

Doctoral Dissertation

博士論文

Systematic study of the fungal family Lachnaceae (Helotiales, Ascomycota)
(シロヒナノチャワソウ科 (菌界、子囊菌門、ビョウタケ目) の系統分類学
的研究)

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Abstract

The fungal family Lachnaceae (Helotiales, Ascomycota) now contains > 300 species distributed in 13 genera, and is known as a group of saprotrophytes forming small fruit bodies (= apothecia) on various plant substrates. Its taxonomy has been mainly based on micromorphology of apothecia. Apothecia of Lachnaceae have stipes and external hairs with granules in common. Lachnaceae is phylogenetically monophyletic at family level. On the other hand, since morphological characters are highly convergent, not all lachnaceous genera have been supported by phylogeneny. Currently, type genus *Lachnum* is not monophyletic and morphological delimitations with its related genera *Erioscyphella* and *Lachnopsis* are still uncertain. Additionally, the monophyly of *Incrucipulum* is not verified based on abundantly sampled taxa. In the present study, I attempted to delimit the four genera using molecular phylogenetic analyses, and to redefine the genera morphologically based on detailed morphological observation, using abundant specimens collected by myself from all over Japan in addition to those already housed in the TNS herbarium. Evolutional tendency in convergence of taxonomic characters of Lachnaceae were discussed. Since Japanese mycobiota of Lachnaceae has not been exhaustively searched, I also aimed to describe lachnaceous species new to Japan, in addition to new species and new combinations.

Erioscyphella has long been morphologically ill-delimited from *Lachnum*. In this study, *Erioscyphella* was morphologically redefined mainly based on shapes and the presence of amorphous materials on hairs and the length of ascospores (Chapter I). In *Erioscyphella*, I tentatively recognized species boundaries based on morphology/host plants/phylogeny. For more objective species recognition, I validated the species boundaries based on 5 methods of species delimitation analyses using fungal DNA barcode (ITS region). When a species was supported by majority of species delimitation methods (> 3 methods), I regarded it as “reasonable species”. Based on species delimitation analyses, some species were turned out to form species complex or to be synonymized under previously known species (Chapter I). In this study, species-level taxonomy based on ITS-analyses was carried out only in *Erioscyphella*, but henceforth it should be applied to other genera.

Incrucipulum was defined by thick-walled hairs and ectal excipular cells with granulated surfaces. The monophyly of *Incrucipulum* has been discussed based on very limited taxa (3 species). In this study, I collected much more specimens (11 species), and verified the monophyly of *Incrucipulum* (Chapter II).

Since *Lachnum* and its related genera (*Lachnum sensu lato*; including

Lachnopsis) has not been monophyletic, division of *Lachnum* sensu lato into multiple genera was examined based on phylogeny and/or morphology. As a result, *Lachnum* sensu lato was phylogenetically divided into 3 clades: a) *Lachnum* sensu stricto, b) *Lachnum* spp. having hairs with crystals, and c) *Lachnum* spp., *Lachnopsis* spp., and coelomycetous asexual state. I proposed b) as a new genus *Neolachnum*, while taxonomic treatment to c) was suspended because no common characters were found (Chapter II).

The evolutionary tendency of convergent taxonomic characters of Lachnaceae were discussed based on the 7-gene phylogenetic analyses. Morphological and ecological characters partially reflected phylogeny, but a number of convergences were recognized. Lachnaceae spp. was found to be diversified by flexibly changing their morphology or ecology (Chapter III).

One new genus, 12 new species and 5 new combinations were proposed. Detailed descriptions, figures, and illustrations were also provided for previously less known or undescribed taxa (Chapter IV).

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DISCLAIMER: I do not regard this doctoral dissertation constituting effective publication. No scientific names proposed in this study are to be considered published following the International Code of Nomenclature for algae, fungi, and plants (see Shenzhen Code, Art. 30.9).

List of abbreviations

AIC	Akaike's information criterion
ASAP	Assemble Species by Automatic Partitioning
ASDSF	average standard deviation of split frequencies
BI	Bayesian inference
BPP	Bayesian posterior probabilities
CB	cotton blue
CBLA	cotton blue dissolved in lactic acid
CBS	Central Bureau of Fungal Cultures
CI	consistency index
CTAB	cetyltrimethylammonium bromide
DDBJ	DNA Data Bank of Japan
DOI	digital objective identifier
EDTA	ethylenediaminetetraacetic acid
ESS	effective sample size
GBIF	Global Biodiversity Information Facility
GMYC	generalized mixed Yule-coalescent
IKI	Lugol's iodine
INSDC	International Nucleotide Sequence Database Collaboration
ITS	internal transcribed spacer region of nuclear ribosomal DNA
JC69	Jukes-Cantor
LA	lactic acid
LSU	partial large subunit nuclear ribosomal RNA gene
MAFF	Ministry of Agriculture, Forestry and Fisheries
MCMCMC	Metropolis-Coupled Markov Chains of Monte Carlo
MCM7	minichromosome maintenance complex component 7
ML	maximum likelihood
MLZ	Melzer's reagent
MP	maximum parsimony
MPBP	bootstrap values estimated by MP method
mtSSU	partial mitochondrial small subunit
NBRC	NITE National Biological Resource Center
PCR	polymerase chain reaction
PTP	Poisson Tree Processes
RC	rescaled consistency index

RI	retention index
SDS	Sodium Dodecyl Sulfate
SH	species hypothesis (pl. -es)
SSU	small subunit of nuclear ribosomal DNA
RPB1	largest subunit of the nuclear RNA polymerase II gene
RPB2	section '6–7' of the second largest subunit of the nuclear RNA polymerase II gene
TBR	tree bisection reconnection
TNS	National Museum of Nature and Science

General Introduction

Taxonomic importance of Lachnaceae

Most fungi of the order Helotiales Nannf. (Ascomycota) form minute goblet-shaped fruiting bodies (apothecia; < 2 mm in diameter) on various kinds of plant residues (Korf 1973; Spooner 1987; Baral 2016; Hosoya 2021; Quandt and Haelewaters 2021) and considered as weak saprophytes (Alexopoulos et al. 1996; Hosoya et al. 2021). In my experience, there were many chances to see infinite number of apothecia of Helotiales occurring on plant residues accumulated on the ground in northern wetlands in Japan, where the amount of plant residues originally exceeds the decomposition capacity of microorganisms (Fujita 2017). Although species of Helotiales are considered essential decomposer for such plant substrates, we can not accurately recognize their diversity, because their taxonomy has been confused due to high convergence of taxonomic characters.

Most remarkable saprophytes of Helotiales are known to belong to the family Lachnaceae Raitv., which form goblet-shaped apothecia with stipes and covered by external hairs (Raitviir 2004; Hosoya et al. 2010; Baral 2016; Hosoya 2021). Lachnaceae is known as very diverse family embracing 13 genera and > 300 species (Baral 2016; Index Fungorum 2021). Taxonomy of Lachnaceae has also been confused because of their convergent traits. However, lachnaceous fungi show abundant morphological characters and are defined as phylogenetically monophyletic family unlike other helotialean families (Raitviir 2004; Hosoya et al. 2010; Baral 2016; Ekanayaka et al. 2019; Johnston and Baschien 2020; Hosoya 2021). Therefore, by resolving superficial morphological resemblance based on molecular phylogenetic analyses, taxonomic reassessments based on morphology is realizable in Lachnaceae, and the evolutionary tendency of their convergent characters is presumable.

Historical review of familial delimitation of Lachnaceae

Lachnaceous fungi were disposed to the tribe Lachneae in the family Hyaloscyphaceae Nannfeldt (1932). Tribe Lachneae was morphologically defined focusing on shape of ectal excipulum (= mainly composed of *textura prismatica*, but in few cases *t. angularis*), lanceolate paraphyses, and multiseptate hairs by Nannfeldt (1932). Cantrell and Hanlin (1997) examined taxonomic validity of lachnaceous fungi based on single-gene molecular phylogenetic analyses, and concluded *Lachnum* Retz. and its allied genera formed an independent clade apart from other members of Hyaloscyphaceae. Cantrell and Hanlin (1997) treated the clade as “subfamily Lachnoidae” in

Hyaloscyphaceae. Raitviir (2004) was convinced by the phylogeny by Cantrell and Hanlin (1997), and founded the family Lachnaceae by raising the tribe Lachneae. Lachnaceae sensu Raitviir (2004) included both granulated- and non-granulated-haired species and both stipitate and sessile species.

Hosoya et al. (2010) discussed the delimitation of Lachnaceae based on multigene phylogenetic analyses and morphology, and revealed that most of the lachnaceous fungi with granulated hairs at least at their bases formed a strongly supported clade. They formed a separate clade from *Lasiobelonium*, *Trichopeziza* and *Trichopezizella* with non-granulated hairs and sessile apothecia (Hosoya et al. 2010; Baral 2016; Johnston and Baschien 2020). Hosoya et al. (2010) then regarded only granulate-haired genera as Lachnaceae sensu stricto and emended the familial definition. However, Hosoya et al. (2010) excluded *Capitotricha*, *Neodasyscypha* Spooner, and *Proliferodiscus* J.H. Haines & Dumont in their phylogenetic analysis. Later, I included the three genera in his phylogenetic analyses, and showed they are positioned in Lachnaceae sensu Hosoya et al. (2010) in my master's thesis (Tochihara 2019).

Ekanayaka et al. (2019) also supported the exclusion of *Lasiobelonium* (Sacc.) Sacc. & P. Syd., *Solenopezia* Sacc., *Trichopeziza* Fuckel, and *Trichopezizella* Dennis ex Raitv. from Lachnaceae based on phylogenetic analyses, and this taxonomic treatment was upheld by robust phylogenetic analyses by Johnston and Baschien (2020). Currently, 11 genera *Albotricha* Raitv., *Brunnipila* Baral, *Capitotricha* (Raitv.) Baral, *Erioscyphella* Kirschst., *Incrucipulum* Baral, *Dasyscyphella* Tranzschel, *Lachnellula* P. Karst., *Lachnopsis* Guatim., R.W. Barreto & Crous, *Lachnum* (sensu stricto), *Neodasyscypha*, and *Proliferodiscus* have been phylogenetically confirmed as members of Lachnaceae. *Beloindium* Mont. & Durieu and *Perrotia* have not included in multigene phylogenetic studies in Lachnaceae.

Based on previous studies, Lachnaceae sensu Hosoya et al. (2010), sharing granulated haired- and stipitate- apothecia, can be currently accepted as phylogenetically monophyletic and morphologically identifiable family.

Historical review of generic delimitation in Lachnaceae

In Lachnaceae, type genus *Lachnum* was described by Nannfeldt (1932) in broader sense than now, and a lot of genera have been segregated from *Lachnum* based on the morphological viewpoints, until the appearance of molecular phylogenetic analyses in the late 1990s. Shape, size, or structures of hairs, ascospores, ectal excipulum, paraphyses have been used as important genus-level taxonomic characters. However,

since such characters were highly convergent, different generic delimitations were proposed by different taxonomists (Hosoya et al. 2010). In addition to *Lachnum*, following genera have been morphologically proposed and revised in Lachnaceae (Baral 2016): *Albotricha* (Raitviir 1970), *Belonidium* (Dennis 1962; Raitviir 1987), *Brunnipila*, *Capitotricha*, and *Incrucipulum* (Baral and Krieglsteiner 1985), *Dasyscyphella* (Dennis 1972; Raitviir 1977; 2002), *Lachnellula* (Dharne 1965; Baral 2009), *Lasiobelonium* (Korf 1978; Raitviir 1978), *Neodasyscypha* (Spooner 1987; Suková 2005), *Perrotia* (Spooner 1987), *Proliferodiscus* (Haines 1983), *Trichopeziza* (Raitviir 1987), and *Trichopezizella* (Raitviir 1970; Haines 1974). In addition, the following genera were proposed mainly based on phylogenetic analyses: *Erioscyphella* Kirschst. (Perić and Baral 2014), and *Lachnopsis* (Guatimosim et al. 2016).

After the 1990s, generic boundaries have been reevaluated based on molecular phylogenetic analyses (Cantrell and Hanlin 1997; Hosoya et al. 2010; Perić and Baral 2014; Guatimosim et al. 2016; Ekanayaka et al. 2019; Johnston and Baschien 2020). Based on multigene phylogenetic analyses, Hosoya et al. (2010) revealed that *Albotricha*, *Brunnipila*, ‘long-spored *Lachnum*’ (= *Erioscyphella*), *Incrucipulum*, *Lachnellula*, and *Lachnum* (sensu stricto) formed monophyletic groups respectively. In my master’s thesis, I focused on the morphological confusion of *Albotricha* and *Capitotricha* whose hair-apices lack granules, and redefined each genus based on morphology of hair apices and ectal excipular cells (Tochihara 2019). Hosoya et al. (2010) and my master’s thesis (Tochihara 2019) also mentioned that *Dasyscyphella* was not monophyletic. Thus, delimitation of lachnaceous genera has been revised and their generic concepts have been emended in combination with molecular phylogenetic analyses and morphological reexaminations since the 2010s. However, *Erioscyphella* and *Lachnopsis* remained unexamined neither morphologically nor molecular phylogenetically in depth, and limited members have been examined in *Incrucipulum* in comparison with the range of the genus. Also, some heterogeneous species of *Lachnum* (sensu lato) have not been included in previous phylogenetic studies.

