Estonia	Pärtel et al. (2017)
Rutstroemia bolaris	1825	KC533546	-	Norway	Aynardi et al. (2016)
Rutstroemia bolaris	1525	Z80894	-	Norway	Holst-Jensen et al. (1997)
Rutstroemia bulgarioides	TNSF 40005	AB926053	AB926122	Japan	Zhao et al. (2016)
Rutstroemia bulgarioides	TAAM 165289	LT158483	KX090797	Estonia	Pärtel et al. (2017)
Rutstroemia bulgarioides	TAAM 198322	LT158469	KX090836	Estonia	Pärtel et al. (2017)
Rutstroemia bulgarioides	HB 6899	KJ941086	KJ941062	Switzerland	Johnston et al.(2014)
Rutstroemia conformata	F145906	KJ941075	KJ941057	Spain	Johnston et al.(2014)
Rutstroemia conformata	CBS518.75	AB926156	AB926156	Japan	Zhao et al. (2016)
Rutstroemia conformata	C-F26964	LT158453	-	Denmark	Pärtel et al. (2017)
Rutstroemia calopus	F148155	KF588373	-	Spain	Johnston et al. (2014)
Rutstroemia elatina	15858	JF908711	-	Italy	Osmundson et al. (2013
Rutstroemia elatina	ANK Akata 7020	MN263048	-	Turkey	Akata & Erdoğdu (2020)
Rutstroemia firma	CBS 115.86	MH861930	MH873619	Netherlands	Vu et al. (2019)
Rutstroemia firma	CBS 341.62	MH858174	MH869768	France	Vu et al. (2019)
Rutstroemia firma	TU 104481	LT158450	KX090832	Estonia	Pärtel et al. (2017)
Rutstroemia fruticeti	KL 590	MK501759	-	Montenegro	Perić & Baral (2017)
Rutstroemia fruticeti	F163001	KF588370	-	Spain	Johnston et al.(2014)
Rutstroemia luteovirescens	TU 104450	LT158431	KX090814	Estonia	Pärtel et al. (2017)
Rustroemia maritima	F159519	KJ941084	KJ941064	Spain	Johnston et al.(2014)
Rustroemia maritima	F118839	KF588372	KJ941063	Spain	Johnston et al.(2014)
Rutstroemia paludosa	CBS 46473	AB926158	-	USA	Zhao et al. (2016)
Rutstroemia pruni-serotinae	TNSF 40119	AB926083	AB926173	Japan	Zhao et al. (2016)
Rutstroemia punicae	KL 497	MK501758	MK501758	Montenegro	Perić & Baral (2017)
Rutstroemia sydowiana	CBS 115928	AB904507	AB904507	Japan	Zhao et al. (2016)
Rutstroemia sydowiana	CBS 115975	AB904506	AB904506	Japan	Zhao et al. (2016)
Rutstroemia sydowiana	KL 289	LT158447	-	Estonia	Pärtel et al. (2017)
Rutstroemia sydowiana	KL 318	LT158457	-	Estonia	Pärtel et al. (2017)
Torrendiella ciliata	F132996	KC412008	KJ627220	Spain	Baral et al. 2013
Torrendiella setulata	HB 9775	KF588367	KJ941052	Canada	Johnston et al. (2014)

[a] AH, AHsn: University of Alcalá, Spain; ANK Akata: Ankara University, Ankara, Turkey; C: University of Copenhagen, Denmark; CBS: Central Bureau voor Schimmelcultures, Utrecht, The Netherlands; F: Fundación Medina's Fungal Culture collection, Spain; GM: G. Marson collection, Luxembourg; HB, HOB: Hans Otto Baral collection, Germany; ICMP: International Collection of Microorganisms from Plants, New Zealand; KL: Landesmuseum für Kärnten, Austria; KUS: Korea University Herbarium, Seoul, Korea; MFLU: Mae Fah Luang University Herbarium, Chiang Rai, Thailand; OSC: Oregon State University, Corvallis, USA; PDD: Manaaki Whenua - Landcare Research, New Zealand Fungarium, New Zealand; TAAM: Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences, Estonia; TNS: National Museum of Nature and Science, Tsukuba, Japan; TU: University of Tartu, Estonia; Z.S.: Zuzana Sochorova collection, Czech Republic; WU: University of Vienna, Austria. Type strains are labeled by (T), newly generated sequences are in bold, "–" indicates unavailable sequences.

Sequence alignments and phylogenetic analyses

The sequence quality of ITS and LSU was checked and assembled with SeqMan V. 7.0.0 (DNASTAR, Madison, WI) and edited in BioEdit v 7.0.9.0 (Hall, 1999). The new sequences were searched in the NCBI BLASTn search engine and downloaded reference sequences from related literature (Table 1). Each data set was aligned in MAFFT v. 7 (Katoh et al., 2019), trimmed using trimAl v1.2 (Capella-Gutiérrez et al., 2009), and adjusted manually where necessary in BioEdit v. 7.2 (Hall, 1999). The alignment and tree were deposited in Tree base (http://purl.org/phylo/treebase/phylows/study/TB2:S30498). Phylogenetic analyses of single-gene alignments and the combined genes (ITS and LSU) were performed based on maximum likelihood and Bayesian inference (BI) analyses. Maximum likelihood analysis was performed in the IQ tree with 1000 replications on the IQ tree web server with TIM2e + I + G4 as the model of evolution (Trifinopoulos et al., 2016).

For BI analysis, substitution models were selected for ITS and LSU sequence data using JModeltest 2.3 in the CIPRES platform: TIM2e + I + G4 and TN + F + I + G4 respectively (Nylander, 2004). The BI analysis was conducted in MrBayes v. 3.2.2 with six simultaneous Markov Chain Monte Carlo (MCMC) chains, run for 2 million generations, and sampled the trees at every 1000th generation (Ronquist &Huelsenbeck, 2003). From the resulting trees, 25% were discarded as 'burn-in', and the remaining were used to calculate posterior probabilities of the majority rule consensus tree. Phylogenetic trees were viewed in FigTree v. 1.4.4 and edited in Adobe Illustrator CS v. 6 (Adobe Systems, USA; Rambaut, 2012).

Results

Phylogenetic analyses

The combined alignment of ITS and LSU contains 81 fungal taxa, including the six newly collected specimens. The best maximum likelihood tree with a final optimization likelihood value of -11044.3737 is shown in Fig. 1. The matrix comprises 541 distinct alignment patterns with 31.8% of gaps and undetermined characters (ITS = 500 bp, LSU = 848 bp). Base frequencies were estimated as follows: A = 0.242311, C = 0.226593, G = 0.271005, T = 0.26009 with substitution rates AC = 2.074553, AG = 3.110679, AT = 1.710131, CG = 1.257967, CT = 6.065717, GT = 1.00; gamma distribution shape parameter α = 0.240679. The BI analysis generated 1000 trees after 2,000,000 generations. The burn-in phase in the analysis discarded the first 250 trees and retained the remaining results to determine the posterior probability distribution.

Multigene phylogenetic analyses based on the ITS and LSU gene regions of *Rutstroemiaceae* taxa depicted in the phylogram (Fig. 1). The *Lambertella* clade (which was described as *Lambertella sensu stricto*) by Zhao et al. (2016), phylogenetically related with the newly collected *Lambertella* specimens encompasses 13 species with available molecular data, including our geographical records species, *Lambertella aurantiaca* (MFLU 23–0090) and five novel species, *Lambertella sessilis* (MFLU 23–0092), *Lambertella fusoidea* (MFLU 23–0086), *Lambertella phanensis* (MFLU 23–0091), *Lambertella takensis* (MFLU 23–0089) and *Lambertella tectonae* (MFLU 23–0087). In the phylogram, *Lambertella sessilis* (MFLU 23–0092) separated from *Lambertella tectonae* (MFLU 23–0087 and MFLU 23–0088) and *Lambertella himalayensis* (CBS 230.77) with 100% maximum likelihood bootstrap support and 1.00 posterior probability support, and *Lambertella tectonae* (MFLU 23–0087 and MFLU 23–0088) separated from the latter by 100% maximum likelihood bootstrap support and 0.99 posterior probability. In comparison, the other four newly collected *Lambertella* form distinct lineages. *Lambertella aurantiaca-L. takensis* clade forms a distinct lineage from *L. himalayensis-L. tectonae* clade with 100% maximum likelihood bootstrap support and 1.00 posterior probability, while *L. aurantiaca* (MFLU 23–0090) forms a sister relationship with *L. takensis* (MFLU 23–0089) with 100% maximum likelihood bootstrap support and 1.00 posterior probability. *Lambertella fusoidea* (MFLU 23–0086) forms a distinct clade from *L. aurantiaca-L. takensis* clade with 74% maximum likelihood bootstrap support and less than 0.90 posterior probability, and *L. phanensis* (MFLU 23–0091) separated from the others with 66% maximum likelihood bootstrap support and less than 0.90 posterior probability (Fig. 1).

Taxonomy

Lambertella sensu stricto Höhn. amend. Y.J. Zhao amend A.S. Lestari

Saprobic or *parasitic* on leaves, twigs, woody substrates and fruits. *Stroma* Dark zones on the substrates. **Sexual morph**: Discomycetous. *Apothecia* sessile, substipitate to stipitate. *Receptacle* sometimes with hair or not. *Disc* cupulate to applanate. *Ectal excipulum* composed of hyaline to brown cells of *textura prismatica, textura angularis, textura porrecta*, and rarely *textura intricata*. *Medullary excipulum* composed of hyaline to pale brown cells of *textura intricata. Paraphysis* filiform, numerous, unbranched to branched. *Asci* inoperculate, 4–5 to 8-spored, unitunicate, cylindric to cylindrical clavate, with rounded apex, ascal tip turning blue or not when tested with MLZ agent with or without KOH treatment. *Ascospores* uniseriate, partially biseriate to biseriate, fusoid, ellipsoid, sometimes ventricose, and guttulate. *Stroma* see Galán et al. (1994); Zhao et al. (2013); Zhao et al. (2015). **Asexual morph**: Undetermined. *Type species: Lambertella corni-marris* Höhn. Sber.Akad. Wiss. Wien, Math. -naturw. Kl., Abt. 1 127: 375 [47 repr.] (1918) *Notes*: Based on the combined ITS–LSU sequence data, our phylogenetic analyses support the inclusion of *Lambertella tectonae* and *L. sessilis* in the *Lambertella sensu stricto*, as those two new species form

a sister relationship to *L. himalayensis* with 100% maximum likelihood bootstrap support and 0.99 posterior probability (Fig. 1). The phylogram also supports the inclusion of *L. aurantiaca, L. takensis*, *L. fusoidea*, and *L. phanensis* in the *Lambertella sensu stricto*, as *L. aurantiaca* and *L. takensi*s form a sister relationship to the cluster comprising *L. himalayensis*, *L. tectonae*, and *L. sessilis* with 100% maximum likelihood bootstrap support and 1.00 posterior probability. Furthermore, *L. fusoidea* forms a distinct lineage to the cluster containing *L. takensis* and *L. aurantiaca* with 4 % maximum likelihood bootstrap support and 0.62 posterior probability, and *L. phanensis* forms a distinct branch to *L. fusoidea* with 6 % maximum likelihood bootstrap support and 0.64 posterior probability. Moreover, *Lambertella palmeri* forms a sister relationship with *L. phanensis* with 99% maximum likelihood bootstrap support and 0.99 posterior probability. Therefore, according to the morphology and multi-gene phylogeny, *L. aurantiaca, L. takensis*, and *L. tectonae* fit the criteria of *Lambertella sensu stricto*. However, the morphology of *L. sessilis* with its sessile apothecia and *L. phanensi*s with its mature hyaline ascospores as it is shown in (Figs. 7–8) ,do not fit to the description of *Lambertella sensu stricto* established by Zhao et al. (2015). Therefore, we emended the morphological description of *Lambertella sensu stricto* to include the characters of *L. phanensis* and *L. sessilis.*

Lambertella aurantiaca V.P. Tewari & D.C. Pant, Mycologia 59(1): 120 (1967), Mycobank number: MB 332943, Facesoffungi number: FoF 14252, (Figs. 2, 3)

Etymology

from aurantius meaning an orange, from the bright orange color of apothecia

Typhus

India, Varanasi, old Botanical Garden B.H.U, on the petioles and veins of decaying leaves of *Madhu*a (*Bassia*) *latifolia* and *Mangifera indica* L. and on an unidentified twig, 19 and 31 August 1964, V. P. Tewari and D.C. Pant (**holotype** BHUPP 223; **paratype** BHUPP 221).