Species recognition in Lachnaceae

In Lachnaceae, species recognition has been based on the combination of morphology, host plants and phylogenetic monophyly. Recently, in some taxonomic studies in microfungi, many new species have been described based on superficial examinations of morphology and molecular phylogenetic analyses (e.g. Dothideomycetes and Sordariomycetes, referred by Koukol and Delgado (2021)), and more objective species recognition is desired. In the present study, to realize more objective species recognition

in Lachnaceae, I focused on sequences of ITS, widely accepted as fungal DNA barcode (Begerow et al. 2010; Schoch et al. 2012; Hosoya 2021), as a tool to reevaluate species boundaries recognized by morphology, host plants and phylogenetic monophyly.

Aim of this study

In the present study, I tried to refine the delimitation and generic definitions of taxonomically problematic genera based on phylogenetic analyses and morphological observations, and to carry out species-level taxonomic treatment based on more objective methods. Additionally, the evolutionary tendency of convergent characters was examined. In Lachnaceae, there may be much more high species diversity in Japan. I therefore documented new findings in Japan.

In Chapter I, *Erioscyphella* was focused on, as a suitable case of cooperation of genus-level taxonomy and species-level taxonomy using ITS-based methods. For *Erioscyphella*, morphological definition from *Lachnum* sensu stricto was carried out. In species-level taxonomy, ITS-based species delimitation analyses were tested to revalidate the species boundaries, because in *Erioscyphella* ITS sequences of 10 species among 11 known species were available.

In Chapter II, phylogenetic and morphological delimitation of *Lachnum* and morphologically related genera (including *Lachnopsis*) and the monophyly of *Incrucipulum* was discussed based on wider range of taxon sampling.

In Chapter III, the tendency of evolution of convergent morphological and ecological characters in Lachnaceae were discussed based on selected members based on 7-gene phylogenetic analyses.

In Chapter IV, species new to Japan were described with figures and line drawings in addition to new species and new combinations proposed in the previous chapters.

In this study, neither *Belolnidium* (Dennis 1962; Raitviir 1987) nor *Perrotia* (Dennis 1981; Spooner 1987) were included, because no specimens are currently available. I also could not discuss the monophyly of *Neodasyscypha* and *Proliferodiscus*, because only one species was available for each genus, and no sequences or specimens of *P. inspersus* (Berk. & M.A. Curtis) J.H. Haines & Dumont [type of *Proliferodiscus*] were available.

Chapter I: Generic delimitation and species boundaries in *Erioscyphella*

Introduction

The genus *Erioscyphella* includes 11 species: *E. abnormis* (Mont.) Baral, Šandová & B. Perić [lectotype of *Erioscyphella* (Haines and Dumont 1984); as '*E. longispora* (P. Karst.) Kirschst.' in the original description (Kirschstein 1938)], *E. alba* Ekanayaka & K.D. Hyde, *E. aseptata* Ekanayaka & K.D. Hyde, *E. bambusina* (Bres.) Kirschst., *E. brasiliensis* (Mont.) Baral, Šandová & B. Perić, *E. curvispora* B. Perić & Baral, *E. euterpes* (S.A. Cantrell & J.H. Haines) Guatim., R.W. Barreto & Crous, *E. fusiformis* (Ekanayaka & K.D. Hyde) Ekanayaka & K.D. Hyde, *E. lunata* (W.Y. Zhuang & Spooner) B. Perić & Baral, *E. lushanensis* (W.Y. Zhuang & Zheng Wang) Guatim., R.W. Barreto & Crous, and *E. sclerotii* (A.L. Sm.) Baral, Šandová & B. Perić. (Index Fungorum 2021).

Most members of *Erioscyphella* were disposed in *Lachnum* and called 'long-spored *Lachnum*' characterized by longer ascospores and the occurrence in tropical areas (Dennis 1954; Haines 1980; Haines and Dumont 1984; Spooner 1987). By molecular phylogenetic analyses, long-spored *Lachnum* was suggested as a monophyletic group apart from *Lachnum* sensu stricto by Cantrell and Hanlin (1997), Hosoya et al. (2010), Perić and Baral (2014), and Guatimosim et al. (2016). Hence, Perić and Baral (2014) segregated long-spored *Lachnum* from *Lachnum*, and assigned an obsolete name *Erioscyphella* (Kirschstein 1938) to the genus.

Erioscyphella was originally proposed by Kirschstein (1938) embracing *E. longispora* [= currently *E. abnormis*; lectotype of *Erioscyphella*] and *E. bambusina*. In the original description by Kirschstein (1938), *Erioscyphella* was defined based on morphology of filiform, colored, and pigmented ascospores and lanceolate paraphyses, but later such features were criticized as taxonomically uninformative (Korf 1978; Perić and Baral 2014). Haines and Dumont (1984) mentioned morphology of *Erioscyphella* (= referred as 'long-spored *Lachnum*') was consecutive with that of *Lachnum* sensu stricto in particular ascospore length and shape of ectal excipular cells. Thus, *Erioscyphella* has been distinguished from *Lachnum* sensu stricto phylogenetically, but Perić and Baral (2014) did not present clear morphological delimitation from *Lachnum* sensu stricto.

To discuss generic boundaries of *Erioscyphella*, I used specimens from TNS (Tsukuba, Japan) as most of them were accompanied by cultures and/or DNA extracts. In TNS, only three identified species of *Erioscyphella* were recognized (*E. abnormis*, *E. brasiliensis*, and *E. sclerotii*); however, since I have found a lot of candidates of

Erioscyphella in TNS specimens (including specimens collected by myself), I presumed that much more unidentified species of *Erioscyphella* were housed therein.

For more objective species-level taxonomy, ITS-based species delimitation analyses were tested to evaluate species boundaries recognized by morphology, ecology, and phylogenetic relationships. To evaluate ITS-based species boundaries more objectively, multiple methods of species delimitation analyses were carried out and clusters supported by majority of the methods were accepted as “reasonable species boundaries”.

Materials and Methods

Taxon sampling

In TNS, specimens labeled as *Erioscyphella* were initially searched, and closely related specimens to *Erioscyphella* were searched based on the sequence similarities of ITS. Specimens were tentatively identified based on morphology following Dennis (1954), Haines (1980), Haines and Dumont (1984), Spooner (1987), and Perić and Baral (2014).

Morphological observation

External appearance of apothecia was examined using SZ61 stereoscopic microscope (Olympus, Tokyo, Japan) and microphotographs were taken using DS-L4 (Nikon, Tokyo, Japan). To examine microscopic characters, dried apothecia were detached from the substrate and rinsed in 70% ethanol followed by rehydration in deionized water for six hours, and then observed in CBLA (0.5 g CB and 99.5 mL LA) using a BX51 microscope equipped with a Nomarski interference contrast device (Olympus). Microphotographs were taken using DS-L3 with the camera head DS-Fi2 (Nikon). Line drawings were prepared using drawing device U-DA (Olympus) equipped with BX51. To check the ascal apex iodine reaction, MLZ (0.5 g I₂, 1.5 g KI, 20 g chloral hydrate, and 20 g water) was initially used without KOH pretreatment, and IKI (1 g I₂ and 1 g KI, and 100 mL H₂O) and MLZ with 3% KOH pretreatment were used when necessary.

DNA extraction and sequencing

When isolates were available, 2 mL of 2% malt extract broth was inoculated with isolates and cultivated at room temperature for two weeks. The cultivated mycelia were frozen at -80 °C for two hours, dried by a Bulk Tray Dryer (Labconco, Kansas, USA) and then crushed using TissueLyser (Quiagen, Hilden, Germany). Powdered fungal samples were incubated in a CTAB buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA) at 65 °C for one hour, and protein was removed using the mixture

of chloroform/isoamylalcohol (24:1). The solution was purified using 6M sodium iodine buffer (6 M NaI, 50 mM Tris-HCl pH 7.4, 10 mM EDTA, 0.1 M Na₂SO₃) (Hosaka and Castellano 2008) with GLASSMILK (Funakoshi, Tokyo, Japan) and washed using ethanol/buffer solution (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 100 mM NaCl, 50% EtOH). Purified DNA was eluted and dissolved in Tris-EDTA buffer (TE buffer; 10 mM Tris-HCl, 1 mM EDTA).

When isolates were not available, DNA was extracted directly from a fresh apothecium using a DNA extraction buffer (SDS 0.35 mM, Proteinase K 0.1mg/ml, Tris-HCl 10 mM, MgCl₂ 1.5 mM, KCl 50 mM, dH₂O to adjust to total volume 50 mL). An apothecium was put in a 1.5 mL Eppendorf tube and smashed manually using 1.5 mL pellet pestle (Fisher Scientific, Hampton, USA). 50 µl DNA extraction buffer was added and incubated for 90 min. at 40 °C followed by 10 min. incubation at 90 °C. After centrifuged, the supernatant was preserved as extracted DNA.

The obtained isolates were deposited in NBRC, except for isolates with restriction on transition by Japanese laws and those unavailable because of contracts with private companies. Extracted DNA samples were deposited to Molecular Biodiversity Research Center in the National Museum of Nature and Science (Tsukuba, Ibaraki, Japan) and available for DNA-based works.

PCR was used to amplify the following regions: ITS (= ITS1-5.8S-ITS2), LSU, mtSSU, and RPB2. Primer pairs for PCR reactions of ITS, LSU and mtSSU were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) or ITS1 (5'-TCCGTAGGTGAACCTCGGG-3') (White et al. 1990) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), LR0R (5'-ACCCGCTGAACCTTAAGC-3') and LR5 (5'-TCCTGAGGGAACTTCG-3') (Vilgalys and Hester 1990), and mrSSU1 (5'-AGCAGTGAGGAATATTGGTC-3') and mrSSU3R (5'-ATGTGGCACGTCTATAGCCC-3') (Zoller et al. 1999) respectively. The PCR program consisted of an initial denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 35 s, 51 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. When appropriate PCR products were not obtained, a modified PCR program was applied first, and then alternative primer pairs were tested. For RPB2, an alternative forward primer fRPB2-5F (5'-GAYGAYMGWGATCAYTTYGG-3') (Liu et al. 1999) or RPB2-P6Fa (5'-TGGGGRYTK GTBTGYCCKGCHGA-3') (Hansen et al. 2005) and a reverse primer bRPB2-7.1R2 (5'-CCCATNGCYTGYTTVCCCATDGC-3') (modified from bRPB2-7.1R) (Matheny 2005; Matheny et al. 2007; Gelardi et al. 2015) were used.

Sequencing was conducted on an ABI PRISM 3500xL Genetic Analyzer

(Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) with a BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems). The obtained sequences were assembled using ATGC 7 (Genetyx, Tokyo, Japan). Assembled ITS sequences were deposited in the UNITE database (<https://unite.ut.ee/>) via the PlutoF workbench (<https://plutof.ut.ee/>) (Abarenkov et al. 2010). Assembled LSU, mtSSU, and RPB2 sequences were deposited in INSDC via DDBJ.

Phylogenetic analyses

The specimens obtained from TNS were included in the phylogenetic analyses as candidate members of *Erioscyphella* (‘†’ in Table 1). From other genera of the family Lachnaceae, four species of *Lachnum*, two species of *Albotricha*, *Brunnipila*, *Capitotricha*, *Dasyscyphella*, *Incrucipulum*, and *Lachnellula*, and one species of *Neodasyscypha* and *Proliferodiscus* were used (‘†’ in Table 1). Among the eight genera, seven of them (except *Proliferodiscus*) included type species. Three species of Helotiales were selected as outgroups following Hosoya et al. (2010) (Table 1).

A concatenated dataset of ITS, LSU, mtSSU, and RPB2 was used in the phylogenetic analyses. Each region was aligned separately using MAFFT 7 (Katoh and Standley 2013). The Q-INS-i option was used for ITS, LSU, and mtSSU to accommodate the secondary structures of RNA, and the G-INS-1 option was used for RPB2 to assume global alignment using the entire region. The aligned sequences were edited manually using BioEdit 7.0.5.2 (Hall 1999). For ITS, LSU, and mtSSU, all insertions/deletions were deleted.

Phylogenetic conflicts among gene partitions were checked before the phylogenetic analyses using the concatenated matrix. ML trees with 1,000 bootstrap replications (Felsenstein 1985) using the ITS, LSU, mtSSU, and RPB2 datasets separately were constructed using MEGA X (Kumar et al. 2018) with the GTR+G model; branches with bootstrap values > 70% were compared among trees. For mtSSU and RPB2, specimens containing missing data were excluded from the analyses.

The concatenated dataset was analyzed using ML, MP, and BI. For the ML and BI analyses, substitution models were estimated for each partition (ITS, LSU, mtSSU, and each codon position of RPB2) based on AIC (Akaike 1974) using Modeltest-NG 0.1.6 (Darriba et al. 2019).

ML tree search (Felsenstein 1984) and bootstrapping (Felsenstein 1985; Lemoine et al. 2018) was performed using RAXML-NG 0.9.0 (Kozlov et al. 2019) with 1,000 bootstrap replications under the substitution model SYM+I+G4 for ITS, TIM1+I+G4 for LSU, TPM1uf+I+G4 for mtSSU and RPB2 third codon position,

GTR+I+G4 for RPB2 first codon position, and TPM3uf+I+G4 for RPB2 second codon position.

MP analysis was conducted using PAUP* 4.0a 167 (Swofford 2002). All substitutions were treated as unordered and of equal weights. All gaps were treated as missing data. A heuristic parsimony search was carried out with 1,000 replicates of random step addition, with a TBR branch swapping algorithm, Multrees option on, Steepest descent modification option on, and branch collapse option set to MinBrLen. MPBP (Felsenstein 1985) were estimated from 1,000 replicates of heuristic searches, with random taxon addition, TBR branch swapping, and Multrees options off.

BI analysis was based on MrBayes 3.2.7a (Ronquist 2012) under the substitution model SYM+I+G4 for ITS, GTR+I+G4 for LSU and RPB2 first codon positions, HKY+I+G4 for mtSSU and RPB2 third codon positions, and F81+I for RPB2 second codon position. Two separate MCMCMC ran simultaneously starting from random trees for 20 million generations, and trees were sampled every 500 generations. ASDSF and ESS were checked using Tracer 1.7.1 (Rambaut 2018a) as an indication of convergence. Using post-burn-in trees, a 50% majority rule consensus tree was generated, and BPP were calculated to evaluate node supports.

Trees were visualized using FigTree 1.4.4 (Rambaut 2018b) based on the ML, MP, and BI analyses respectively. Branches with MLBP and MPBP > 90% and BPP > 0.95 were regarded as strongly supported.

ITS-based species delimitation analyses (Fig. 1)

To maximize the number of ITS sequences, I used the UNITE SH system provided by the UNITE database (Kõljalg et al. 2013; Nilsson et al. 2015; GBIF 2018; Kõljalg et al. 2020). In the UNITE SH system, all fungal ITS sequences are periodically divided into species-level clusters (= SH) at optional sequence-distance thresholds (0%–3% in 0.5% steps), each of which is assigned to a unique UNITE SH code represented by a DOI accessible from internet (Nilsson et al. 2015; Kõljalg et al. 2016; 2020).

Based on the UNITE SH system, I collected ITS sequences of *Erioscyphella* in the following process: a) selectivity of closely related sequences: for every ITS sequence newly obtained from TNS specimens (= query sequences, 49 sequences), UNITE SH code at the 3% threshold value were searched in the UNITE database to gather sequences in wider scope, and all sequences within the UNITE SH code were downloaded. b) selectivity based on taxon names: using the UNITE search page, ITS sequences named *Erioscyphella* were searched, because only closely related sequences to query sequences are filtered under the a) criterion. Sequences with synonyms of

Erioscyphella species were also searched, because the UNITE lookup function is not supported by any backbone taxonomies to integrate synonyms. Sequences satisfying criterion a) or b) were downloaded for ITS-based species recognition.

Species delimitation analyses were carried out based on an OTU clustering method, hierarchical clustering method, and two phylogeny-based methods using the selected ITS sequences. In advance of species delimitation analyses, ITS1, 5.8S, and ITS2 regions were extracted using ITSx (Nilsson et al. 2010) to construct an accurate ITS dataset, because the inclusion of segments of adjacent regions (such as small subunit of 18S rRNA or LSU) may decrease the accuracy of the calculation of ITS distances (Nilsson et al. 2010).

As OTU clustering method, classification in the UNITE SH system was checked at 3% and 1.5% threshold values. However, in UNITE SH system, not any UNITE SH code was assigned to a sequence MG434782 (*Erioscyphella lushanensis*). I therefore additionally tested an alternative OTU-clustering method VSEARCH, in which is based on the same algorithm to the UNITE SH system, to check which SHs MG434782 belong to. An alternative OTU clustering analysis was executed using VSEARCH v2.17.2 (Rognes et al. 2016) implemented in the Qiime 2 microbiome analysis platform (Bolyen et al. 2019). The concatenated dataset of extracted ITS1, 5.8S, and ITS2 was incorporated into VSEARCH, and OTU clustering at 3% and 1.5% sequence-thresholds were performed using the ‘-cluster_fast’ option.

Hierarchical clustering based on pairwise sequence distances was executed using ASAP method (Puillandre et al. 2021). The datasets of extracted ITS1, 5.8S, and ITS2 were separately aligned using MAFFT 7 under the Q-INS-i option and edited using trimAl v1.2 (Capella-Gutiérrez et al. 2009) under the ‘-gappyout’ option. The concatenated dataset of the three aligned partitions was analyzed using ASAP web (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>). JC69 was selected as a substitution model for computing pairwise distances of sequences.

As phylogeny-based species delimitation methods, the GMYC model (Pons et al. 2006; Fujisawa and Barraclough 2013) and the PTP model (Zhang et al. 2013) were used. In both models, speciation (species-level differentiation) and coalescence (population-level differentiation) are identified based on the length of phylogenetic trees. GMYC requires the use of phylogenetic trees following the molecular clock model (= ultrametric tree) because it detects transition points from speciation to coalescence focusing on the time axis, while PTP does not require ultrametric tree as it focuses on the number of nucleotide substitutions. Ultrametric trees were estimated using BEAST v2.6.3. (Bouckaert et al. 2019). The ITS dataset was divided into ITS1,

5.8S, and ITS2, and suitable substitution models GTR+G for ITS1 and JC+G for 5.8S and ITS2 estimated using Modeltest-NG 0.1.6. were applied. To estimate branch length, a Yule model and a relaxed clock with a log-normal distribution were selected. MCMC chains were run for 1.5×10^8 generations and sampled every 1,000 generations. After each run, convergence was checked using Tracer 1.7.1, and the first 10% were discarded as burn-in. A consensus tree was generated using TreeAnnotator v1.10.4 in BEAST package, from 150,000 generated trees except for the first 10% regarded as burn-in. A single-threshold species delimitation analysis based on GMYC was conducted using the R package 'splits' (Fujisawa and Barraclough 2013).

For the species delimitation analyses using PTP, an unrooted ML phylogenetic tree was constructed using RAxML-NG 0.9.0. The analysis used ITS1, 5.8S, and ITS2 partitions, aligned as previously described, under the substitution models TIM2+G4 for ITS1, TPM2+I+G4 for 5.8S, and GTR+I+G4 for ITS2, estimated using Modeltest-NG 0.1.6. based on the AIC. The species delimitation analysis was executed using the generated ML best-scored tree with the bPTP web server (<https://species.h-its.org/>). The MCMC run was set to 500,000 generations and burn-in rate was set to 0.1. The convergence of MCMC runs were visually checked. In ML and Bayesian results, a result generating fewer SHs were adopted to avoid excessive species division.

Species recognition

I initially delimit species based on traditional methods. Namely, morphological and/or ecological features, with the result of phylogenetic analyses. In this chapter, species boundaries were cross-checked based on the results of ITS-based species delimitation analyses. When the species boundaries were supported by the majority (= more than three methods) of the five species delimitation methods (UNITE SH at 3% threshold, UNITE SH at 1.5% threshold, ASAP, GMYC, and PTP) , I regarded the species as acceptable and carried out taxonomic treatments if necessary.

Results

Gathered specimens from TNS

Forty-nine specimens in TNS were picked up as candidates of *Erioscyphella* and morphologically identified as *E. abnormis*, *E. brasiliensis*, *E. sclerotii*, *Lachnum hainanense* W.Y. Zhuang & Zheng Wang, *L. mapirianum* (Pat. & Gaillard) M.P. Sharma, *L. mapirianum* var. *sinense* Z.H. Yu, W.Y. Zhuang, *L. novoguineense* var. *yunnanicum* W.Y. Zhuang, and *L. palmae* (Kanouse) Spooner (Table 1), together with six undescribed species candidates of *Erioscyphella*, and their detailed description are

provided in Chapter IV in the present paper ([*E. boninensis*, *E. insulae*, *E. otanii*, *E. papillaris*, *E. paralushanensis*, and *E. sasibrevispora*], Table 1). *Lachnum mapirianum* var. *sinense* and *Lachnum novoguineense* var. *yunnanicum* are newly described from Japan (Chapter IV).

Phylogenetic analyses

The molecular phylogenetic analyses were based on 70 specimens selected from TNS (Table 1). The concatenated sequence matrix was composed of 2488 bp (sites 1–332 for ITS, 333–1108 for LSU, 1109–1828 for mtSSU, and 1829–2488 for RPB2). In the matrix, the following parts were treated as missing data: TNS-F-17245, 17249, and 81229 for mtSSU, and TNS-F-17567 for RPB2.

Among the four ML trees based on each region, no conflicts were found in clades with > 70% support (Appendix 1). Therefore, I considered these four regions to be combinable, and phylogenetic analyses were based on the concatenated sequence matrix. In the MP analysis, 766 nucleotide substitution sites were detected, 601 of which were parsimony informative. A total of 182,630 equally parsimonious trees were generated with 2,985 steps, CI = 0.38, RI = 0.73, and RC = 0.28. In the BI analysis, when two runs reached 20 million generations and the first 10,000 trees (25%) of generated trees were excluded, ASDSF was observed to fall below 0.004 and ESS of all parameters was over 200. The first 10,000 trees were discarded as burn-in. A 50% majority rule consensus tree was constructed and BPP was calculated based on the remaining 30,000 trees.

As no topological contradictions occurred among the ML best-scored tree, MP strict consensus tree, and BI 50% majority-rule consensus tree, only ML tree was illustrated, and MLBS, MPBS, and BPP were plotted on its branches (Fig. 2).

Based on the phylogenetic analyses, 49 candidates of *Erioscyphella* formed a strongly supported clade (= Clade A, MLBP = 100%/MPBP = 100%/BPP = 1.00), apart from the clade of *Lachnum* sensu stricto (= *L. asiaticum* (Y. Otani) Raitv., *L. pudibundum* (Quél.) J. Schröt., *L. rachidicola* J.G. Han, Raitv. & H.D. Shin, and *L. virgineum* (Batsch) P. Karst.) [type of *Lachnum*] (Fig. 1). Clade A and *Proliferodiscus alboviridis* formed a relatively strongly supported clade (Clade B, MLBP = 78%, MPBP = 82%, BPP = 1.00).

Within Clade A, each morphologically identified species and variety formed strongly supported monophyletic groups of their own (Fig. 2), and five strongly supported subclades were recognized (Clades I–V, Fig. 2). *Lachnum mapirianum* (TNS-F-17545, 17249) and *E. insulae* (TNS-F-26500, 39720) did not belong to any subclade. Clade I was

composed of *E. boninensis*, *E. paralushanensis*, *L. hainanense*, and *L. mapirianum* var. *sinense*. Within Clade I, only *E. paralushanensis* occurred on bamboo sheaths, while others occurred on fallen leaves of broad-leaved trees. Clade II was composed only of *L. palmae*, which occurred on the palm petioles. Clade III was composed of *E. otanii* and *E. papillaris* occurring on bamboo leaves. Clade IV was composed of *L. novoguineense* var. *yunnanicum*, and *E. sasibrevispora*, occurring on bamboo sheaths. Clade V was composed of *E. abnormis*, *E. brasiliensis*, and *E. sclerotii*, occurring on wood.

Morphological characters within Clade A

Members of Clade A had totally and densely granulated, hyaline to brown, thin-walled hairs, fusiform to long filiform ascospores, ectal excipulum composed of *textura prismatica* to *textura angularis*, asci lacking croziers at the bases, and smooth walled ectal excipulum cells. Exceptionally, *E. sasibrevispora*, *L. hainanense* (Hosoya et al. 2013), and *L. novoguineense* var. *yunnanicum* had croziers and *E. boninensis* had granulated ectal excipulum.

Moreover, hairs of Clade A lacked crystals, but equipped with apical amorphous materials and/or resinous materials. In the present study, “crystals” refers to amber colored materials that positioned near the hair apices and regular-shaped (e.g. tetrahedral materials, masses of needle-like materials, or cross-shaped materials), described by Raitviir (2002) and Suková (2005). “Resinous materials” refers to colored, refractive, irregular-shaped materials attached on any parts of hairs, described by Spooner (1987). Crystals and resinous materials are easily detached from hairs and broken into fragments in the squash mount. “Apical amorphous materials” is termed uniquely in this study, and refers to hyaline to brown, refractive, irregular-shaped materials positioned outside the hair apices. They are usually small and inconspicuous cap-like shaped, and conspicuously globular in some species. Apical amorphous materials do not grow to big masses and are not easily detached from hairs in the squash mount.

In Clade A, members except for *E. boninensis*, *E. sasibrevispora* and *L. novoguineense* var. *yunnanicum* had apical amorphous materials, and *E. boninensis*, *E. paralushanensis*, and *L. palmae* complex also had resinous materials.