Material examined: **Thailand**, Chiang Rai, Phan District, Mae Ao, Ang Kep Nam Huai Tonyang Reservoir, 19.67568037694373, 99.79873968293234, on unidentified the petioles of decaying leaves, 27 September 2021, Anis S. Lestari, NHT1 (MFLU 23–0090). Sequences derived from sexual morph: OP967479 (ITS), OP965344 (LSU).

Diagnosis: Stroma visible on the surface of leaf petioles, blackened zones. **Sexual morph**: *Apothecia* 0.5–1.7 × 304–452 mm ($\bar{x}$ = 1.2 × 0.9 mm, n = 10), arising solitary, stipitate. *Stipe* 0.3–1.2 × 0.2–0.4 mm ($\bar{x}$ = 0.8 × 0.3 µm, n = 10), concolorous to the receptacle. *Receptacle* cupulate to discoid, golden brown or deep cadmium yellow. *Margin* concolorous to the receptacle. *Disc* saucer shaped. *Hairs* absent. *Ectal excipulum* 33–44 µm ($\bar{x}$ = 39.2 µm, n = 10) at lower flanks, composed from thin-walled cells of *textura prismatica* to *textura porrecta*. *Medullary excipulum* 37–79 µm ($\bar{x}$ = 54.1 µm, n = 10) in lower flanks, composed of thin-walled, hyaline cells of *textura intricata*. *Hymenium* 75–88 µm ($\bar{x}$ = 80.9 µm, n = 15), hyaline when immature, becomes dark brown at maturity. *Paraphyses* 1.6–2.2 µm wide ($\bar{x}$ = 1.9 µm, n = 10), numerous, filiform, septate, similar length with asci, branched to 2–3 branches. *Asci* 66–81 × 4.1–7.3 µm ($\bar{x}$ = 75.7 × 5.8 µm, n = 10), 8-spored, unitunicate, cylindric, rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 7.3–9.5 × 4.1–4.5 µm ($\bar{x}$ = 8.3 × 4.3 µm, n = 10), uniseriate, hyaline when immature, becoming yellowish brown to dark brown at maturity, with 1–2 guttules. **Asexual morph**: Undetermined.

Notes

The newly collected isolate (MFLU 23–0090) was identified as *Lambertella aurantiaca*, previously described by Tewari and Pant (1967), based on similar morphology and chemical tests as sequence data are unavailable. Our isolate (MFLU 23–0090) resembles the type species of *Lambertella aurantiaca* (BHUPP 223) by encompassing stalked apothecia with deep cadmium yellow color receptacle, cylindrical asci, and brown, ellipsoid ascospores (Tewari and Pant 1967, Fig. 2–3). The identity was also confirmed by a chemical test using 2% KOH on the apothecial receptacle of MFLU 23–0090, which resulted in a color change from cadmium to purple, concise with the description of the type species (Tewari and Pant 1967). Hence, based on the morphology comparison and chemical test result, we confirmed our newly collected discomycetes specimen (MFLU 23–0090), *Lambertella aurantiaca*, as a new geographical record. Additionally, our study provided the sequence data of *Lambertella aurantiaca* for the first time.

Phylogenetically, our newly collected *L. aurantiaca* form a distinct lineage from the cluster of *L. himalayensis* (CBS 230.77) and *L. tectonae* (MFLU 23–0087 and MFLU 23–0088) with 100% maximum likelihood support and 1.00 posterior probability. Morphologically, *L. aurantiaca* (MFLU 23–0090) is characterized by a cadmium yellow receptacle with 8-spored asci distinct from *L. himalayensis* with its 4–5-spored asci and pale olivaceous to vinaceous brown receptacle (Tewari and Pant 1967). Furthermore, *Lambertella aurantiaca* is distinguished from *L. tectonae* based on its apothecial and ascospore morphology. *Lambertella tectonae* has white, grey to pale brown receptacle and pale brown ascospores, in contrast to the cadmium yellow receptacle and dark brown ascospores of *L. aurantiaca* (Fig. 2–3, Fig. 12–13).

Lambertella fusoidea Lestari & Chethana, sp. nov. Mycobank number: MB 849025, Facesoffungi number: FoF 14253 (Figs. 4, 5).

Etymology

Epithet represent the ascospores' shape

Typhus: **Thailand**, Chiang Rai Province, Forest nearby Huay Mae Sai Waterfall, 20.005648312575723, 99.71441705353928, on unidentified rotten leaf surface, 24 June 2020, Anis S. Lestari, (**holotype** MFLU 23–0086, original code: HMS1). Sequences derived from sexual morph: OP967481 (ITS), OP965340 (LSU)

Diagnosis: Stroma visible on the leaf surface, blackened zones. **Sexual morph**: *Apothecia* 442–894 × 413–724 µm ($\bar{x}$ = 686.1 × 563.5 µm, n = 10), arising solitary, stipitate. *Stipe* 350–478 × 160–185 µm ($\bar{x}$ = 429.3 × 173.8 µm, n = 10), concolorous to the receptacle. *Receptacle* discoid to applanate, brown. Margin concolorous to the receptacle. *Disc* brown, slightly concave. *Hairs* absent. *Ectal excipulum* 25–34 µm ($\bar{x}$ = 30.1 µm, n = 12) in lower flanks, composed from thick-walled cells of *textura angularis* to *textura prismatica*. *Medullary excipulum* 31–49 µm ($\bar{x}$ = 39,1 µm, n = 15) in lower flanks, composed of hyaline to light brown, thick-walled cells of *textura porrecta* to *textura angularis*. *Hymenium* 73–81 µm ($\bar{x}$ = 76.2 µm, n = 10), hyaline to pale brown. *Paraphyses* 1.6–3 µm ($\bar{x}$ = 2.1 µm, n = 10) wide at the terminal cells, numerous, filiform, septate, similar with asci in length, guttulate. *Asci* 76–86 × 7.3–8.7 µm ($\bar{x}$ = 81.6 × 7.6 µm, n = 10), unitunicate, 8-spored, cylindric, with rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 12–15 × 3.2–3.6 µm ($\bar{x}$ = 13.5 × 3.4 µm, n = 10), uniseriate to biseriate, fusoid, hyaline, smooth, 1–3 guttules, with rounded ends. **Asexual morph**: Undetermined.

Notes

In the phylogram, *L. fusoidea* (MFLU23-0086) forms a distinct lineage from *L. aurantiaca-L. takensis* cluster with a 78% maximum likelihood bootstrap support and 0.62 posterior probability bootstrap support. Morphologically, *L. fusoidea* (MFLU23-0086) is distinct with its hyaline, fusoid ascospores (Fig. 4–5), in contrast to dark brown ascospores of *L. aurantiaca* and *L. takensis*. Additionally, the ectal excipulum of *L. fusoidea* comprises *textura angularis-prismatica* cells, whereas they are *textura prismatica–porrecta* in *L. aurantiaca* and *L. takensis*. Hence, based on morphology and phylogeny, *L. fusoidea* (MFLU 23–0086) is introduced as a new species.

Lambertella phanensis Lestari & Chethana, sp. nov. Mycobank number: MB 849026, Facesoffungi number: FoF 14254 (Figs. 6, 7)

Etymology

Epithet represents the district where the specimen was found

Typhus: **Thailand**, Chiang Rai, Phan District, Mae Ao, Ang Kep Nam Huai Tonyang Reservoir, 19.67734726710035, 99.79890061546585, on leaf petioles of an unidentified plant, 27 September 2021, Anis S. Lestari (**holotype** MFLU 23–0091, original code NHT2). Sequence derived from apothecia : OP967478 (ITS), OP965343 (LSU).

Diagnosis: Stroma visible on the surface of leaf petioles, blackened zones. **Sexual morph**: *Apothecia* 0.6–1.3 × 0.5–0.9 mm ($\bar{x}$ = 0.9 × 0.7 mm, n = 8), arising solitary, stipitate, brown in dried condition. *Stipe* 181–578 × 162–302 µm ($\bar{x}$ = 286.7 × 203.9 µm, n = 10), concolorous to the receptacle. *Receptacle* cupulate to discoid, brown. *Margin* concolorous to the receptacle. *Disc* convex to applanate. *Hairs* absent. *Ectal excipulum* 30–45 µm ($\bar{x}$ = 37.7 µm, n = 11) at lower flanks, composed from brown to hyaline cells of *textura prismatica* to *textura porrecta*. *Medullary excipulum* 19–31 µm ($\bar{x}$ = 25 µm, n = 12) in lower flanks, composed of thin-walled, brown cells of *textura prismatica* to *textura angularis*. *Hymenium* 75–85 µm ($\bar{x}$ = 80 µm, n = 15), hyaline. *Paraphyses* 1.1–1.7 µm wide ($\bar{x}$ = 1.3 µm, n = 10), numerous, filiform, aseptate, similar length with asci, branched at 1/3 of the length and at the base. *Asci* 59–82 × 3.7–6.1 µm ($\bar{x}$ = 70.2 × 5.2 µm, n = 10), 8-spored, unitunicate, cylindric, rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 7.3–9.5 × 4.1–4.5 µm ($\bar{x}$ = 8.3 × 4.3 µm, n = 10), uniseriate to partially biseriate, fusoid, 1–2 guttules, hyaline, becoming brown after discharge. **Asexual morph**: Undetermined.