ITS-based species delimitation analyses

In UNITE v8.3, 86 ITS sequences (excluding MG434782) were clustered into 23 SHs at 3% and 26 SHs at 1.5% threshold values (Table 2, Fig. 3). The UNITE SH code for each SH is presented in Table 2. In an alternative OTU clustering method (VSEARCH), 87

ITS sequences were clustered into 25 SHs at 3% threshold and 28 SHs at 1.5% threshold (Table 2, Appendix 3). VSEARCH SH codes (allocated in this study uniquely; VSH97_1 to VSH97_25, VSH985_1 to VSH985_28) are shown in Table 2. In VSEARCH clustering at 3% and 1.5% thresholds, a sequence MG434782 (*E. lushanensis*) formed a cluster together with two sequences of *E. lushanensis* (AF505515, JF937582) (Fig. 3, Table 2). Hereafter, the results of VSEARCH at 3% and 1.5% thresholds were adopted as OTU clustering results of *E. lushanensis* (AF505515, JF937582, MG434782) instead of UNITE SH at 3% and 1.5% thresholds.

The extracted and aligned ITS sequences were composed of three partitions, ITS1 (162 bp), 5.8S (157 bp), and ITS2 (142 bp). In the ASAP analysis, the concatenated dataset of these partitions (461 bp) was input, and 87 ITS sequences were clustered into 18 SHs with the lowest asap-score, reflecting better partitioning (Appendix 4). In the GMYC analysis, 29 SHs were delimited (Appendix 5). For the PTP analyses, PTP analyses delimited 23 SHs in the Bayesian support and 26 SHs in the ML support (Appendix 6), and the former was adopted.

Comparing the number of SHs generated by different clustering methods and applied thresholds, 18 SHs by ASAP, and 23 SHs by UNITE SH at 3% threshold represented the lowest SH numbers (Fig. 3; Table 2). The ASAP results were too rough to delimit the boundaries of *E. abnormis*, *E. boninensis*, *E. brasiliensis*, *E. curvispora*, and *E. sclerotii*.

Comparing the results of five species delimitation methods (UNITE SH at 3% threshold, UNITE SH at 1.5% threshold, ASAP, GMYC, and PTP), sequences labeled as *E. alba*, *E. brasiliensis*, *E. curvispora*, *E. euterpes*, *E. fusiformis*, *E. lunata*, *E. sclerotii*, *L. mapirianum*, *L. mapirianum* var. *sinense*, *L. novoguineense* var. *yunnanica*, and six new species candidates were distinguished as separate clusters by majority (≥ 3) of species delimitation methods (Fig. 3). These species clusters did not contradict with morphological/ecological or phylogenetic relationships (Fig. 2). Seven sequences labeled as *L. hainanense* were clustered into one SH by four species delimitation analyses, and part of the SHs included a sequence labeled as *Lachnum albidulum* (Fig. 3).

Three species (*Erioscyphella abnormis*, *E. aseptata*, and *L. palmae*) did not form separate clusters supported by majority of four species delimitation analyses (Fig. 3). Sequences labeled as *E. abnormis* were clustered into one to four SHs, and some SHs included sequences labeled as *Chapsa patens* (Nyl.) Frisch, *E. aseptata*, *E. brasiliensis*, and *E. sclerotii* (Fig. 3). Twelve sequences labeled as *L. palmae* were clustered into four to six SHs (Fig. 3).

Discussion

Generic delimitation and generic concept of *Erioscyphella*

I accepted Clade A as a monophyletic unit for *Erioscyphella* supported by morphology. Although Clade B comprised Clade A together with *P. alboviridis*, Clade B should not be regarded as a genus delimitation of *Erioscyphella*, because *Proliferodiscus* differs from members of Clade A in having apothecia proliferating from the margins continuously and thick-walled and coarsely warted hairs (Haines and Dumont 1983; Spooner 1987).

All members of Clade A are distinguishable from the other lachnaceous genera. In contrast to *Erioscyphella*, *Albotricha* and *Dasyscyphella* are distinguished by hair apices with no granulation (Hosoya et al. 2010), *Brunnipila*, *Capitotricha*, and *Incrucipulum* by hair-crystals (Baral and Krieglsteiner 1985), and *Lachnellula* by ectal excipulum composed of *textura globose* to *textura oblita* (Dharne 1965). Typical members of Clade A can be easily segregated from *Neodasyscypha*, because the characteristic features of *Neodasyscypha*, such as dark-brown hairs, ectal-excipulum structure, and ellipsoid to fusoid ascospores < 10 µm long (Spooner 1987) are rare in Clade A. Among members of Clade A and *Lachnum* sensu stricto, the shape and length of ascospores were continuous (Fig. 4), as indicated by Haines and Dumont (1984). However, ascospores longer than 15–20 µm were restricted to Clade A (Fig. 4). Moreover, most members of Clade A have hairs with apical amorphous, which are not seen in *Lachnum* sensu stricto. Members of Clade A usually also have hairs not swelling at the apices and distantly septate, as Perić and Baral (2014) pointed out for three tropical members, while members of *Lachnum* have swelling apices. The combination of such characters allows me to differentiate typical members of *Erioscyphella* from *Lachnum*.

In summary, *Erioscyphella* is still difficult to define solely based on morphology because of multiple exceptional characters continuous to other genera, but its typical members could be recognizable mainly by the hair structures and ascospore length. Based on members of Clade A, *Erioscyphella* is tentatively described as follows: apothecia occurring on dead hardwood leaves, rotten wood, bamboo sheaths, bamboo leaves or palm leaves; asci mostly arising from simple septa, but occasionally from croziers; ascospores fusiform to long needle-shaped, aseptate to multi-septate; paraphyses filiform to narrowly lanceolate, not exceeding the asci long, but rarely lanceolate and long exceeding the asci; hairs straight or irregularly curved, usually not swollen at the apices, thin-walled, hyaline, but sometimes brown, totally and densely granulated, usually distantly septate, without needle-like or three-dimensional shaped crystals but mostly

equipped with hyaline to brown apical amorphous materials, and/or resinous materials at any part of hairs; walls of ectal excipulum cells smooth but granulated in one species.

Perić and Baral (2014) pointed out that “yellow hymenium derived from carotenoid” is one of the common characters of *Erioscyphella*. This feature was not discussed in this study because some specimens were not observed when fresh; the hymenium color is variable (usually white hymenium becomes yellow) between fresh and dried states in lachnaceous species.

Host selectivity of *Erioscyphella*

In *Erioscyphella*, the tendency of selectivity of species to host plants or parts occurs across the genus. Each subclade within *Erioscyphella* (Clade I–V) generally shared tendencies toward host selectivity as follows: Clade I on leaves of broad-leaved trees, except for *E. paralushanensis* occurring on bamboo sheaths, Clade II on palm leaves, Clade III on bamboo leaves, Clade IV on bamboo sheaths, and Clade V on rotten wood (Fig. 2). The results showed that selectivity to host plants and parts of *Erioscyphella* were acquired as apomorphic characters during speciation.

Is *Erioscyphella* limited to ‘tropical’ zones?

Erioscyphella (long-spored *Lachnum*) has long been known as the tropical genus in Lachnaceae (Dennis 1954; Spooner 1987; Guatimosim et al. 2016). Most of long-spored species were described from tropical areas of Latin America (Dennis 1954) and tropical to temperate areas of Australasia (Spooner 1987). However, the new species or new combinations proposed in this study were reported from Japan in subtropical areas (*E. boninensis* and *E. insulae*), temperate area (*E. hainanensis*, *E. palalushanensis*, and *E. sinensis*) and cool-temperate to subarctic areas (*E. otanii*, *E. papillaris*, and *E. sasibrevispora*), showing that *Erioscyphella* is not limited to tropical zones, but is also distributed in temperate to subarctic zones in the northern hemisphere.

Species-level taxonomic treatment of *Erioscyphella*

In this study, I carried out taxonomic treatment for species which were distinguished by morphology/ecology and phylogenetic analyses and formed single clusters in species delimitation analyses. Based on this criteria, six undescribed species of *Erioscyphella* have been proposed as new species of *Erioscyphella* [*E. boninensis*, *E. insulae*, *E. otanii*, *E. papillaris*, *E. paralushanensis*, and *E. sasibrevispora*], and *Lachnum hainanense* and *L. mapirianum* var. *sinense* have been proposed as new members of *Erioscyphella*. Interpretation of species boundaries of *L. hainanense* was discussed in

the taxonomy chapter. For new species and new combinations, Japanese names were also denominated for wider use of Japanese mycologists or amateurs.

In the phylogenetic analyses, Malaysian materials of *L. mapirianum* (TNS-F-17245, 17249) and Japanese materials of *L. novoguineense* var. *yunnanicum* (TNS-F-16442, 16642) were also found to be members of *Erioscyphella* (Fig. 2). However, I hesitate to transfer the two species into *Erioscyphella*, as I cannot guarantee the identification accuracy of the materials, because of inadequate type information of the two species.

Taxonomic assessments of *E. abnormis*, *L. aseptata*, and *L. palmae*, which were not accepted as independent species in species delimitation analyses, are discussed below.

Taxonomy of *E. abnormis* and its related species

In the species delimitation analyses, sequences labeled as *E. abnormis* formed a single SH at UNITE SH 3% threshold (DOI: SH1155612.08FU) and divided into two to four SHs at UNITE SH 1.5% threshold, and GMYC (Fig. 3).

In ASAP, sequences labeled as *E. abnormis* belong to a single SH, but the SH also contained sequences labeled as *Chapsa patens*, *E. aseptata*, *E. brasiliensis*, *E. curvispora*, and *E. sclerotii* (Fig. 3). However, the phylogenetic analyses revealed that *E. brasiliensis*, and *E. sclerotii* are separate from the clade of *E. abnormis* (Fig. 2), suggesting that the two species are different from *E. abnormis*. Although *E. curvispora* was not included in the phylogenetic analyses (Fig. 2), the apparent morphological and ecological differentiation (Perić and Baral 2014) and low similarity of ITS (< 97%) with members of *E. abnormis* (Fig. 3) suggest that *E. curvispora* is different from *E. abnormis*.

Erioscyphella aseptata was originally described in Thailand and characterized by having aseptate ascospores, unlike *E. abnormis* or *E. sclerotii* with septate ascospores (Ekanayaka et al. 2019). However, the species delimitation analyses in this study suggested the difficulty of delimiting *E. aseptata* (MK584957) from *E. abnormis* (Fig. 3), suggesting that *E. aseptata* is a morphologically atypical (aseptate-ascospored) individual of *E. abnormis*.

Although two ITS sequences of *C. patens* (MT995055 = specimen no. FJ19131 and MW007918 = specimen no. FJ19049) were positioned in SHs dominated by *E. abnormis*, LSU and mtSSU sequences of FJ19131 and LSU sequence of FJ19049 were closely related to *Chapsa* spp. [Graphidaceae, Ostropales]. Since Lachnaceae and Graphidaceae are phylogenetically distant, the two ITS sequences MT995055 and

MW007918 have been misidentified.

Considering that the monophyly of *E. abnormis* is strongly supported (Fig. 2) and members of the species share high ITS similarities (> 97%, compiled into SH1155612.08FU) (Fig. 3, Table 2), *E. abnormis* is accepted here as a species with some intraspecific morphological and phylogenetic variation.

Taxonomy of '*Lachnum*' *palmae*

Lachnum palmae formed a strongly supported clade in the phylogenetic analyses (Clade II in Fig. 2). They also shared strong selectivity to palm leaves and characteristic morphology such as thick-walled asci, hairs with resinous materials and apical amorphous materials (Appendix 2) and ectal excipulum composed of thick-walled prismatic cells and interwoven hyphae. However, sequences labeled as *L. palmae* were divided into 4 to 7 SHs in all species delimitation analyses (Fig. 3), indicating that *L. palmae* is a species complex that includes multiple potential sister species. At present, I avoid creating new species from the complex, because the morphological and ecological differences detected among SHs are not enough to delimit species boundaries, although the size of asci and ascospores considerably differ among some SHs, as shown in Fig. 4.

Phylogenetic analyses revealed that members of the *L. palmae* complex belonged to *Erioscyphella* (Fig. 2). However, as I could not judge which SH within the complex is equivalent to *L. palmae* as originally described from Honduras by Kanouse (1941) and redescribed by Spooner (1987) from the type plus another specimen from New Zealand. There are no *L. palmae* sequences from the tropical American type locality, so phylogenetic characterization and recombination of the species was avoided in the present study.

Chapter II: Generic reassessment of *Lachnum sensu lato* and *Incrucipulum*

Introduction

In Chapter I, *Erioscyphella* was refined as a phylogenetically monophyletic genus with substantial morphological support. However, morphological and phylogenetic delimitation of other members of *Lachnum sensu lato* (= species morphologically resembling *Lachnum sensu stricto*; including *Lachnopsis*) and monophyly of *Incrucipulum* remain problematic as discussed in detail below.

Based on the molecular phylogenetic analysis, Hosoya et al. (2010) elucidated that *Lachnum*, the largest genus in Lachnaceae, was not monophyletic, but contains four major elements as follows: 1) monophyletic group including the type species *L. virgineum* (= *Lachnum sensu stricto*), 2) long-spored *Lachnum* (= *Erioscyphella*, redefined in Chapter I), 3) *Lachnum* clade with no certain morphology in common, and 4) *Lachnum varians* (Rehm) Spooner, whose phylogenetic position was uncertain.

The third clade consisted of *L. rhytismatis* and undescribed species of *Lachnum* (= *Lachnum* sp. 14 in Hosoya et al. (2010)), but in my master's thesis (Tochihara 2019) I revealed the clade was falsely generated based on a cross-contaminated sequence (RPB2 sequence of *Lachnum* sp. 14 (AB481364) from unknown origin in Hosoya et al. (2010)). As a result of phylogenetic analyses using proper sequence instead of AB481364, the clade was not formed (Tochihara 2019).

Based on his morphological reexamination, I concluded that “*L. rhytismatis*” was misidentified *Capitotricha fagicola* (Yas. Ono & Hosoya) Tochihara & Hosoya, and “*Lachnum* sp. 14” was a non-lachnaceous member having smooth hairs (Tochihara 2019). The proper taxonomic position of *L. rhytismatis* is still uncertain.

Generic position of *L. varians* was discussed by Guatimosim et al. (2016). Guatimosim et al. (2016) found *Lachnum* spp. forming apothecia on tropical tree fern were phylogenetically distinct from *Lachnum sensu stricto*, and founded the new genus *Lachnopsis*. *Lachnopsis* consists of *Ls. catarinensis* Guatimosim, R.W. Barreto & Crous [type species] and *Ls. dicksoniae* Guatimosim, R.W. Barreto & Crous, and *Lm. varians* and *Lm. pteridophyllum* (Rodway) Spooner were suggested as possible additional members of *Lachnopsis* (Guatimosim et al. 2016). However, *Lachnopsis* was morphologically not distinct from *Lachnum sensu stricto*, because the four species do not have common morphological features in shape and size of ascospores (containing both short-fusiform ones and long-filiform ones) and hair-structures (containing both species with resinous materials and lacking any materials) (Guatimosim et al. 2016).

In addition to above, phylogenetic consideration on *Lachnum nipponicum* J.H.

Haines & S. Kaneko has not been carried out. The fungus is known as forming both sexual and asexual fruit bodies on dead leaves of host plants (Haines and Kaneko 1984). The asexual state of *L. nipponicum* is a coelomycetes forming tetrapod-shaped conidia (= formerly identified as *Eriosporella calami* Niessl ex Höhn. based on morphology) (Haines and Kaneko 1984). Since asexual state is very rare in Lachnaceae (only seen in *Lachnellula* (Oguchi 1981; Baral 2009) and *L. nipponicum*) and tetrapod-shaped conidia is unique to *L. nipponicum* in Lachnaceae (Haines and Kaneko 1984; Baral 2016), generic position of *L. nipponicum* should be phylogenetically discussed.

In this way, *Lachnum* sensu lato is still thought as heterogeneous. In this chapter, I tried to taxonomically reexamine *Lachnum* sensu lato based on phylogeny and/or morphology using abundant taxon sampling. Many specimens of *Lachnum* sensu lato were gathered from TNS, including correctly identified specimens of *L. nipponicum*, *L. rhytismatis*, and *L. varians*. Since two coelomycete species *Crucellisporium umtamvunae* Marinc., Gryzenh. & M.J. Wingf. and *C. marquesiae* Crous, which form tetrapod-shaped conidia collected in Africa, were suggested as members of Lachnaceae based on molecular phylogenetic analyses (Marincowitz et al. 2010; Crous et al. 2014), sequences of the two species were also incorporated in these analyses.

Incrucipulum was founded by Baral (Baral and Krieglsteiner 1985) focusing on “thick-walled (0.5–1.3 μm), cubic ectal excipular cells with densely granulated surface” (Baral and Krieglsteiner 1985). Thin to thick-walled hairs with crystals and thick-walled apices were also mentioned as common characters (Baral and Krieglsteiner 1985). The granulation of ectal excipulum was originally noted by Le Gal (1939) for *I. ciliare* and *I. capitatum* Baral and has been known as a unique character to *Incrucipulum*. *Incrucipulum capitatum*, *I. ciliare* [type species], *I. sulphurellum* Baral and *I. virtembergense* (Matheis) Baral were originally included (Baral and Krieglsteiner 1985), and six species were later added to the genus: *I. densiseptatum* (Raitv. & R. Galán) Raitv., *I. longispineum* (Hosoya & Issh. Tanaka) Sasagawa & Hosoya, *I. radiatum* Sasagawa & Hosoya, *I. saccardoi* (Raitv. & Sacconi) Raitv., *I. sinegoricum* (Raitv.) Raitv. and *I. uralense* Chlebicki & Suková (Baral and Krieglsteiner 1985; Raitviir 2006; Index Fungorum 2021).

Incrucipulum was suggested as a monophyletic genus by Hosoya et al. (2010) based on molecular phylogenetic analyses based on *I. ciliare*, *I. longispineum* and *I. radiatum*. However, all the three species have narrowly filiform paraphyses, though *Incrucipulum* also contains members with widely lanceolate paraphyses such as *I. sulphurellum*. Therefore, monophyly of the genus was not still inspected based on

abundant taxon sampling. In this chapter, the monophyly of the genus was checked by phylogenetic analyses based on wider range of taxon sampling mainly collected by me from all over Japan, including both members with filiform paraphyses and members with lanceolate ones.

Material and methods

Taxon sampling

Collection, isolation, morphological observations, and DNA work followed the methods mentioned in Chapter I. To examine the monophyly of *Incruciulum*, specimens of *Incrucipulum* were gathered from TNS focusing on scientific names. Additionally, I searched unrecorded species of *Incrucipulum* in Japan by visiting habitats of their host plants, because members of *Incrucipulum* have strong host selectivity (Hosoya et al. 2010). Specimens were morphologically identified following Raitviir (1977), Baral and Krieglsteiner (1985), Suková (2005), Raitviir (2006), and Hosoya et al. (2010).

Twelve species of *Lachnum* (sensu lato) were included in the phylogenetic analyses (Table 3). Among them, correctly identified specimen of *L. rhytismatis*, morphologically similar species to *L. rhytismatis* (= *Lachnum* sp. 1, TNS-F-81438), *L. varians*, and *L. nipponicum* were included. Isolates of *L. nipponicum* was purchased from MAFF in Japan, and those of *Crucellisporium umtamvunae* and *Crucellisporiopsis marquesiae* were purchased from CBS in Netherlands. ITS and LSU sequences of *Lachnopsis* were downloaded from the INSDC database. From other lachnaceous genera, more than two specimens from each genus of *Albotricha*, *Brunnipila*, *Capitotricha*, *Dasyscyphella*, *Erioscyphella*, *Incrucipulum*, *Lachnellula*, and *Lachnum* were incorporated. For *Neodasyscypha* and *Proliferodiscus*, only one species was included due to the availability. All genera except for *Proliferodiscus* included type species. Four species of Helotiales were selected as outgroup.

Phylogenetic analyses

Methods of molecular phylogenetic analyses followed those of Chapter I. For known species of *Lachnopsis*, only ITS and LSU sequences were accessible on the INSDC database. To discuss generic delimitation of *Lachnum* sensu lato and *Incrucipulum* based on robust phylogenetic trees, phylogenetic analyses based on four DNA regions (ITS, LSU, mtSSU, and RPB2) were initially tested, excluding the *Lachnopsis* sequences. Later phylogenetic analyses based on ITS+LSU (= ITS+LSU dataset) were carried out including the *Lachnopsis* sequences to elucidate the phylogenetic delimitation of *Lachnopsis*. Comparing phylogenetic trees based on the two datasets, the

generic delimitation was discussed. The two datasets were constructed and analyzed as bellow (Table 3).

ITS+LSU+mtSSU+RPB2 dataset:

This dataset was composed of 56 specimens (46 species including outgroup; Table 3). Each region was aligned separately. The Q-INS-i option was used for ITS, LSU, and mtSSU, and the G-INS-1 option was used for RPB2. The aligned sequences were edited manually using BioEdit.

After checking that there are no topological contradictions among partitions, the concatenated dataset was analyzed using ML, MP, and BI analyses following the programs mentioned in Chapter I. For ML analyses, substitution models SYM+I+G4 for ITS, TIM1+I+G4 for LSU, TIM3+I+G4 for mtSSU and RPB2 second codon position, GTR+I+G4 for RPB2 first codon position, TrNef+I+G4 for RPB2 third codon position were used. For BI analysis, substitution models SYM+I+G4 for ITS, GTR+I+G4 for LSU, mtSSU and RPB2 first codon positions, HKY+I+G4 for RPB2 second and third codon positions were used. MCMCMC ran for 1.5 million generations, sampling every 500 generations. After convergence and burn-in, a 50% majority rule consensus tree was generated.

ITS+LSU dataset:

This dataset was composed of reduced members (43 specimens; 38 species including outgroup) containing two species of *Lachnopsis* (Table 3). Based on the result of ITS+LSU+mtSSU+RPB2 phylogenetic analyses, some members of *Lachnum* sensu stricto and *Incrucipulum* were excluded from this analysis (Table 3), because they are considered not necessary to the generic boundaries of *Lachnopsis*. The Q-INS-i option was used for each partition. The aligned sequences were edited manually using BioEdit.

After checking that no topological contradictions among partitions occurred using MEGA X, the concatenated dataset was analyzed using ML, MP, and BI analyses following the programs mentioned in Chapter I. Both ML and BI analyses were carried out under the substitution model SYM+I+G4 for ITS and TIM1+I+G4 for LSU. MCMCMC ran for 1.5 million generations, sampling every 500 generations. After convergence and burn-in, a 50% majority rule consensus tree was generated.

Results

Gathered members of *Incrucipulum*

As a result of collecting and survey in the present study, three new records in Japan (= *I. capitatum*, *I. sulphurellum*, and *I. virtembergense*) and five undescribed species (= *I. aurantiacum*, *I. brevistipitatum*, *I. foliicola*, *I. hakonechloae-macrae*, and *I.*

pseudosulphurellum, see Chapter IV) were found. In total, 11 species of *Incrucipulum* were collected (Table 3). Five species of these had lanceolate paraphyses: *I. brevistipitatum*, *I. capitatum*, *I. foliicola*, *I. pseudosulphurellum*, and *I. sulphurellum*.

Phylogenetic analyses

For both datasets, no topological conflicts were found in clades with > 70% support among ML tree based on each region (Appendix 7, 8). Therefore, I considered these four regions to be combinable, and phylogenetic analyses were based on the concatenated sequence matrix.

ITS+LSU+mtSSU+RPB2 dataset:

The concatenated sequence matrix was composed of 2682 bp (sites 1–472 for ITS, 473–1325 for LSU, 1326–1997 for mtSSU, and 1998–2682 for RPB2). In the ML analysis, a best-scored tree was yielded with loglikelihood –21134.140982. In the MP analysis, a most parsimonious tree was generated with scores as follow: parsimony informative sites = 628, tree length = 3912, CI = 0.32, RI = 0.60, RC = 0.19. In the BI analysis, A 50% majority rule consensus tree was constructed and BPP was calculated based on the post-burn-in trees. As no topological contradictions occurred among the ML best-scored tree, MP 50% majority-rule consensus tree, and BI 50% majority-rule consensus tree, only ML tree was illustrated, and MLBS, MPBS, and BPP were plotted on its branches (Fig. 5).

For *Albotricha*, *Brunnipila*, *Capitotricha*, *Erioscyphella* and *Lachnellula*, members of each genus formed strongly supported clades of their own, and specimens of *Neodasyscypha* and *Proliferodiscus* belonged to no clades of other genera. Five members of *Dasyscyphella* did not form a strongly supported clade of their own, similarly to the result of Hosoya et al. (2010) and my master's thesis (Tochihara 2019).

While eight species of *Lachnum* including *L. virgineum* formed a strongly supported clade (= *Lachnum sensu stricto*, in Fig. 5), two species of *Lachnum* (*L. rhytismatis* and *Lachnum* sp. 1) formed a strongly supported clade (= Clade C, in Fig. 5) apart from *Lachnum sensu stricto*. *Lachnum nipponicum* and *L. varians* formed strongly supported clade (= Clade D) with *Crucellisporium umtamvunae* and *Crucellisporiopsis marquesiae* apart from *Lachnum sensu stricto* and any other genus-level clades.

All 18 specimens of 11 species morphologically identified as *Incrucipulum* formed strongly supported clade (= Clade *Incrucipulum*, in Fig. 5). Within *Incrucipulum*, a strongly supported clade “Clade I-A” including 6 species was found also supported by filiform paraphyses, which is regarded as a synapomorphic character. Other members of *Incrucipulum* shared lanceolate paraphyses (Fig. 6).

ITS+LSU dataset:

The concatenated sequence matrix was composed of 1323 bp (sites 1–494 for ITS, 495–1323 for LSU). In the ML analysis, a best-scored tree was yielded with loglikelihood –7002.932545. A most parsimonious tree was generated with the following scores: parsimony informative sites = 209, tree length = 1037, CI = 0.44, RI = 0.62, RC = 0.27. In the BI analysis, A 50% majority rule consensus tree was constructed and BPP was calculated based on the post-burn-in trees. As no topological contradictions occurred among the ML best-scored tree, MP 50% majority-rule consensus tree, and BI 50% majority-rule consensus tree, only ML tree was illustrated, and MLBS, MPBS, and BPP were plotted on its branches (Fig. 7).