Notes

Based on the multi-gene phylogeny, *Lambertella phanensis* (MFLU 23–0091) forms a distinct lineage basal to the *L. fusoidea* (MFLU 23–0086) with a maximum likelihood bootstrap value of 66% and Bayesian posterior probability of 0.64. Morphologically, *L. phanensis* (MFLU 23–0091) differs from *L. fusoidea* (MFLU 23–0086) by having ellipsoid ascospores in contrast to fusoid ascospores of the latter (Fig. 5, 7). Hence, based on morphology and phylogeny, *Lambertella phanensis* (MFLU 23–0091) was introduced as a new species.

Lambertella sessilis Lestari & Chethana, sp. nov. Mycobank number: MB 849027, Facesoffungi number: FoF 14255, (Figs. 8, 9)

Etymology

Epithet represents the sessile apothecia of this species

Typhus: **Thailand**, Tak province, Omkoi district, Sop Khong, 17.639554620869404, 98.24246141202575, on a wood bark.16 October 2019, Anis S. Lestari (**holotype** MFLU 23–0092, original code OMK2). Sequence derived from sexual morph: OQ650294 (ITS), OQ650293 (LSU).

Diagnosis: Stroma visible on the surface of wood bark, irregular blackened zones. **Sexual morph**: *Apothecia* 311–848 × 303–708 µm ($\bar{x}$ = 589.8 × 529.7 µm, n = 5), arising solitary, sessile, greyish brown when fresh, brown in dried condition. *Receptacle* cupulate to discoid, brown when it is dried. *Margin* concolorous to the receptacle. *Disc* discoid to cupulate. *Ectal excipulum* 55–62 µm ($\bar{x}$ = 59.5 µm, n = 10) at lower flanks, composed of brown to hyaline cells of *textura angularis* to *textura prismatica*. *Medullary excipulum* 62–171 µm ($\bar{x}$ = 115.5 µm, n = 10) in lower flanks, composed of thin-walled, brown cells of *textura epidermoidea*. *Hymenium* 74–81 µm ($\bar{x}$ = 78.7 µm, n = 10), grey, pale brown to brown. *Paraphyses* 1.7–3.5 µm wide ($\bar{x}$ = 2.6 µm, n = 12) at the terminal cells, numerous, filiform, septate, similar length with asci, branched at 1/2 length and at the base. *Asci* 74–88 × 6.9–10.2 µm ($\bar{x}$ = 81.5 × 8.4 µm, n = 10), 8-spored,

unitunicate, cylindric to cylindrical clavate, rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 8–10 × 2.8–4.9 µm ($\bar{x}$ = 8.9 × 4.1 µm, n = 10), uniseriate to partially biseriate, ellipsoid, 1–2-guttulate, hyaline, slightly brown to brown. **Asexual morph**: Undetermined.

Notes

Newly collected *Lambertella sessilis* (MFLU 23–0092) form a sister relationship with the cluster of *Lambertella himalayensis* (CBS 230.77) and *L. tectonae* (MFLU 23–0087 and MFLU 23–0088) with 100% maximum likelihood bootstrap support and 0.98 posterior probability. Morphologically, *Lambertella sessilis* (MFLU 23–0092) is characterized by sessile apothecia, medullary excipulum comprising *textura epidermoidea* cells, and brown ascospores, which are distinct from *L. tectonae* (MFLU 23–0087), which is characterized by stipitate apothecia, medullary excipulum of *textura intricata* cells and pale brown ascospores (Fig. 8–9, 12–13). *Lambertella sessilis* (MFLU 23–0092) is also distinguished from *L. himalayensis* by having sessile apothecia, while the latter has stipitate apothecia (Tewari and Pant 1967, Fig. 14), hence, *L. sessilis* introduced as a new *Lambertella* species.

Lambertella takensis Lestari & K.D. Hyde, sp. nov. Mycobank number: MB 849028, Facesoffungi number: FoF 14256, (Figs. 10, 11).

Etymology

Epithet represents the province where the holotype was found.

Typhus: **Thailand**, Tak province, Tha Song Yang district, Mae Wa Luang, 17.712253372145504, 97.99567621471674, on leaf petioles of *Tectona grandis*, 17 October 2019, Anis S. Lestari (**holotype** MFLU 23–0089, TAK1). Sequence derived from sexual morph: OP967480 (ITS), OP965339 (LSU).

Diagnosis: Stroma visible on the surface of leaf petioles, blackened zones. **Sexual morph**: *Apothecia* 0.7–2.6 × 0.5–2.5 mm ($\bar{x}$ = 1.7 × 0.3 mm, n = 10), arising solitary, brown surface, stipitate. *Stipe* 0.3–1.4 × 0.2–0.5 mm ($\bar{x}$ = 0.6 × 0.3 mm, n = 10), yellowish brown. *Receptacle* cupulate to discoid, white or beige color. *Margin* concolorous to the receptacle. *Disc* saucer-shaped to applanate. *Hairs* absent. *Ectal excipulum* 31–46 µm ($\bar{x}$ = 39.9 µm, n = 15) at lower flanks, composed from thin-walled, hyaline to light brown cells of *textura prismatica*. *Medullary excipulum* 44–58 µm ($\bar{x}$ = 48.8 µm, n = 10) in lower flanks, composed of thin-walled, hyaline cells of *textura intricata*. *Hymenium* 61–79 µm ($\bar{x}$ = 65.2 µm, n = 15), hyaline when immature, becomes dark brown at maturity. *Paraphyses* 2.6–3.3 µm wide ($\bar{x}$ = 2.9 µm, n = 10), numerous, filiform, septate, similar length with asci, some branched at 1/3 of the length from the base. *Asci* 56–71 × 5–6 µm ($\bar{x}$ = 64.6 × 5.6 µm, n = 10), 8-spored, unitunicate, cylindric, rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 6–7.6 × 3.4–4 µm ($\bar{x}$ = 6.8 × 3.7 µm, n = 10), uniseriate, hyaline when immature, becoming yellowish brown to dark brown at maturity, ellipsoid, with 2–3 guttules. **Asexual morph**: Undetermined.

Notes

In the phylogeny, our new specimen, *Lambertella takensis* (MFLU 23–0089), forms a sister relationship with *L. aurantiaca* (MFLU 23–0089) with 100% maximum likelihood bootstrap support and 1.00 Bayesian posterior probability. *Lambertella takensis* (MFLU 23–0089) has a creamy to pale brown receptacle that differs from the deep cadmium yellow of *L. aurantiaca* (MFLU 23–0090). Another distinct character was revealed via a chemical test using 2% KOH on the *Lambertella* receptacle as described by Tewari & Pant (1967) to differentiate *L. aurantiaca* from other *Lambertella* species. The receptacle of *L. takensis* (MFLU 23–0089) tested negative (no color change), whereas *L. aurantiaca* (MFLU 23–0090) showed a color change to purple.

Lambertella tectonae Lestari & Chethana, sp. nov. Mycobank number: MB 849029, Facesoffungi number: FoF 14257, (Figs. 12, 13).

Etymology

Epithet represents the host *Tectonae*.

Typhus: **Thailand**, Chiang Rai, Phan District, Sai Khao, Phra That Wang Chom Thong Monastery, 19.67835471370441, 99.74994242011047, on leaf petioles of *Tectona grandis*, 31 August 2021, Anis S. Lestari (**holotype** MFLU 23–0087, original code: PTW1). Sequences derived from sexual morph: OP967476 (ITS), OP965342 (LSU).

Additional material examined: **Thailand**, Huai Sak, Ang Kep Nam Nong Buak Tao Reservoir, 19.792211684477675, 99.87910786250163, on leaf petioles of unidentified leaves, 27 September 2021, Anis S. Lestari, BTR5 (MFLU 23–0088). Sequences derived from sexual morph: OP967477 (ITS), OP965341 (LSU).

Diagnosis: Stroma visible on the surface of leaf petioles, inconspicuous on the leaves' mid rib, blackened zones. **Sexual morph**: *Apothecia* 0.9–2.6 × 0.9–2.6 mm ($\bar{x}$ = 1.9 × 1.8 mm, n = 10), arising solitary, short stipitate, white to pale brown when fresh, brown in dried condition. *Stipe* 400–778 × 267–640 µm ($\bar{x}$ = 566.3 × 424.1 µm, n = 10). *Receptacle* cupulate, brown to grey when it is dried. *Margin* concolorous to the receptacle. *Disc* convex to applanate. *Hairs* present. *Ectal excipulum* 74–138 µm ($\bar{x}$ = 100.9 µm, n = 10) at lower flanks, composed from pale brown to hyaline, thin-walled cells of *textura prismatica* to *porrecta*. *Medullary excipulum* 95–366 µm ($\bar{x}$ = 223.1 µm, n = 10) in lower flanks, composed of thin-walled, hyaline cells of *textura intricata*. *Hymenium* 94–99 µm ($\bar{x}$ = 95.1 µm, n = 11), hyaline to pale brown. *Paraphyses* 1.9–2.8 µm wide ($\bar{x}$ = 2.3 µm, n = 12) at the terminal cell, numerous, filiform, septate, similar length with asci, branched at 1/2 and 1/3 length. *Asci* 74–82 × 6.4–8.1 µm ($\bar{x}$ = 77.9 × 7.4 µm, n = 11), 8-spored, unitunicate, cylindric, rounded apex, J+ (eu-amyloid, in MLZ with and without KOH treatment), arising from croziers. *Ascospores* 8.5–12 × 3.2–4.8 µm ($\bar{x}$ = 10.7 × 4.2 µm, n = 15), uniseriate to partially biseriate, hyaline to pale brown, fusoid to ellipsoid, smooth. **Asexual morph**: Undetermined.

Notes

Newly collected *Lambertella tectonae* (MFLU 23–0087 and MFLU 23–0088) form a sister relationship with *Lambertella himalayensis* (CBS 230.77) with 100% maximum likelihood bootstrap support and 1.00 Bayesian posterior probability. The morphological differences in paraphyses and ascospores distinguish *Lambertella tectonae* (MFLU 23–0087) from *Lambertella himalayensis* (CBS 230.77). *Lambertella tectonae* (MFLU 23–0087 and MFLU 23–0088) differs by having up to 4-branched paraphyses, 8-spored asci, and hyaline ascospores, which develop a pale brown edge after discharge (the brown color can be seen at the wall of the germinating ascospores) (Figs. 12–13), while the isotype of *L. himalayensis* (CUP-050013) exhibits simple, unbranched paraphyses, and 4–5-spored asci with brown ascospores (Fig. 14; Tewari & Pant 1967).

Discussion

Previous studies have reported *Lambertella* on various substrates, such as rotten or mummified fruits (Wiseman et al., 2015), and dead twigs and leaves (Tewari & Pant, 1967). Geographically, *Lambertella* has been found in various regions in the tropics, northern and southern hemispheres (Whetzel, 1943; Cash, 1958; Tewari, 1963; Dumont, 1971; Dumont, 1974; Korf & Zhuang 1985; Hosoya & Otani, 1997; Zhao et al., 2013) but not in Thailand (Phanichapol, 1986; Ekanayaka et al., 2019; Phutthacharoen et al., 2022). This study reported six *Lambertella* specimens from Northern Thailand (Chiang Mai, Chiang Rai and Tak provinces), among which five are novel species, and one is a geographical record. The new species are *L. fusoidea* (MFLU 23–0086), *L. phanensis* (MFLU 23–0091), *L. sessilis* (MFLU 23–0092), *Lambertella takensis* (MFLU 23–0089), and *L. tectonae* (MFLU 23–0087), while *L. aurantiaca* (MFLU 23–0090) is a geographical record.

Phylogenetic affinities of six newly collected *Lambertella* species are shown to be clustered in *Lambertella sensu stricto*, similar to the previous study by Zhao et al. (2016) based on LSU and *RPB2* sequences. The topology of our *Lambertella sensu stricto* clade is similar to that of Zhao et al. (2016), even though phylogenetic positions for some species, such as *L. pruni* that showed slight differences in our phylogram based on ITS and LSU sequence data and with more *Lambertella* species and other representative genera in *Rutstroemiaceae* (Fig. 10). *Lambertella* species in our phylogram are polyphyletic, similar to the ITS, LSU and *RPB2* combined phylogeny of Zhao et al. (2016). Additionally, other members of *Rutstroemiaceae*, like *Rutstroemia* and *Lanzia*, also showed polyphyly (Fig. 1), hence, re-evaluating the family *Rutstroemiaceae* based on morphology and phylogeny with more fungal collections is necessary to address these confusing taxonomic placements.

Based on ITS and LSU phylogram (Fig. 1), two newly collected *Lambertella*, *L. sessilis* (MFLU 23–0092) and *L. tectonae* (MFLU 23–0087), form a sister relationship with *L. himalayensis* (CBS 230.77) with 100% maximum likelihood bootstrap support and 1.00 Bayesian posterior probability. The only available sequence data for *L. himalayensis* originated from the culture CBS 230.77 isolated from *Cassia siamea* in Myanmar. However, this culture is neither the holotype nor the isotype of *L. himalayensis* (CUP-050013) described in Tewari & Pant (1967) and lack complete morphological descriptions (Salgado-Salazar et al., 2013). Hence, in this study, we observed the isotype of *L. himalayensis* (CUP-050013) to confirm the presence of any characters similar to our newly introduced species, especially the sexual forms of our *Lambertella* specimens. We found that the morphology of apothecia and ascospores of *L. himalayensis* (CUP-050013) were distinct from *L. tectonae* (MFLU 23–0087) and *L. sessilis* (MFLU 23–0092). *Lambertella himalayensis* (CUP-050013) has long stipitate apothecia (up to 2 mm) with 4–5-spored asci and brown ascospores (Tewari & Pant, 1967), while *L. tectonae* have substipitate to shorter apothecia (up to 0.8 mm), 8-spored asci and pale brown ascospores (Fig. 13–14; Table 2), and *L. sessilis* (MFLU 23–0092) characterized by sessile apothecia, *textura angularis-prismatica* cells of ectal excipulum and 8-spored asci which differ from long stipitate apothecia, *textura porrecta* cells and 4–5-spored asci of *L. himalayensis* (CUP-050013) (Fig. 8; 14; Table 2).

The phylogenetic relationship between the four newly collected *Lambertella* species (*L. tectonae, L. sessilis, L. takensis*, and *L. tectonae*) is depicted in the phylogram (Fig. 1). *Lambertella tectonae* (MFLU 23–0087) and *L. sessilis* (MFLU 23–0092) form a basal lineage to *L. takensis* (MFLU 23–0089) and *L. aurantiaca* (MFLU 23–0090), confirming their affinities at the species level. However, *Lambertella aurantiaca, L. takensis, L. tectonae*, and *L. sessilis* differ based on apothecial and the ascal ring asci. *Lambertella aurantiaca* (up to 1.4 mm) and *L. takensis* (up to 5 mm) have longer stipitate apothecia than *L. tectonae* (up to 0.8 mm), whereas *L. sessilis* has no stipe (sessile apothecia) (Table 2). The ascal rings of *L. aurantiaca* and *L. takensis* show no vertical lines at the ascal tips as they appear on the asci of *L. sessilis* and *L. tectonae* (Fig. 3; 9; 11; 13).

A distinct lineage was formed by *L. aurantiaca* (MFLU 23–0090) and *L. takensis* (MFLU 23–0089) in *Lambertella sensu stricto* with 100% maximum likelihood bootstrap support and 1.00 posterior probability (Fig. 1) between the two species. Morphology between the two species is similar except for the color of the receptacle, which is considered an interspecies variation. Additionally, a chemical test with 2% KOH on the *L. aurantiaca* (MFLU 23–0090) receptacle changed the color from cadmium yellow (original receptacle color) to purple, which fits the description of *L. aurantiaca* (BHUPP 211, **holotype**) in Tewari & Pant (1967), while the same test resulted in a negative reaction on the receptacle of *L. takensis*. In the case of morphology, *L. takensis* is similar to the isotype of *L. himalayensis* (CUP-050013) except for the number of ascospores inside the asci (Tewari & Pant 1967). *Lambertella takensis* (MFLU 23–0089) is characterized by 8-spored asci, whereas *L. himalayensis* (CUP-050013) has 4–5-spored asci (Table 2). Based on ITS and LSU phylogeny, the association between *L. himalayensis* (CUP-050013) and *L. takensis* (MFLU 23–0089) is not also closely related (Fig. 1). There are no doubts that *L. takensis* (MFLU 23–0089) has been presumably described in previous studies (Dumont, 1971; Whetzel, 1943) under different species, however, most extant *Lambertella* species were already old, and morphological descriptions are not complete for character comparison and identification. Among 66 *Lambertella* species registered in the Species Fungorum (2023), only 17 have sequence data (Table 2). In this study, morphological description coupled with chemical testing confirmed the identity of *L. aurantiaca*, which was previously found in India without any sequence data. Our study successfully added molecular data for the extant species, *L. aurantiaca*. Therefore, field explorations to find new and extant *Lambertella* specimens are important to update the morphology and sequence data in public databases.

In our study, four new *Lambertella* species, *L. aurantiaca* (MFLU 23–0090), *L. takensis* (MFLU 23–0089), *L. fusoidea* (MFLU 23–0086) and *L. phanensis* (MFLU 23–0091) form basal lineages to *L. palmeri* (AHsn and AH 7576) with 99% maximum likelihood bootstrap support and 0.99 posterior probability, which suggest a close phylogenetic relationship with the extant *Lambertella* species. However, distinct morphologies of *L. palmeri* with ventricose ascospores in contrast to fusoid to ellipsoid ascospores of our four newly introduced *Lambertella* species confirmed that our specimens are different species. Another two new *Lambertella* from Thailand, *L. fusoidea* and *L. phanensis*, also form distinct lineages within *Lambertella sensu stricto* clade, however, they do not cluster with other extant *Lambertella* species (Fig. 1). Morphologically, *L. fusoidea* is characterized by its hyaline, fusoid ascospores distinct from other newly collected *Lambertella* (*L. aurantiaca, L. sessilis, L. takensis*) which are mostly dark brown and ellipsoid except for *L. tectonae* with its pale brown ascospores and *L. phanensis* with its hyaline but ellipsoid ascospores (Fig. 10–13, Table 2). Zhao et al. (2016) stated that *Lambertella sensu stricto* clade consists of *L. corni-maris*, *L. hicoriae*, *L. himalayensis*, *L. pruni*, and *L. pyrolae* and redefined it exclusively based on the formation of brown ascospores before ascal discharge which differs from previous *Lambertella* generic descriptions given by Dumont (1971), and Korf & Zhuang (1985). Based on the phylogenetic analysis in our study, *L. tetrica* (F142281) and *L. palmeri* (AHsn and AH7576), which were not included in the phylogeny of Zhao et al. (2016), grouped with our newly collected specimens in *Lambertella sensu stricto* clade (Fig. 1; Zhao et al. 2016). The formation of brown ascospores of *L. palmeri* and *L. tetrica* fits the morphological description of *Lambertella sensu stricto* clade in Zhao et al. (2016). However, some of our new species, especially *L. phanensis* (MFLU 23–0091) characterized by the hyaline, ellipsoid ascospores and its mature ascospores confirmed by its germinated ascospores, do not fit the morphological description of *Lambertella sensu stricto* clade. Hence, we propose to include hyaline ascospores and sessile apothecia (as described in *L. sessilis*) and amend the generic description of *Lambertella sensu stricto.*

Lambertella is found mostly on lignocellulose substrates, such as *Acer*, *Andromeda, Archontophoenix, Artemisia, Artocarpus, Aster, Astronia, Berberis, Calophyllum, Carya, Cassia, Cephalanthus, Citharexylum, Coccolobis, Coptis, Cornus, Cryptomeria, Dalbergia, Damnacanthus, Elaeocarpus, Eriobotrya, Eugenia, Euphorbia, Euterpe, Fraxinus, Gayrya, Gunnera, Guttifera, Hedera, Ilex, Ixora, Jasminum, Madhuca, Maesa, Malus, Mangifera, Melasomataceae, Myrica, Myrtaceae, Pachysandra, Palmae, Pinus, Prunus, Pyrola, Pyrus, Quercus, Rhamnus, Rosaceae, Rhubus, Shorea* and *Viburnum* (Korf & Zhuang, 1985; Schumacher & Holøs, 1989; Salgado-Salazar et al., 2013; Zhao et al., 2013). In addition, this study recorded a new host, *Tectona grandis*, for *L. tectonae* (MFLU 23–0087) and *L. takensis* (MFLU 23–0089), which was not previously reported for any *Lambertella* species. Doilom et al. (2017) documented a new species and some microfungi on *Tectona grandis* in northern Thailand, but no *Lambertella* species was found. It is remarkable that five new species in a new single conspicuous genus were found in a small area of northern Thailand, indicating that novel species discoveries are far from reaching asymptote (Hyde et al., 2020) and that the tropics is an unexplored cache of novel taxa (Hyde et al., 1997, 2019).