Two species of *Lachnopsis* formed strongly supported clade together with all members of Clade D in Fig. 5 (= Clade D' in Fig. 7). Other strongly supported clades of the ITS+LSU tree (Fig. 7) did not contradict with those of the ITS+LSU+mtSSU+RPB2 tree (Fig. 5).

Morphology of Clade C (Fig. 5)

Clade C and *Lachnum* sensu stricto are not distinguishable based on size or shape of ascospores, asci, and paraphyses (= lanceolate throughout), and host plants and parts (= not common within each clade). However, two members of Clade C have hairs with crystals at the apices. Since the presence of crystals is rare in *Lachnum* sensu stricto (= only seen in *L. nudipes*), the two clades are generally distinguishable focusing on the presence/absence of hair crystals.

Morphology and ecology of Clade D (Fig. 5) and D' (Fig. 7)

Based on the phylogenetic analyses (Fig. 5, 7), Clade D and D' were apart from *Lachnum* sensu stricto and any other genera. Since Clade D' in Fig. 7 is corresponding to Clade D in Fig. 5 and contains more members, the delimitation of Clade D was discussed based on Clade D' in the following part.

Clade D' was composed of two known species of *Lachnopsis* (*Ls. catarinensis* and *Ls. dicksoniae*), and *Lm. nipponicum*, *Lm. varians*, *Crucellisporium umtamvunae*, and *Crucellisporiopsis marquesiae*. Apothecia (= sexual states) of *Ls. catarinensis*, *Ls. dicksoniae*, *Lm. nipponicum*, and *Lm. varians* had filiform to narrowly lanceolate (= not conspicuous lanceolate) paraphyses, and hairs without crystals and not swollen at their apices (Haines and Kaneko 1984; Spooner 1987; Guatimosim et al. 2016). However, common morphological features were not found among apothecia of Clade D', and Clade D' are not still distinguishable from *Lachnum* sensu stricto in morphological

aspects. For instance, while *Ls. catarinensis* and *Lm. nipponicum* had long (> 20 µm), multiseptated, fusiform ascospores, *Ls. dicksoniae* and *Lm. varians* had much shorter, aseptate ascospores. The presence or absence of apical amorphous materials observed in *Erioscyphella* were not seen in *Lm. nipponicum* and *Lm. varians*.

On the other hand, *Lachnum nipponicum*, *Crucellisporium umtamvunae* (sexual state not found), and *Crucellisporiopsis marquesiae* (sexual state not found) had coelomycetous asexual states which form tetra- to polyradiate conidia. The presence of coelomycetous asexual states producing tetra- to polyradiate conidia is unique to these three species in Lachnaceae.

Within Clade D', two known species of *Lachnopsis* and a potential member (*Ls. catarinensis*, *Ls. dicksoniae*, and *Lm. varians*) form apothecia on dead fern petioles, and other members form apothecia and/or asexual states on other types of plant residues. Fernicolous members are characteristic to Clade D', because in Lachnaceae fernicolous species are limited to members of Clade D' and *L. pteridophyllum*, which is a potential species of *Lachnopsis* (Spooner 1987; Guatimosim et al. 2016) and not analyzed in this study.

Discussion

Monophyly of *Incrucipulum*

With the addition of five new species, *Incrucipulum* now contains 15 species. *Incrucipulum capitatum*, *I. sulphurellum*, and *I. virtembergense* were newly collected from Japan. Based on the multigene phylogenetic analyses (Fig. 5), *Incrucipulum* was revealed as a monophyletic group containing both lanceolate and filiform paraphyses. Thus, *Incrucipulum* is distinguishable based on the combination of granulated ectal excipulum and thick-walled hairs with crystals.

Within *Incrucipulum*, filiform paraphyses are presumed to be synapomorphic character because filiform paraphyses were shared only in Clade IA (Fig. 5). However, since most of the species of Helotiales have filiform paraphyses and lanceolate paraphyses, which are seen in many genera of Lachnaceae (see Table 4), are mostly limited to Lachnaceae (Korf 1973; Baral 2016; Hosoya 2021), members of Clade IA could possibly show a morphological throwback in paraphysis shape, indicating the strong convergence of morphological characters.

All members of *Incrucipulum* have been reported from north of the temperate zone of the northern hemisphere (Dennis 1949; Otani 1967; Tanaka and Hosoya 2001; Raitviir 2006; GBIF 2021; this study). Additionally, *Incrucipulum foliicola*, *I. pseudosulphurellum* and *I. sulphurellum* have strong host selectivity to *Myrica gale*

subsp. *tomentosa*, endemic plants to the subarctic zone in the Far East (GBIF 2021), and *I. virtembergense* has strong host selectivity to *Vaccinium uliginosum* (Matheis 1977; Baral and Krieglsteiner 1985; this study), mainly distributed in the subarctic to arctic zones (GBIF 2021). Therefore, species of *Incrucipulum* are considered to have been diversified in north of the temperate zone of the northern hemisphere by flexibly using plants in cold zones as host, in contrast to species of *Erioscyphella*, mainly diversified in tropical zones.

Taxonomic treatment of Clade C

Species of Clade C had hairs with crystals in common and this was a distinguishing character from most of *Lachnum* sensu stricto, although exceptional species (*L. nudipes*) is still included. Because molecular phylogenetic analyses evidently showed Clade C was a different at genus-level from *Lachnum* sensu stricto, I propose a new genus (*Neolachnum*) for Clade C. The proposal of new genus is accompanied with a proposal of a new combination. Taxonomic treatment is presented in the Chapter IV.

Taxonomic treatment of Clade D and D'

Based on the phylogenetic analyses (Figs. 5, 7), while the monophyly of Clade D and D' were strongly supported, phylogenetic relationships within Clade D and D' were not revealed, partly because mtSSU and RPB2 sequences of *Ls. catarinensis* and *Ls. dicksoniae* were unavailable. So I can not judge that Clade D and D' should phylogenetically be treated as a genus, or divided into multiple genera. Since common morphological or ecological features are also not found in Clade D, I withhold any taxonomic treatments for Clade D at this moment. However, so far some members of Clade D' have unique characters in Lachnaceae, such as apothecia on dead tree-fern petioles and/or the presence of coelomeycete asexual state forming tetradiate conidia. By collecting much more specimens, carrying out multigene phylogenetic analyses and more detailed morphological and ecological examinations, genus-level taxonomy of Clade D and D' could possibly be realized. Henceforth, focused taxon sampling should be carried out mainly in tropical and subtropical zones, where tree ferns are mainly distributed (GBIF 2021) and potential members of Clade D could possibly be highly diversified.

Chapter III: Convergence of taxonomic characters within Lachnaceae

Introduction

Based on previous studies and Chapter I and II, *Albotricha*, *Brunnipila*, *Capitotricha*, *Erioscyphella*, *Incrucipulum*, *Lachnellula*, *Lachnum* sensu stricto, and *Neolachnum* were now defined as monophyletic. A number of convergent characters were recognized through the analyses (Hosoya et al. 2010; Tochihara 2019; Chapters I and II in this study, see Table 4). It was also recognized that *Dasyscyphella* and members of Clade D (Chapter II) could not be treated as genera supported by both molecular phylogeny and morphological/ecological characters (Table 4).

In this chapter, I aimed to recognize evolutionary tendency of convergent characters of Lachnaceae based on the phylogenetic relationships. To recognize the process of acquiring/losing characters, constructing robust phylogenetic trees is desirable. Since four-gene phylogenetic analyses in Chapters I and II did not reveal intergeneric relationships at high resolution (Figs. 2, 5), I therefore tested seven-gene phylogenetic analyses to construct more robust phylogenetic trees based on representative members.

Materials and methods

Taxon sampling

Taxon sampling were conducted to thoroughly cover lachnaceous genus-level clusters based on Chapters I and II and previous studies. Members of *Albotricha*, *Brunnipila*, *Capitotricha*, *Erioscyphella*, *Lachnellula*, *Lachnum* sensu stricto, *Neodasyscypha*, *Neolachnum*, *Proliferodiscus*, 5 species of *Dasyscyphella*, members of Clade D recognized in Fig. 5 and Fig. 7 were included (Table 5). *Hymenoscyphus varicosporoides*, *Trichopeziza mollissima* Fuckel, and *Trichopezizella otanii* J.H.Haines were selected as outgroup (Table 5).

PCR, phylogenetic analyses

ITS, LSU, SSU, RPB1, RPB2, MCM7, and mtSSU were used for phylogenetic analyses. For ITS, LSU, RPB2, primer pairs ITS1F and ITS4, LR0R and LR5, and RPB2-P6F and RPB2-P7R were used respectively. For SSU, a forward primer NS1 (5'–GTAGTCATATAGCTTGTCTC–3') (White et al. 1990) and a reverse primer NS4 (5'–CTTCCGTC AATTCCTTTAAG–3') (White et al. 1990) or NS41 (5'–CCCGTGTTGAGTCAAATTA–3') (Hibbett 1996) were used. For RPB1, forward primers gRPB1-A (5'–GAKTGTCCCKGGWCATTTTGG–3') (Matheny et al. 2002) or

RPB1-Af (5'–GARTGYCCDGGDCAYTTYGG–3') (modified from Stiller and Hall (1997) by Giho Sung in the Hall lab), and reverse primers fRPB1-C (5'–CCNGCDATNTCRTRTCCATRTA–3') (Matheny et al. 2002) or RPB1-Cr (5'–CNGCDATNTCRTRTCCATRTA–3') (modified from fRPB1-Cr by Giho Sung in the Hall lab) were used. For MCM7, a forward primer Mcm7-709F (5'–ACIMGIGTITCVGAYGTHAARCC–3') and a reverse primer Mcm7-1348R (5'–GAYTTDGCACICCCIGGRTCWCCCAT–3') (Schmitt et al. 2009) were used. PCR was carried out under the program mentioned in Chapter I, and under modified programs when not enough PCR products were gained.

The dataset consisted of 31 specimens (Table 5). Each region was aligned separately. The Q-INS-i option was used for ITS, LSU, SSU and mtSSU, and the G-INS-1 option was used for RPB1, RPB2, and MCM7. The aligned sequences were edited manually using BioEdit. For ITS, LSU, SSU, and mtSSU, all insertions/deletions were deleted.

After checking that there are no topological contradictions among partitions, the concatenated dataset was analyzed using ML, MP, and BI analyses following the programs mentioned in Chapter I. For ML analyses, substitution models GTR+I+G4 for MCM7 second codon position, TIM1ef+I+G4 for MCM7 second codon position, TIM2ef+I+G4 for ITS and RPB2 second codon position, TIM1+I+G4 for SSU, RPB1 second codon position and RPB2 first codon position, TIM2+I+G4 for LSU and RPB1 third codon position, TIM3+I+G4 for RPB2 third codon position, TPM1uf+I+G4 for mtSSU, HKY+I+G4 for RPB1 first codon position and MCM7 third codon position were used.

For BI analyses, substitution models SYM+G4 for RPB2 second codon position, SYM+I+G4 for ITS, RPB1 second codon position, GTR+G4 for RPB1 third codon position, GTR+I+G4 for LSU, mtSSU, RPB2 first codon position, RPB2 third codon position, MCM7 first and second codon position, K80+I+G4 for SSU, HKY+I+G4 for RPB1 first codon position, MCM7 third codon position were used. Two MCMCMC ran for 1.5 million generation, sampling every 500 generations. After convergence and burn-in, a 50% majority rule consensus tree was generated.

After analyses, each taxonomic character (Table 4) was compared to the phylogenetic relationships, and the evolutionary tendency of each character was discussed.

Results

Among the four ML trees based on each region, no conflicts were found in clades with

support > 70% (Appendix 9). The concatenated sequence matrix was composed of 5155 bp (sites 1–502 for ITS, 503–1352 for LSU, 1353–2129 for mtSSU, 2130–3149 for SSU, 3150–3849 for RPB1, 3850–4528 for RPB2, 4529–5155 for MCM7). In the ML analysis, a best-scored tree was yielded with loglikelihood –35447.281666. In the MP analysis, a most parsimonious tree was generated with scores as follows: parsimony informative sites = 1233, tree length = 6887, CI = 0.35, RI = 0.43, RC = 0.15. In the BI analysis, a 50% majority rule consensus tree was constructed and BPP was calculated based on the post-burn-in trees. As no topological contradictions occurred among the the ML best-scored tree, the MP tree, and the BI 50% majority-rule consensus tree, only the ML tree was illustrated, and MLBS, MPBS, and BPP were plotted on its branches (Fig. 8).

The resulting molecular phylogenetic tree (Fig8) did not conflict with the previous result in generic groupings, but gave better support in more number of clades. Five species of *Dasyscyphella* did not form a monophyletic group as shown in my master's thesis (Tochihara 2019). *Erioscyphella*, *Proliferodiscus*, and Clade D formed a strongly supported clade (= Clade E), and *Incrucipulum* and *Neolachnum* formed another strongly supported clade (= Clade F). Clade F+*Albotricha*+*Lachnellula* formed a strongly supported clade in ML and BI analyses, but the clade was not strongly supported in MP analysis.

Members of Clade E had filiform to narrowly lanceolate paraphyses and not hairs with crystals in common, but these characters did not help clade E in distinguishing it from *Lachnum* sensu stricto.

Members of Clade F had hairs with crystals. However, while *Incrucipulum* has thick-walled hairs and granulated ectal excipulum, *Neolachnum* has thin-walled hairs and non-granulated ectal excipulum.

Discussion

Phylogenetic analyses

Seven-gene phylogenetic analyses partially revealed intergeneric relationships in Lachnaceae (Clade E and Clade F, Fig. 8), but some intergeneric relationships remained unsolved.

Evolutional tendency of morphological/ecological characters

Morphologically, *Incrucipulum* was considered to closely relate to *Capitotricha* in the past, because both have thick-walled hairs with crystals, and thick-walled ectal excipulum (Table 4). In fact, Otani (1967) misidentified a specimen of *Incrucipulum* (*I.*

sinegoricum) as a member of *Capitotricha* (= *C. bicolor*, Raitviir 1977). Raitviir (1987) convinced that both genera were closely related based on morphology and proposed “subgenus *Capitotricha*” consisting of “tribe *Capitotricha*” and “tribe *Incrucipulum*”. However, based on the 7-gene phylogenetic analyses in this study, *Incrucipulum* was found to be a sister genus of *Neolachnum* (= Clade F in Fig. 8). *Incrucipulum* and *Neolachnum* shared hairs with crystals, but considerably differ in hair-thickness and ectal excipular structure (Table 4). Thick-walled hairs are characteristic acquired independently between *Capitotricha* and *Incrucipulum*.

Likewise, hair crystal was presumed to have been acquired multiple times in Lachnaceae, and has currently shared within *Brunnipila*, *Capitotricha*, and members in Clade F. In contrast, only *L. nudipes* has hair-crystals in *Lachnum* sensu stricto, and only one undescribed species has hairs without crystals in *Capitotricha* (Table 4) as shown in my master’s thesis (Tochihara 2019). Therefore, the crystals could have also been acquired/lost multiple times in Lachnaceae.

Granulated ectal excipulum has been found and shared in *Incrucipulum*. A species of *Erioscyphella* (*E. boninensis*) has ectal excipular cells with granulated surfaces, although its closely related species (= *E. paralushanensis*) did not have this character.

In Lachnaceae, many species strongly select types (e.g. herbs, hardwoods, grass, palm, bamboo...) and parts (e.g. leaves, twigs/wood, sheath, petiole...) of host plants (Haines and Kaneko 1984; Hosoya et al. 2010, Figs 2, 5, 7). Host types are not generally shared within genera (Table 4), and within *Incrucipulum* miscellaneous host types were seen in intragenus clade (Clade IA) (Fig. 2). On the other hand, *Lachnellula* spp. occur on residues of Pinaceous plant in common (Table 4). Additionally, within *Erioscyphella*, tendency of host selectivity was shared within each intragenus clade (Clade I-VI; discussed in Chapter I, Fig. 2), indicating host types partly reflect the phylogenetic relationships.

In conclusion, states of morphological and ecological traits were considered to be partly shared within phylogenetic groups, indicating that such characters are usable as taxonomic characters in combination with morphological characters. On the other hand, such characters could flexibly be change in every part of lachnaceous phylogeny, indicating such morphological or ecological flexibility has helped the high species-level diversification of Lachnaceae.

Chapter IV: Descriptions

I provided various taxonomic treatment as described in the previous chapters. Besides those I made miscellaneous discovery including new reports (7 species), new combinations (5 species) and discovery of new species (12 species). In this chapter, detailed descriptions were provided for such species.

For the previously known distribution, the database of GBIF (<http://www.gbif.org/occurrence>, accessed 30 Oct 2021) was searched, and countries with occurrences of the given species are shown with asterisks (*). Distributions known only in the literature are shown with double asterisks (**). Those with both information are indicated with triple asterisks (***). World Geodetic System 84 was used for the geographic coordinates.

***Brunnipila clandestina* (Bull.) Baral**, Beih. Z. Mykol. 6: 49. 1985. [new to Japan]
MycoBank No: MB104069

Figs. 9, 10

Peziza clandestina Bull., Herb. Fr. Champ., Hist. Champ. Fr. (Paris) 1: 251. 1791.

Peziza clandestina var. *clandestina* Bull., Herb. Fr. Champ., Hist. Champ. Fr. (Paris) 1: 251. 1791.

Dasyscyphus clandestinus (Bull.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 305. 1870 [1869–70].

Lachnea clandestina (Bull.) P. Karst., Not. Sällsk. Fauna et Fl. Fenn. Förh. 11: 250. 1871 [1870].

Erinella clandestina (Bull.) Quél., Enchir. fung. (Paris): 302. 1886.

Lachnella clandestina (Bull.) W. Phillips, Man. Brit. Discomyc. (London): 236. 1887.

Attractobolus clandestinus (Bull.) Kuntze, Revis. gen. pl. (Leipzig) 3(3): 445. 1898.

Peziza clandestina var. *connivens* Alb. & Schwein., Consp. fung. (Leipzig): 326. 1805.

Peziza clandestina var. *patula* Alb. & Schwein., Consp. fung. (Leipzig): 326. 1805.

Peziza clandestina var. *discoidea* Pers., Mycol. eur. (Erlanga) 1: 263. 1822.

Description. Apothecia gregarious, superficial, forming urceolate cup to almost flat disc, 0.3–1 mm in diameter, stipitate, up to 1 mm high, dark brown, externally covered with brown hairs mostly capitate. Disc concave, white to pale yellow when fresh and dry. Ectal excipulum *textura angularis* to *prismatica* composed of hyaline to pale brown

cells; cells $3\text{--}17 \times 3\text{--}12 \mu\text{m}$, slightly thick-walled. Medullary excipulum *textura intricata* of hyaline hyphae up to $2.5 \mu\text{m}$ wide. Hairs straight, cylindrical with slightly rounded apices, up to $120 \times 3.5\text{--}5 \mu\text{m}$, brown with pale brown or hyaline apices, totally granulate, thin-walled except for the apical cell slightly thick-walled, septate every $10\text{--}30 \mu\text{m}$ except for one or two apical cells with $3\text{--}5 \mu\text{m}$ septation; apex crowned by crystal masses easily detached in squash mounts. Asci $41\text{--}47 \times 3.2\text{--}4.5 \mu\text{m}$, 8-spored, cylindrical-clavate; apical pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa; basal cells usually form more than two asci. Ascospores $(5.8\text{--})6.3\text{--}7.7(9.3) \times (1.3\text{--})1.5\text{--}1.9(2) \mu\text{m}$ (av. $\pm$ s.d. $7 \pm 0.7 \times 1.7 \pm 0.2 \mu\text{m}$, $n = 20$), $Q = (3.2\text{--})3.6\text{--}4.7(5.2)$ (av. $\pm$ s.d. 4.1 ± 0.6 , $n = 20$), ellipsoid to fusiform, aseptate. Paraphyses straight, lanceolate, up to $5 \mu\text{m}$ wide, septate, exceeding the asci $20\text{--}30 \mu\text{m}$.

Specimen examined. JAPAN, Hokkaido, Engaru, Mt. Hirayama, on dead twigs of *Rubus idaeus* subsp. *melanolasius*, 14 Jul 2018, 43.772153, 143.04095, 1030 m, Y. Tochiwara (TNS-F-81486).

Ecology. Saprotrophic on dead twigs of *R. idaeus*, sometimes on dead twigs or stems of other plants.

Distribution. Europe***, KAZAKHSTAN*, KYRGYZSTAN*, MOROCCO*, NEW ZEALAND*, North America***, RUSSIA ***, TAJIKISTAN* (Dennis 1949; Raitviir 1991).

Notes. *Brunnipila clandestina*, the type species of *Brunnipila*, is widely distributed corresponding to the distribution of its host plant *R. idaeus*. In Japan, two occurrences of *B. clandestina* (TNS-F-50486 and TNS-F-50462, registered as '*Dasyscyphus clandestinus*') collected by Yoshio Otani, were found in GBIF. However, based on my re-examination, TNS-F-50486 and 50462 were found to be *Trichopeziza* sp. and *L. pseudocannabinum* (Raitv.) Raitv., respectively. TNS-F-81486 is therefore the first record of *B. clandestina* from Japan.

Baral and Krieglsteiner (1985) reported *B. clandestina* coexisted with *Capitotricha rubi* (Bres.) Baral and *Lachnum virgineum* (Batsch) P. Karst., on the same substrate (*R. idaeus*). I also observed *B. clandestina* shared the twig of *R. idaeus* with *C. rubi* in TNS-F-81486 (Fig. 9B).

***Brunnipila pseudocannabinina* (Raitv.) Tochiwara, Sasagawa & Hosoya comb. nov.**
[new to Japan]

MycoBank No: MB829699

Figs 11, 12

Dasyscyphus pseudocannabinus Raitv., Folia cryptog. Estonica 9: 6. 1977.

Lachnum pseudocannabinum (Raitv.) Raitv., Folia cryptog. Estonica 17: 3. 1985.

Description. Apothecia gregarious, superficial, cup-shaped, 0.6–1.4 mm in diameter, stipitate, up to 1 mm high, dark brown, externally covered with capitate hairs. Disc concave, white when fresh and dry. Ectal excipulum *textura prismatica* to *t. angularis*; cells $2\text{--}13 \times 2\text{--}12 \mu\text{m}$, hyaline, thick-walled up to $2 \mu\text{m}$ thick. Medullary excipulum *textura intricata* of hyaline hyphae up to $3 \mu\text{m}$ wide. Hairs straight, cylindrical with slightly rounded apices, up to $200 \times 4\text{--}5 \mu\text{m}$, brown with pale brown or hyaline apices, totally granulated, thin-walled, slightly thick-walled in one or two cells at the apices; apex crowned by crystal masses easily detached in squash mounts. Asci $57\text{--}70 \times 5.5\text{--}6 \mu\text{m}$, 8-spored, cylindrical-clavate; pore blue in MLZ without 3 % KOH pretreatment; croziers present at the base. Ascospores $10\text{--}15 \times 2\text{--}3 \mu\text{m}$, slightly long fusiform, sometimes irregularly curved, aseptate or rarely one-septate, containing some small lipid bodies. Paraphyses straight, lanceolate with acute apices, up to $5 \mu\text{m}$ wide, septate, exceeding the asci by $25\text{--}30 \mu\text{m}$.

Specimens examined. JAPAN, Hokkaido, Otaru, Zenibako, 43.146985, 141.147294, 40 m, on dead herb stems, 1 Jul 1967, Y. Otani (TNS-F-50462) [as '*Dasyscyphus clandestinus*']. JAPAN, Hokkaido, Mombetsu, Engaru, Mt. Hirayama, 43.29528, 141.316897, 1090 m, on dead stems of *Filipendula camtschatica* (Pall.) Maxim., 12 Jul 2017, Y. Tochiara (TNS-F-81252). Akita, Kosaka, on dead stems of *Fallopia* sp., 26 May 2006, R. Sasagawa (TNS-F-16690 and TNS-F-16691). JAPAN, Aomori, Aomori, Sugayu, 40.649556, 140.849167, 910 m, on dead stems of herbaceous plants, 23 Jun 2005, T. Hosoya (TNS-F-17748, 17751). JAPAN, Aomori, Towada, Lake Towada, 40.415472, 140.86125, 420 m, on dead stems of herbaceous plants, 26 May 2006, T. Hosoya (TNS-F-18049).

Ecology. Saprophytic on dead stems of tall herbaceous plants. Forming apothecia in May to July.

Distribution. Kunashir Island***, Sakhalin**, Kamchatka *** (Raitviir 1977; 1991).

Notes. TNS-F-16691 was examined in Hosoya et al. (2010) and determined as an unidentified lachnaceous species '*Lachnum* sp. 4'. My re-examination revealed the six specimens of '*Lachnum* sp. 4' were *B. pseudocannabinum*, in agreement with the original description of Raitviir (1977).

Raitviir (1991) included *L. pseudocannabinum* in section *Brunnipila* (Baral) Raitv., subgenus *Belonidium*, genus *Lachnum* Retz. together with *L. cannabinum* (=

currently *B. cannabina*), *L. clandestinum* (= currently *B. clandestina*) and *L. fuscidulum* focusing on stipitate apothecia and brown hairs equipped with crystals. Since molecular phylogenetic analysis (Hosoya et al. 2010) and phylogenetic analyses in this study concluded that '*Lachnum* sp. 4' was positioned in the *Brunnipila* clade, *L. pseudocannabinum* was transferred to *Brunnipila*. As I have not examined specimens of *L. fuscidulum*, I hesitate to propose a new combination for *L. fuscidulum*.

Brunnipila pseudocannabina differs from *B. cannabina* (Rehm) Raitv. & Järv in having larger asci of $> 55 \mu\text{m}$ (Raitviir 1991). Raitviir (1991) pointed out this fungus was endemic to the Far East while *B. cannabina* was widely distributed throughout Russia and Europe. *Brunnipila pseudocannabina* distribution probably includes also the subarctic zone, from north of Central Honshu to Kamchatka.

Brunnipila pseudocannabina usually shares the same substrate with other lachnaceous fungi, such as *Lachnum nudipes* (Fuckel) Nannf. (Fig. 11B), which is found on decaying stems of tall herbs. Species of *Trichopezizella* (Solenopezizeaceae) superficially resemble *B. pseudocannabina* in external impression, but differ in having sessile to substipitate apothecia and smooth hairs lacking crystals.