Table 2
Synopsis of *Lambertella* species with molecular data

Species	Origin	Host	Apothecia	Paraphyses	Ectal excipulum	Medullary excipulum	Asci	Ascospore characters	Notes
							Size (µm)	Size (µm)	
Lambertella aurantiaca	India, Thailand **(this study)**	Petioles and veins leaves of *Madhuca latifolia, Mangifera indica* and unknown dicotyledon petiole leaves	Up to 4 mm diam., 5 mm high, orange (cadmium yellow), stipitate	Filiform with 2–3 branches, septate	*Textura prismatica* to *porrecta*	*Textura intricata*	66–105 × 4–8 µm, cylindrical, rounded apex, J+ (without KOH 5% treatment), arising from croziers, 8 spored	6.4–9.6 × 3.2–4 µm, ellipsoid, hyaline to brown at maturity, guttulate, uniseriate	Chemical reaction with KOH 2% (+) turn receptacle to purple cells
Lambertella corni-maris	Australia, Austria, Europe, Japan	On blacken fruits of *Cornus mas, Malus domestica, Prunus domestica, Sorbus aucuparia* and *Cyttaria* galls	1.5–7.5 mm diam., up 0.5–20 mm high, pale pink to flesh colored, stipitate	Filiform, septate, branched	*Textura prismatica*	Interwoven thin walled, septate hyphae	70–108 × 5.5–8 µm, cylindric, rounded apex, J+, arising from croziers, 8 spored	6–10 × 3.5–5 µm, fusoid to ellipsoid, hyaline to brown at maturity, biguttulate, uniseriate	
Lambertella fusoidea sp nov.	Thailand	On a rotten leaf surface	413–894 µm, 160–478 µm high, brown, stipitate	Filiform, septate, branched near the base	*Textura angularis* to *prismatica*	*Textura porrecta* to *angularis*	76–86 × 7.3–8.7 µm cylindric, rounded apex, J+ (with and without KOH treatment), arising from croziers, 8-spored.	12–15 × 3.2–3.6 µm, fusoid, hyaline with rounded ends, guttulate, uniseriate to biseriate.	
Lambertella hicoriae	USA	On *Carya ovata*	N/A	N/A	*Textura porrecta* to *intricata*	N/A	cylindric-clavate	Fusoid, hyaline to brown at maturity, guttulate,	
Lambertella himalayensis	India, Myanmar	On *Cassia siamea,* dead twigs of *Quercus leucotrichophora* and other unidentified herbaceous stems	2–3 mm diam., up to 2 mm high, stipitate.	Filiform (simple), septate	*Textura porrecta*	*Textura intricata*	86.5–102 × 6.5–18 µm, cylindrical, rounded apex, J+, 4–5 spored	9.6–14.5 × 4.5–6.5 µm, ellipsoid, hyaline to brown at maturity, guttulate, uniseriate	
Lambertella langei	Norway	Overwintered leaves of *Andromeda polifolia*	0.5–2.5 mm diam., 1–2.2 mm high, stipitate	Filiform, branched, septate	*Textura prismatica*	*Textura intricata*	130–180 × 11.5–15.5 µm, cylindrical, rounded apex, J+ (with KOH 5% treatment), 8 spored	14.5–21 × 5.6–8 µm, fusoid to ellipsoid, guttulate uniseriate to partly biseriate	
Lambertella palmeri	Mexico	On fallen leaves of *Quercus agrifolia*	Up to 1 mm diam, 1.5 mm high, stipitate	Cylindric	*Textura prismatica* to *porrecta*	*Textura intricata*	115–140 x 12–18 µm, cylindric to cylindrical clavate, truncate apex, J-, arising from simple septa, 8 spored	28–38 x 10–12 µm, fusoid-ellipsoid in dorsal view, ventricose in lateral view, hyaline to brown at maturity, guttulate, biseriate	

Species	Origin	Host	Apothecia	Paraphyses	Ectal excipulum	Medullary excipulum	Asci	Ascospore characters	Notes
							Size (µm)	Size (µm)	
Lambertella phaniensis sp nov	Thailand	On petiole leaves of an unidentified plant	0.6–0.9 mm diam., 0.1–0.5 mm high, stipitate	Filiform, aseptate, branched near the base	*Textura prismatica* to *porrecta*	*Textura prismatica* to *angularis*	59–82 × 3.7–6.1 µm, cylindric, rounded apex, J+ (with and without KOH treatment), arising from croziers, 8-spored.	7.3–9.5 × 4.1–4.5 µm, fusoid, hyaline, guttulate, uniseriate to partially biseriate.	
Lambertella pruni	USA	On mummified fruits of *Prunus avium*	N/A	N/A	N/A	N/A	cylindric, J+, arising from croziers, 8-spored	13–19 × 7–10 µm, almond shaped, hyaline to brown at maturity	
Lambertella pyrolae	Japan	On decayed and petioles of *Pyrola*	0.5–1.6 mm diam., 1.5–2 mm high, stipitate	Filiform, branched near the base	*Textura prismatica* adorned with hairs	*Textura intricata*	81–145 × 6–9.5 µm, clavate, rounded apex, J-, arising from simple septa, 8 spored	14–22 × 3–4.5 µm, fusoid to ellipsoid, hyaline to brown at maturity, guttulate, uniseriate to biseriate	-
Lambertella sessilis sp nov	Thailand	On decayed wood barks	0.3–0.8 mm diam., 0.2–0.5 mm high, sessile	Filiform, branched at the 1/2 length, sometimes at the base	*Textura angularis* to *prismatica*	*Textura epidermoidea*	74–88 × 6.9–10.2 µm, cylindrical clavate, rounded apex, J+ (with and without KOH treatment), arising from croziers, 8-spored	8–10 × 2.8–4.9 µm, ellipsoid, hyaline to brown at maturity, guttulate, uniseriate to partially biseriate	
Lambertella subrenispora	Japan, India	On stems of *Artemisia* sp, *Aster ageratoides*, unidentified *Rosaceae*	Up to 2 mm diam., 1–1.5 mm high, substipitate or short stipe, adorned with hairs	Filiform	*Textura prismatica*	*Textura intricata*	80–95 × 8–9.5 µm, cylindric to cylindrical clavate, J+ (with KOH 10% pretreatment)	11–13 × 4.8–6.4 µm, sub-reniform, hyaline to brown at maturity, guttulate, uniseriate to biseriate	-
Lambertella takensis sp nov	Thailand	On petioles of *Tectonae grandis*	0.7–2.6 mm diam., 0.2–1.4 mm high, light brown to brown, stipitate	Filiform, septate, branched near the base	*Textura prismatica*	*Textura intricata*	56–71 × 5–6 µm, cylindric, rounded apex, J+ (with and without KOH treatment), arising from croziers, 8-spored	6–7.6 × 3.4–4 µm, ellipsoid, hyaline to dark brown at maturity, guttulate, uniseriate.	
Lambertella tectonae sp nov	Thailand	On petioles of *Tectonae grandis*	0.9–2.6 mm diam., 0.2–0.8 mm high, white when fresh, substipitate or short stipe	Filiform, septate, branched 3–4 times	*Textura prismatica* to *porrecta*	*Textura intricata*	81–95 × 5.6–8.8 µm, cylindric, rounded apex, J+ (with and without KOH treatment), arising from croziers, 8-spored	7–11 × 3.8–4.8 µm, fusoid to ellipsoid, hyaline to pale brown, guttulate, uniseriate to partially biseriate	

Species	Origin	Host	Apothecia	Paraphyses	Ectal excipulum	Medullary excipulum	Asci	Ascospore characters	Notes
							Size (µm)	Size (µm)	
Lambertella tetrica	Spain	On leaves of *Hedera helix*	Up to 1 mm, stipitate	Filiform, septate	*Textura prismatica*	*Textura intricata*	cylindric, J-, arising from croziers, 8 spored	Up to 20 µm, in length, up to 5 µm in width, fusoid enveloped with gelatinous sheath, hyaline to brown, guttulate	
Lambertella tubulosa	UK	On decaying twigs of *Acer pseudoplatanus*	0.5–0.7 mm diam., stipitate	Filiform, branched at the base	*Textura prismatica*	*Textura intricata*	50–70 × 6–8 µm, cylindric to cylindrical clavate, rounded apex, J-, 8 spored	6.5–10 × 5–6 µm, subfusoid to broad ellipsoid, hyaline to brown at maturity, uniseriate to biseriate	
Lambertella viburni	India	On fallen fruits of *Viburnum stellatum* and *Rosa macrophylla*	1–3 mm diam., 2–14 mm high, stipitate	Filiform (simple), septate, branched in the lower base	*Textura porrecta*	*Textura porrecta*	125–140.5 × 8–11.5 µm, cylindrical, rounded apex, J+, 8 spored	11–16 × 3–5 µm, ellipsoid, hyaline to brown at maturity, guttulate uniseriate to biseriate	

Declarations

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Data availability

All sequence data generated for this study can be accessed in GenBank. Morphological descriptions are deposited in Mycobank (nomenclature is verified by curator).

Ethical approval This is an original research workand submitted to Mycological Progress for publication. Authors are adhered to discipline-specific rules for acquiring, selecting and processing data. No data or theories by others are presented by others presented in this study.

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Figures

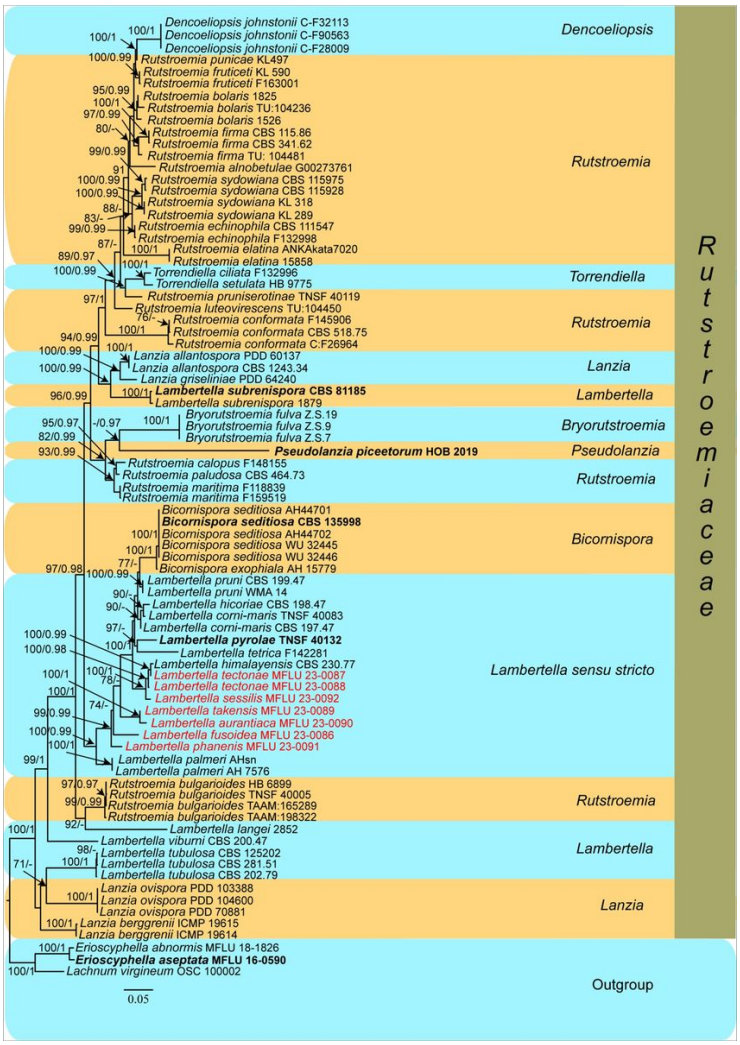

Figure 1

The phylogram of combined ITS and LSU sequence data for genera in *Rutstroemiaceae*. Maximum likelihood bootstrap values greater than 70% and posterior probability values greater than 0.90 given near the nodes. The new geographical record highlighted in red and type strains are in bold. The tree is rooted to member of *Lachnaceae: Erioscyphella abnormis* (MFLU 18-1826), *Erioscyphella aseptata* (MFLU 16-0590) and *Lachnum virgineum* (OSC 100002).

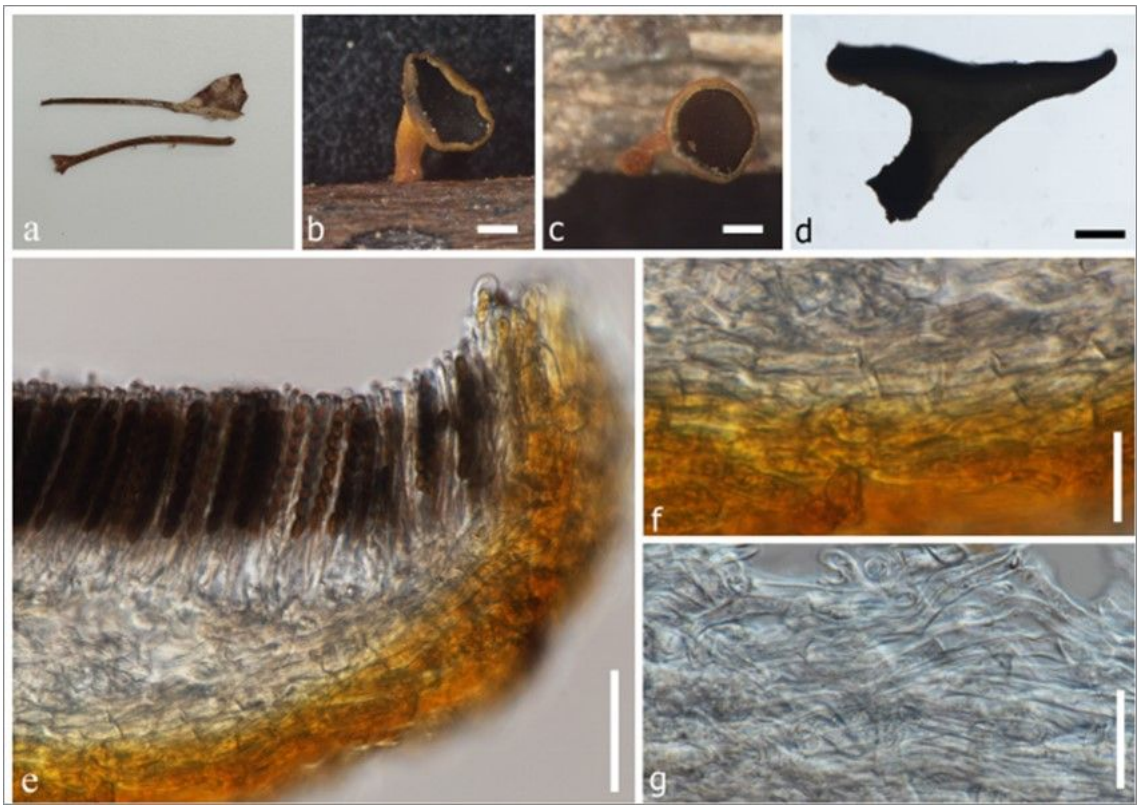

Figure 2

Lambertella aurantiaca (MFLU 23-0090, **a new geographical record**). a. Stems of leaves. b–c. An apothecium on the leaf petiole. d. Close-up of an apothecium cross section. e. Close up of the hymenium at the margin. f. Ectal excipulum cells. g. Medullary excipulum cells. Scale bars: b–c = 500 µm, d = 354 µm, e = 38 µm, f–g= 18 µm.

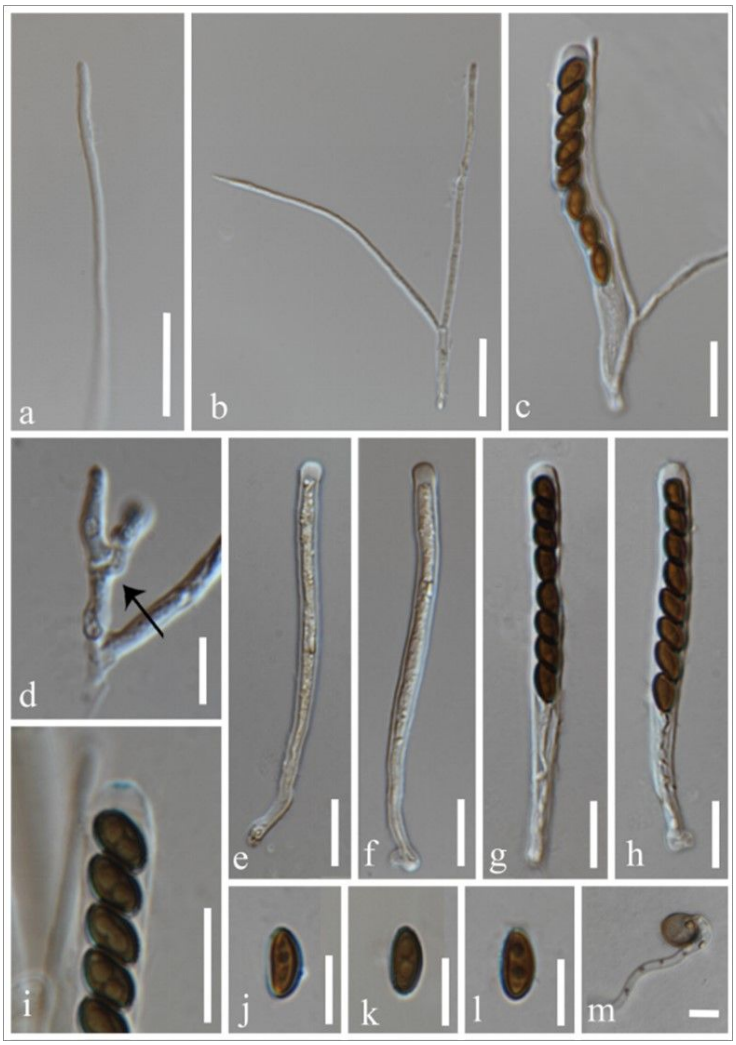

Figure 3

Lambertella aurantiaca (MFLU 23-0090, **a new geographical record**). a–b. Filiform paraphyses. c. Asci with paraphyses. d. Immature ascus with crozier at the base. e–h. Asci. i. Tip of the ascus (mounted in Melzer agent). j–l. Ascospores. m. A germinated ascospore. Scale bars: a–c = 31 µm, e–h = 14 µm, i = 11 µm, d, j–m = 9 µm.

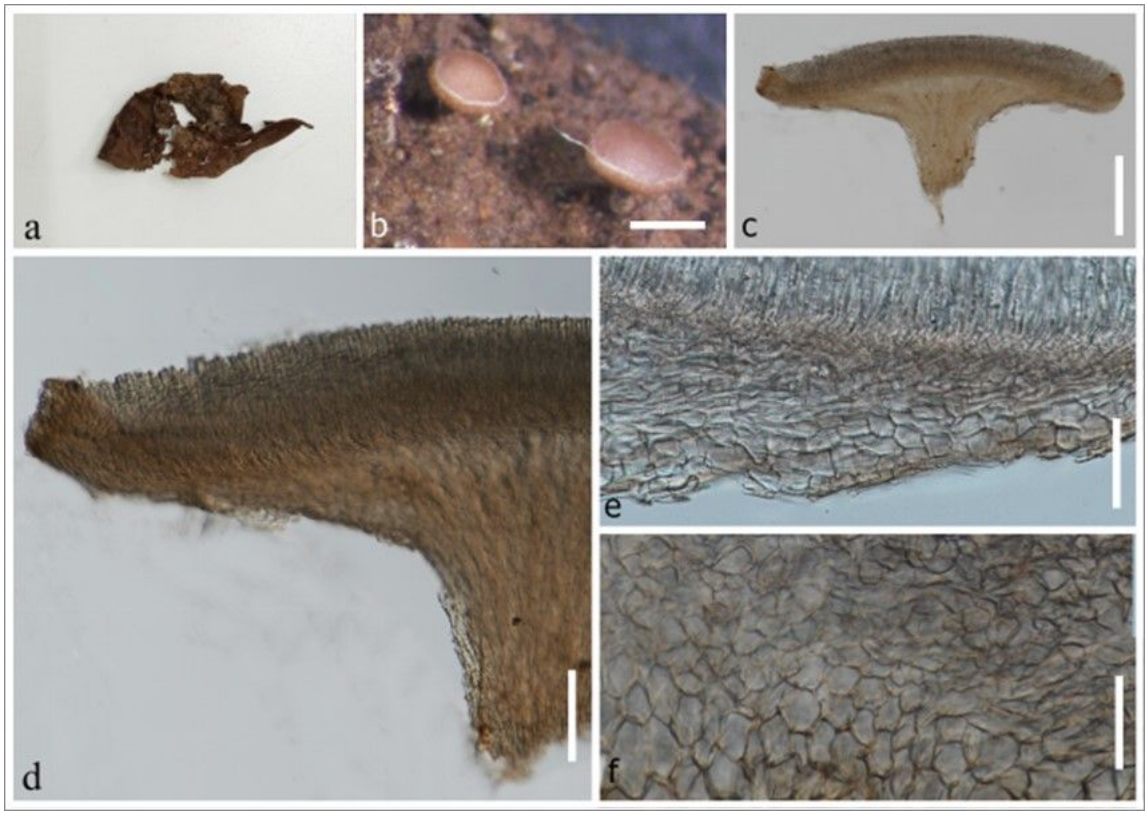

Figure 4

Lambertella fusoidea (MFLU 23-0086, **holotype**). a. Dead leaves. b. Apothecia on the leaf midribs. c. Close-up of an apothecium section. d. Close up of hymenium at the margin. e. Ectal excipulum cells. f. Medullary excipulum cells. Scale bars: b = 550 µm, c = 341 µm, d = 137 µm, e = 40 µm, f= 31 µm.

Figure 5

Lambertella fusoidea (MFLU 23-0086, **holotype**). a–c. Filiform paraphyses. d. Crozier at a ascus's base (arrow pointed). e–h. Asci. i. Tip of an ascus (mounted in Melzer agent without KOH treatment). j–l. Ascospores. Scale bars: a–c = 18 μm, d–h = 13 μm, i = 12 μm, j–l = 11 μm.

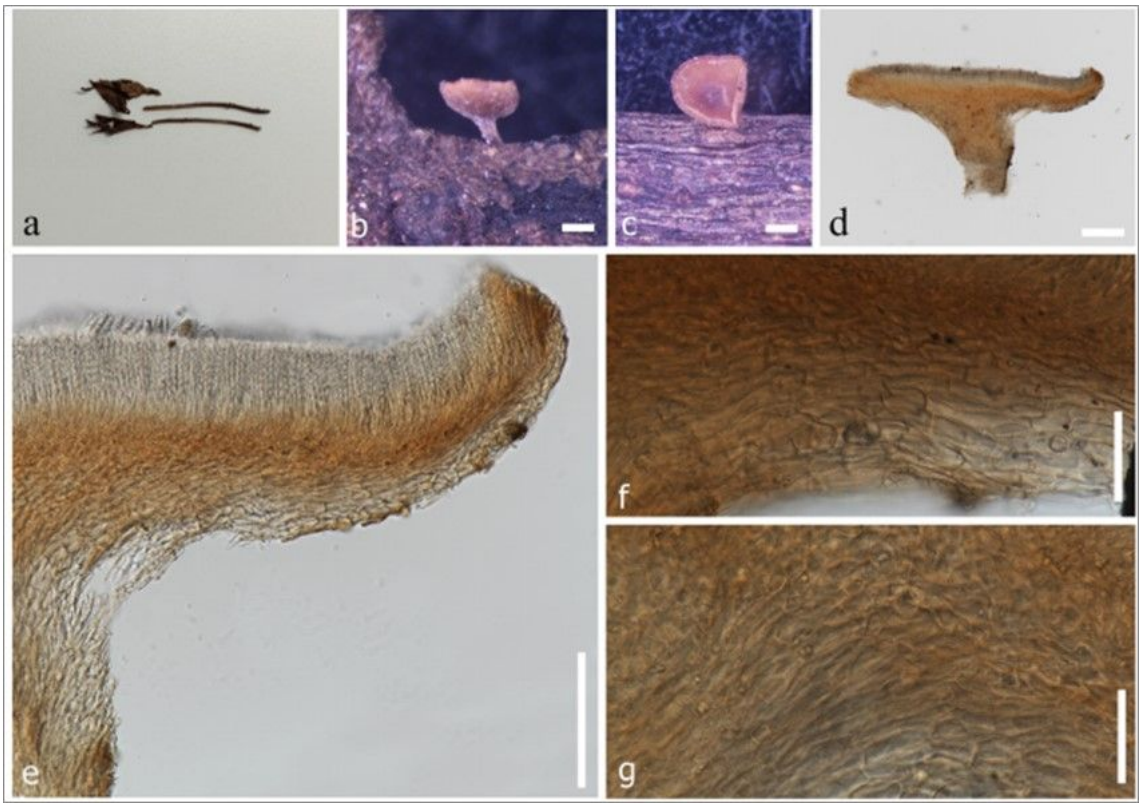

Figure 6

Lambertella phanensis (MFLU 23-0091, **holotype**). a. Dead leaves. b–c. Apothecia on the leaf petioles. d. Close-up of an apothecium section. e. Close up of hymenium at the margin. F. Ectal excipulum cells. g. Medullary excipulum cells. Scale bars: b–c = 323 µm, d = 210 µm, e = 118 µm, f = 37 µm, g= 33 µm.

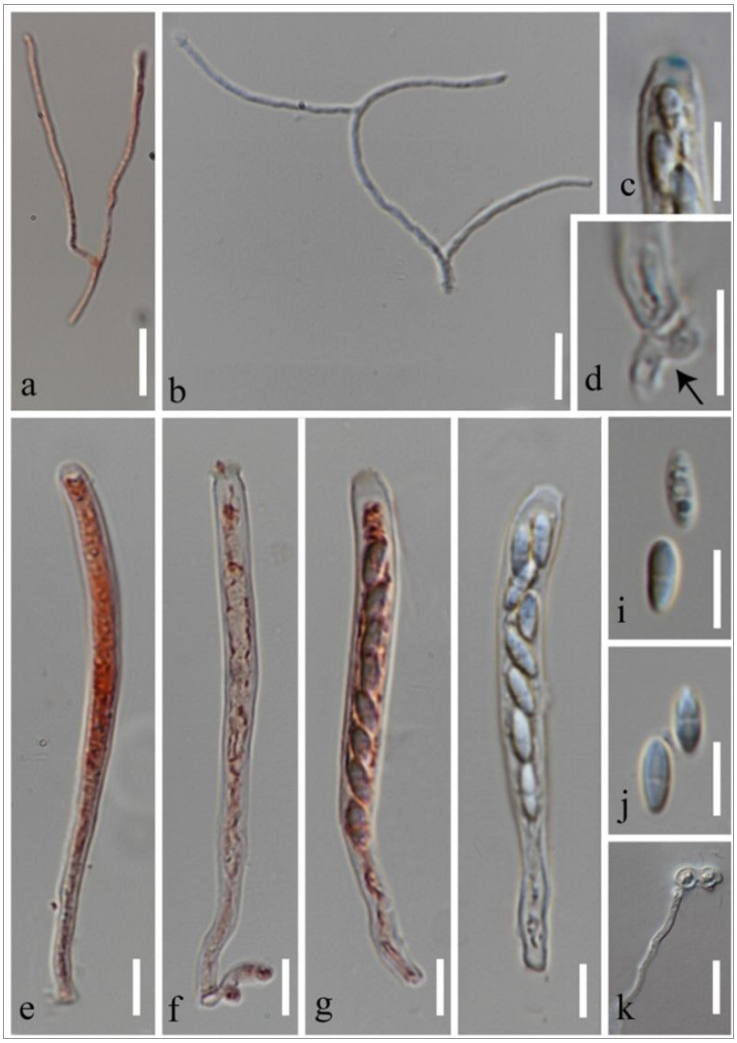

Figure 7

Lambertella phanensis (MFLU 23-0091, **holotype**). a–b. Filiform paraphyses. c. Tip of the ascus (mounted in Melzer agent without KOH treatment). d. Crozier at the ascus's base (arrow pointed). e–h. Asci (e-g. Mounted in Congo red). i–j. Ascospores. k. A germinated ascospore. Scale bars: a–b = 12 µm, c–k = 8 µm.

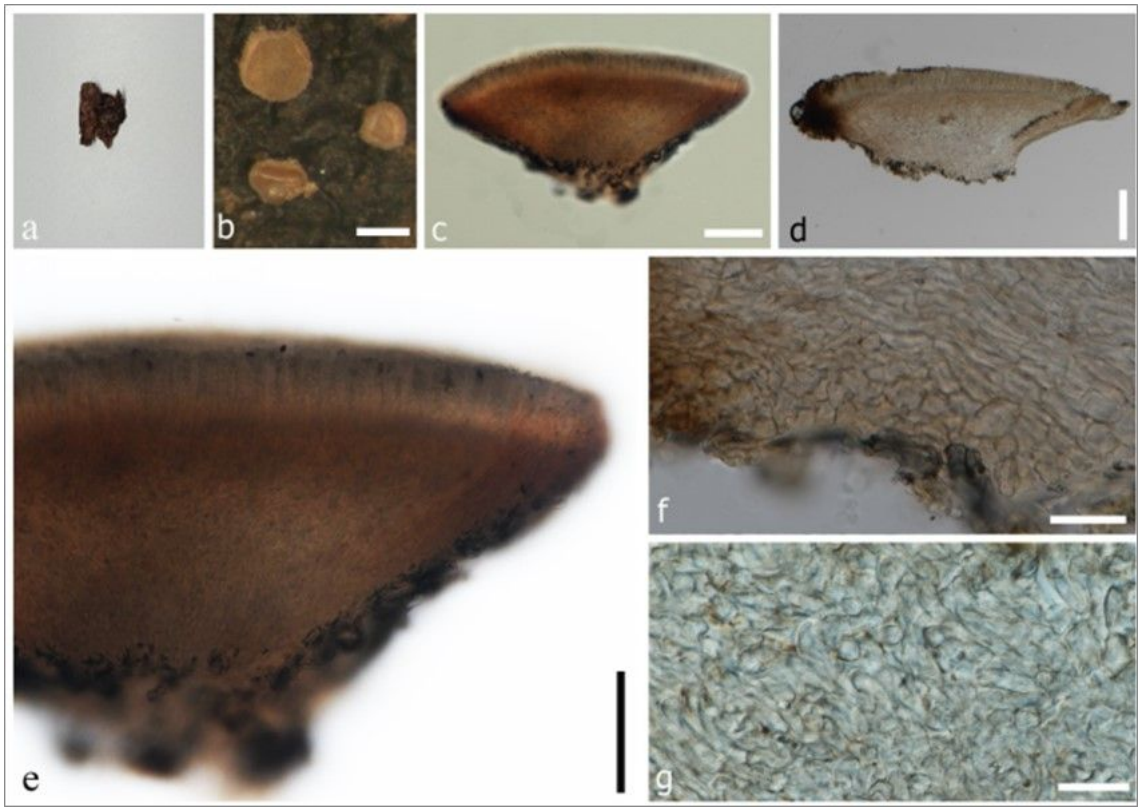

Figure 8

Lambertella sessilis (MFLU 23-0092, **holotype**). a. Wood barks. b. Apothecia on the substrate. c–d. Close-up of an apothecium section. e. Close up of hymenium at the margin. F. Ectal excipulum cells. g. Medullary excipulum cells. Scale bars: b = 500 µm, c = 180 µm, d = 130 µm, e = 96 µm, f = 40 µm, g = 25 µm.

Figure 9

Lambertella sessilis (MFLU 23-0092, **holotype**). a–d. Filiform paraphyses. e–h. Asci. i. Tip of the ascus (mounted in Melzer agent without KOH treatment). j. Crozier at the ascus's base (arrow pointed). k–m. Ascospores (k–l. mounted in Melzer agent without KOH treatment). Scale bars: a–d = 17 μm, e–j = 12 μm, k–m = 8 μm.

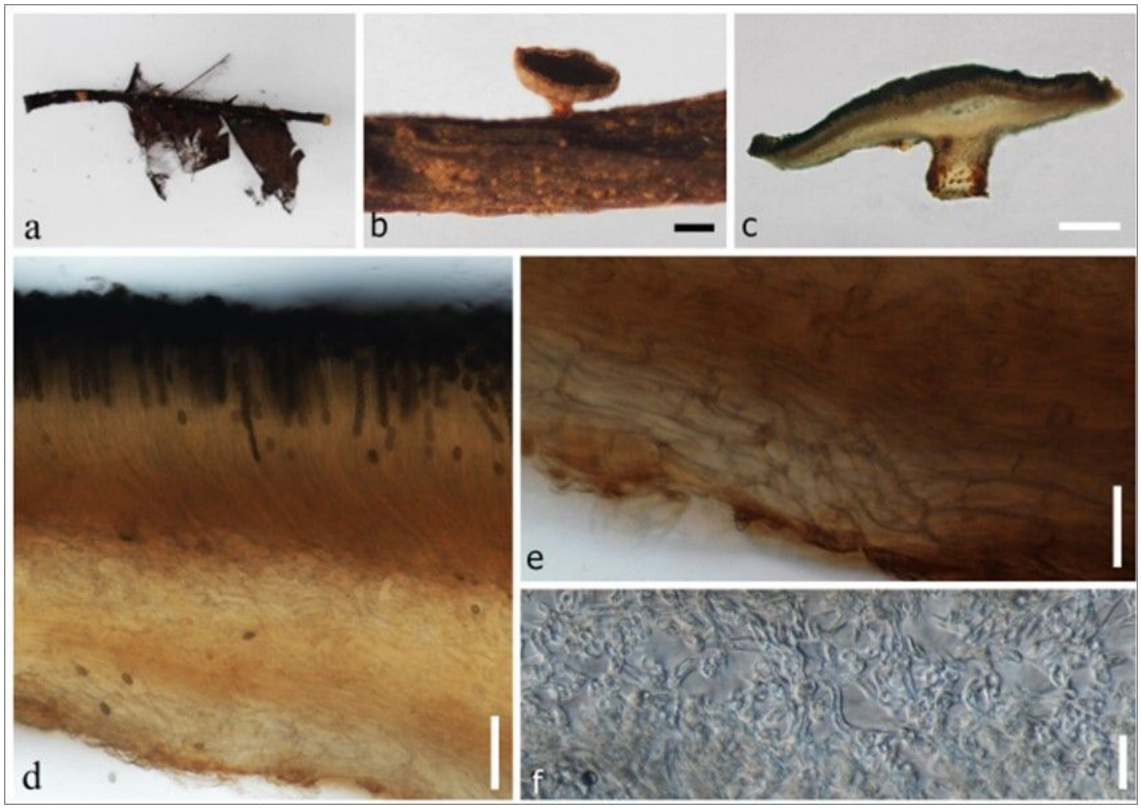

Figure 10

Lambertella takensis (MFLU 23-0089, **holotype**). a. A dead leaf. b–c. Apothecium on the leaf petiole. d. Close-up of an apothecium cross section. e. Close up of hymenium at the margin f. Ectal excipulum cells g. Medullary excipulum cells. Scale bars: b = 450 µm, c = 251 µm, d = 44 µm, e = 23 µm, f = 22 µm.

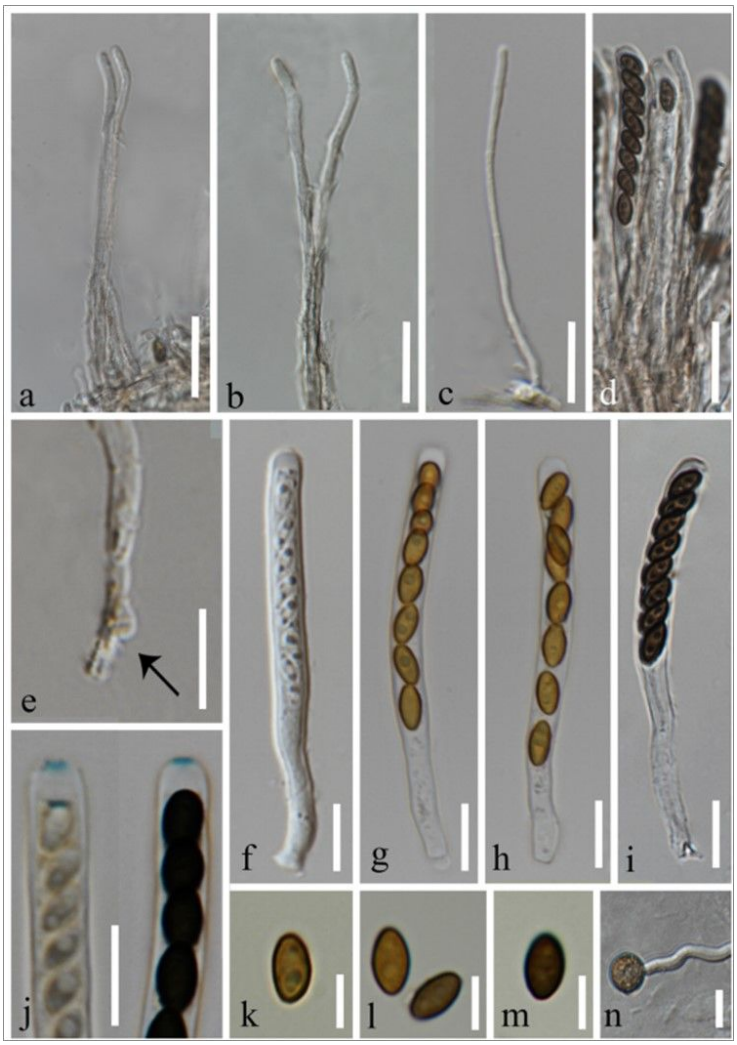

Figure 11

Lambertella takensis (MFLU 23-0089, holotype).a–c. Filiform paraphyses. d. Asci with paraphyses. e. Crozier at the ascus base (arrow pointed). f–i. Asci. j. Tip of an ascus (mounted in Melzer agent). k–m. Ascospores. n. A germinated ascospore. Scale bars: a–d = 22 µm, e = 12 µm, f–j = 9 µm, k–m = 6 µm.

Figure 12

Lambertella tectonae (MFLU 23-0087, **holotype**). a. Dead leaves. b–c. Apothecium on a leaf petiole. d. Close-up of an apothecium cross section. e. Close-up of hymenium at the margin f. Ectal excipulum cells g. Medullary excipulum cells. Scale bars: b = 850 µm, c = 640 µm, d = 480 µm, e = 113 µm, f–g = 27 µm.

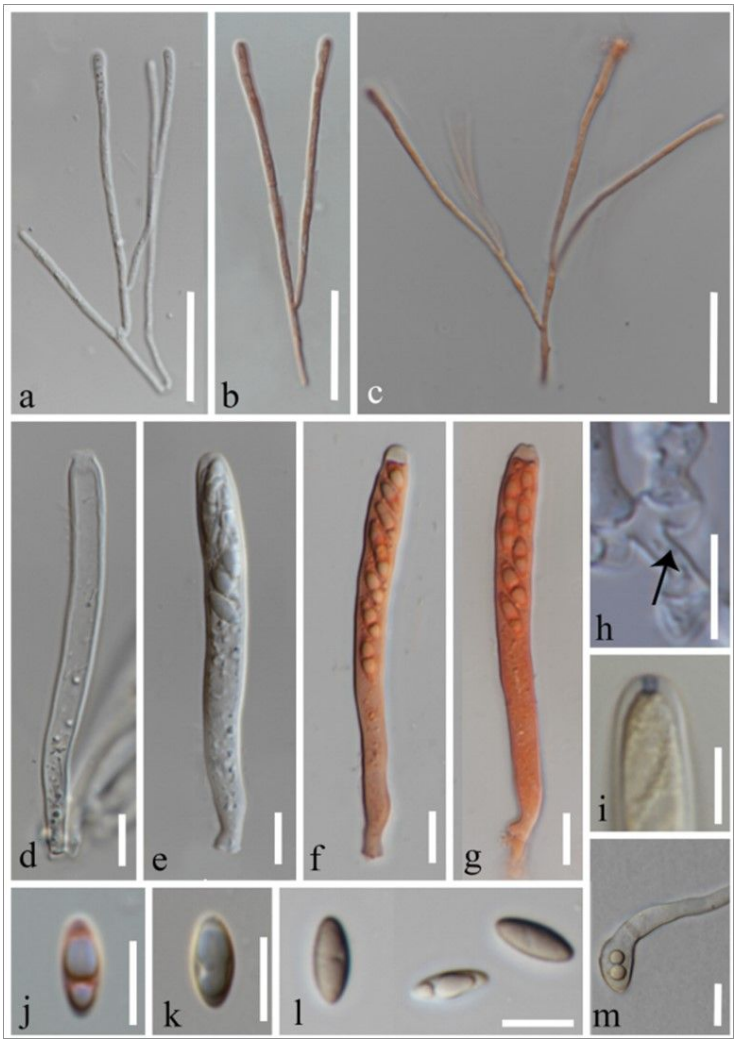

Figure 13

Lambertella tectonae (MFLU 23-0087, holotype). a–c. Filiform paraphyses. d–g. Asci (f–g. mounted in Congo red). h. Crozier at the base of an ascus (arrow pointed). i. Tip of an ascus (mounted in Melzer agent without KOH treatment).j–k. Ascospores (j. Mounted in Congo red). l. An immature hyaline ascospore and pale brown ascospores. m. A germinated ascospore. Scale bars: a–c = 26 µm, d–m = 8 µm.

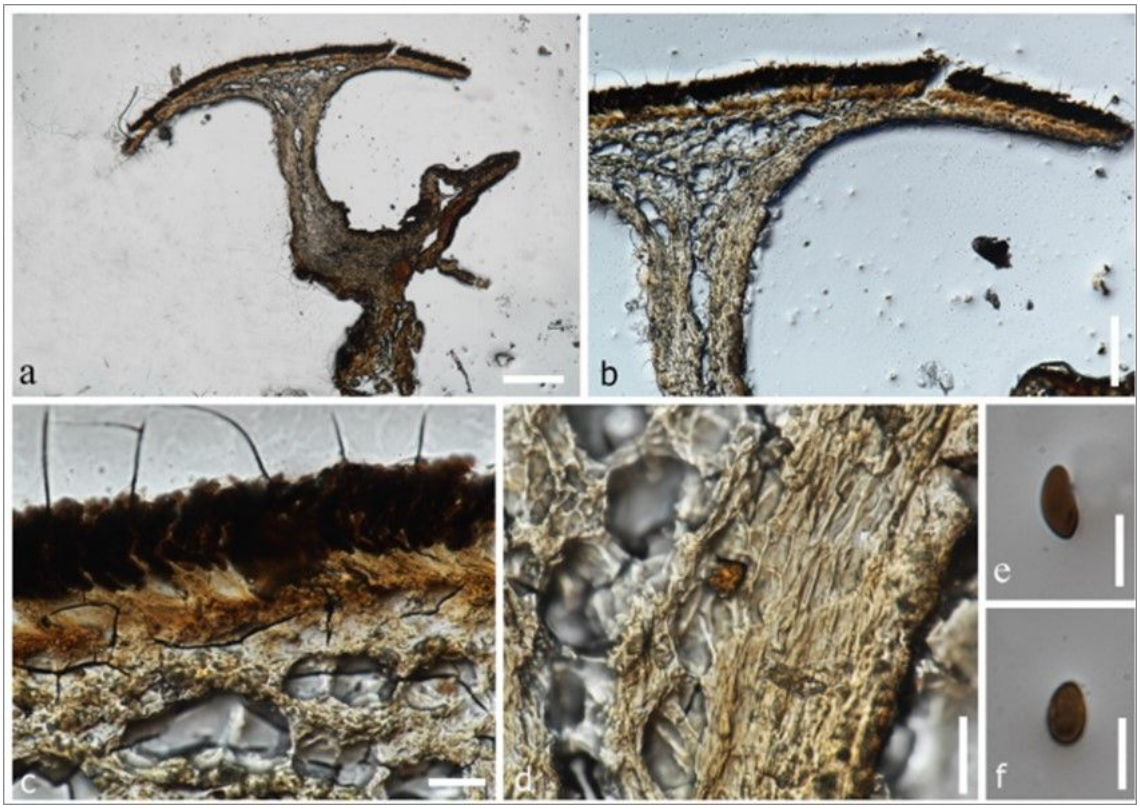

Figure 14

Lambertella himalayensis (CUP-050013,**isotype**). a–b. An apothecium section. c. A close up of hymenium. d. Ectal excipular cells. e–f. Ascospores. Scale bars: a = 330 µm, b = 170 µm, c–d = 35 µm, e–f = 16 µm.