***Erioscyphella boninensis* Tochihara & Hosoya, sp. nov.**

MycoBank No: MB835702

Figs 13, 14

Diagnosis. Differs from all other *Erioscyphella* species by the granulate walls of the ectal excipular cells.

Holotype. JAPAN, Bonin Islands, Chichijima Island, Mt. Tsutsujiyama, 27.060556, 142.222500, 270 m, 28 Jun 2009, on fallen leaves of *Pittosporum boninense*, T. Hosoya (TNS-F-26520).

GenBank/UNITE no. ex holotype. UDB0779049 (ITS), LC533151 (LSU), LC533254 (mtSSU), LC533196 (RPB2).

Etymology. Referring to the type locality Bonin Islands.

Japanese name. Ogasawara-cha-hina-no-chawantake.

Description. Apothecia scattered, superficial, 0.5–1.0 mm in diameter, having well-developed stipes, up to 1.5 mm high, cream to pale brown, externally covered with short and shiny hairs. Disc concave, cream to pale yellow. Ectal excipulum *textura prismatica* composed of long elongated cells to *textura angularis*, $6\text{--}25 \times 5\text{--}13 \mu\text{m}$, hyaline to relatively brown colored, somewhat thick-walled; cell walls covered by granules with a similar appearance to those on hairs. Stipe composed of *textura*

prismatica with a granulate surface as ectal excipular cells. Medullary excipulum *textura intricata* of hyaline hyphae up to 3 μm wide. Hairs straight, cylindrical, 38–62 $\times$ 2.5–4.0 μm , hyaline, completely covered by brown granules, 2–3-septate, thin-walled, arising from swelling cells completely covered by granules; apex lacking crystals or apical amorphous materials, equipped with amber-colored resinous materials dissolvable with CB/LA at a little below the apex. Asci (36–)37.7–44(–46) $\times$ (3.5–)3.6–4.2(–4.5) μm (av. $41 \pm 3.2 \times 3.9 \pm 0.3 \mu\text{m}$, $n = 16$), 8-spored, cylindrical-clavate; pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores (9–)10–12.3(–13) $\times$ 1.2–1.7(–1.8) μm (av. $11 \pm 1.2 \times 1.5 \pm 0.2 \mu\text{m}$, $n = 16$), $Q = (6.3\text{--})6.9\text{--}9.2(10)$ (av. 7.8 ± 1.5 , $n = 16$), fusiform, aseptate. Paraphyses straight, up to 2.5 μm wide, septate, exceeding the asci up to 5 μm , narrowly lanceolate.

Culture characteristics. Colony of NBRC 114447/TNS-F-26520 on PDA umbonate forming a dome-shape, slightly sulcate. Context not shiny, velvety, buff at the center, paler toward the margin, dark buff from the reverse. Sectors and zonation absent. Aerial mycelium white or buff, dense cottony, forming white mycelium strands except in the margin. Margin distinct, entire, flat. Asexual morph absent.

Ecology. Saprotrophic on dead leaves of broad-leaved trees.

Distribution. JAPAN. (Bonin Islands). Known only from the type locality.

Notes. Granulation on the surface of the ectal excipular cells has been observed only in *Incrucipulum* in Lachnaceae (Baral and Kriegelsteiner 1985), and *E. boninensis* is the first report for such a character in *Erioscyphella*. Phylogenetic analysis revealed that *E. boninensis* is closely related to *E. paralushanensis* (Fig. 2). The two species (Clade IA, Fig. 2) have colored granules on hairs and forming red mycelia on PDA. However, granulation of ectal excipulum is seen only in *E. boninensis*.

***Erioscyphella hainanensis* (W.Y. Zhuang & Zheng Wang) Hosoya & Tochiara, comb. nov.**

MycoBank No: MB835707

Lachnum hainanense W.Y. Zhuang & Zheng Wang, Mycotaxon 67: 25.1998.

Diagnosis. Forming apothecia with long stipes and long hairs. Differing *E. sinensis* in much shorter ascospores.

Japanese name. Shii-Kashi-hina-no-chawantake.

Specimens examined. JAPAN, Niigata, Minamiuonuma, 37.056808, 138.80705, 720 m, 14 May 2010, on fallen leaves of *Quercus glauca*, T. Hosoya (TNS-F-35049). Ibid

(TNS-F-35056). JAPAN, Kanagawa, Hiratsuka, 35.33861111, 139.285, 80 m, 12 Apr 2015, on fallen leaves of *Q. myrsinifolia*, M. Nakajima (TNS-F-61775). JAPAN, Kanagawa, Kamakura, 35.30756, 139.51958, 40 m, 24 Apr 2015, on fallen leaves of *Q. serrata*, M. Nakajima (TNS-F-61941). JAPAN, Gunma, Midori, 36.476684, 139.242771, 510 m, 9 May 2016, on fallen leaves of *Q. serrata*, K. Furuya (TNS-F-65722). JAPAN, Kanagawa, Hiratsuka, 35.340139, 139.287167, 60 m, 18 May 2017, on fallen leaves of *Q. glauca*, Y. Tochiara (TNS-F-80356). The same locality, on fallen leaves of *Castanopsis sieboldii*, Y. Tochiara (TNS-F-80371).

Ecology. Saprotrophic on dead leaves of broad-leaved trees. Forming apothecia in April to May.

Distribution. CHINA (Hainan), JAPAN (Honshu: Kanto region).

Notes. ITS sequences of this species were integrated into a single SH (DOI: SH1155844.08FU) at UNITE SH system at a 3% threshold. SH1155844.08FU included sequences labeled as ‘Hyaloscyphaceae’ (JX984680) named by UNITE and ‘*L. albidulum*’ (MK282242) named by INSDC (Table 2). JX984680 was sequenced from air samples in Seoul, South Korea, and was not tied to any fungal specimens or cultures. MK282242 was sequenced from apothecia collected in Korea. *Lachnum albidulum* is common on leathery dicot leaves of the old and new world tropics (Haines 1992). *Erioscyphella hainanensis* resembles *L. albidulum* in morphology, but *L. albidulum* has yellow resinous substances at the tip of apothecial hairs and occurs on dead leaves of Rubiaceae (Haines 1992), whereas *E. hainanensis* lacks resinous substances and occurs on leaves of broad-leaved trees (Zhuang and Wang 1998a; Hosoya et al. 2013). Considering most members of SH1155844.08FU were *L. hainanense*, MK282242 was strongly suggested as a misidentified sequence of *L. hainanense*.

Erioscyphella hainanensis resembles *E. sinensis* in occurring on dead leaves of *Quercus* spp. or *Castanopsis* spp. However, *E. hainanensis* has much shorter ascospores than *E. sinensis*. In this study, presence of minute, hyaline apical amorphous materials and absence of any crystals or resinous materials were confirmed in both species (Appendix 2).

***Erioscyphella insulae* Tochiara & Hosoya, sp. nov.**

MycoBank No: MB835703

Figs 15, 16

Diagnosis. Resembles *Lachnum nothofagi*, but distinguished by pure white apothecia and two-layered ectal excipulum.

Holotype. JAPAN, Okinawa, Yaeyama, Taketomi, Iriomote Island, Otomi, 24.297458, 123.866128, 50 m, 12 Jun 2011, on fallen bark of unidentified tree, T. Fukiharu (TNS-F-39720).

GenBank/UNITE no. ex holotype. UDB0779063 (ITS), LC533177 (LSU), LC533261 (mtSSU), LC533207 (RPB2).

Other specimens examined. JAPAN, Bonin Islands, Hahajima Island, Sekimon, 26.666686, 142.152222, 260 m, 24 Jun 2009, on fallen bark of unidentified tree, T. Hosoya (TNS-F-26485, 26500).

Etymology. Referring to the occurrence of the species on remote islands in Japan.

Japanese name. Shima-hina-no-chawantake.

Description. Apothecia gregarious, superficial, 0.7–1.4(–2.5) mm in diameter, short- and thick-stipitate, up to 0.8 mm high, externally white to cream throughout but sometimes pale brown in the lower parts, covered with white hairs. Disc concave, cream to pale yellow (fresh state not observed). Ectal excipulum composed of two layers: outer layer *textura angularis*, up to 20 μm thick, 3–28 $\times$ 2–8 μm , hyaline, thin to relatively thick-walled, with cell walls smooth; inner layer up to 15 μm thick, *textura porrecta* composed of hyaline hyphae up to 5 μm wide. Medullary excipulum up to 100 μm thick, composed of hyaline hyphae forming *textura intricata*; hyphae up to 3 μm wide. Hairs straight or irregularly curved, cylindrical, sometimes branched, up to 125 $\times$ 2.5–3.0 μm , hyaline, completely granulate, thin-walled; lacking crystals or resinous materials; apex usually equipped with hyaline apical amorphous materials. Asci (88–)92–101(–106) $\times$ 6–7.3(–8) μm (av. 96 $\pm$ 4.5 $\times$ 6.7 $\pm$ 0.6 μm , n = 18), 8-spored, thick-walled, cylindrical-clavate, arising from ascogenous hyphae branching several times; pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores (24–)26.7–34.5(–39) $\times$ (1.8–)1.9–2.3(–2.5) μm (av. 31 $\pm$ 3.9 $\times$ 2.1 $\pm$ 0.2 μm , n = 18), Q = (11–)12.5–17(–20) (av. 14.7 $\pm$ 2.3, n = 18), showing various shapes and lengths, usually long fusiform and sometimes hypsiloid or sigmoid due to bending of both ends, sometimes swelling or constricted irregularly, aseptate or one- to three-septate (usually one-septate). Paraphyses straight, narrowly lanceolate, up to 2.5 μm wide, septate, exceeding the asci up to 7.5 μm .

Culture characteristics. Colony of NBRC 114445/TNS-F-26500 and NBRC 114459/TNS-F-39720 on PDA relatively thick-planar, pruinose, white to cream, ivory at the margin, pale sepia. Sectors and zonation absent. Aerial mycelium white to pale ocher, mainly developed except in the margin, not forming mycelial strands. Soluble pigment amber colored produced at the center. Margin unclear, flat and immersed into agar, radially undulate.

Ecology. Saprotrophic on dead wood. Forming apothecia in June.

Distribution. JAPAN (Bonin Islands, Yaeyama Islands).

Notes. This fungus resembles *Lachnum nothofagi* (Dennis) Spooner in the size and shape of apothecia, ascospores, asci, and hairs. However, *E. insulae* has completely hyaline hairs and ectal excipulum, and hairs are equipped with apical materials (Fig. 15I, J), whereas *L. nothofagi* has partly to totally brown hairs and ectal excipulum (Spooner 1987). *Lachnum nothofagi* is currently known only from New Zealand and Australia and mainly arises from *Nothofagus* spp., which are native in the southern hemisphere (Spooner 1987).

***Erioscyphella otanii* Tochihara, sp. nov.**

MycoBank No: MB835704

Figs 17, 18

Diagnosis. The fungus resembles *L. diminutum* but distinguished by pure white minute apothecia (< 0.3 mm in diameter) while *L. diminutum* has larger and colored apothecia. It has smaller asci compared to similar species *Lachnum minutum*.

Holotype. JAPAN, Hokkaido, Horonobe, Toikambetsu, Teshio Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, 44.993978, 142.130125, 400 m, 11 Jul 2018, on fallen leaves of *Sasa senanensis*, Y. Tochihara & K. Kaneko (TNS-F-81472).

GenBank/UNITE no. ex holotype. UDB0779083 (ITS), LC533179 (LSU), LC533286 (mtSSU), LC533226 (RPB2).

Other specimen examined. JAPAN, Hokkaido, Sapporo, Mt. Moiwa, 43.024718, 141.318427, 530 m, 21 Jun 1965, on fallen leaves of *Sasa kurilensis*, Y. Otani (TNS-F-50482, in poor condition).

Etymology. Referring to the name of Dr Yoshio Otani, the first discoverer of this species.

Japanese name. Kita-sasaba-hina-no-chawantake.

Description. Apothecia scattered, superficial, minute, 0.1–0.3 mm in diameter, at first spherical and later urceolate, having well-developed stipes, up to 0.3 mm high, pure white, externally covered with short white hairs, never colored brown. Disc concave, almost enclosed by an incurving margin when fresh and dry, cream to pale yellow when dry (not observed when fresh). Ectal excipulum *textura prismatica* like stone pavings arranged in rows, 3–25 × 3–8 µm, hyaline, relatively thick-walled; cell walls smooth. Medullary excipulum *textura intricata*; hyphae up to 2.5 µm wide. Hairs straight,

cylindrical or tapering toward the apices, up to 60 μm long, up to 5 μm wide near the bases and 2.5–3.0 μm wide near the apices, arising from swollen ectal excipular cells, hyaline, up to 3-septate (usually 1- or 2-septate), thin-walled, completely granulated; granules dense near the apices and coarse toward the bases; apex sometimes with a hyaline and inconspicuous apical amorphous materials not dissolved with CB/LA, lacking any crystals or resinous materials. Asci (33–)34–38.8(–41) $\times$ 4–5 μm (av. $37 \pm 2.2 \times 4.4 \pm 0.4 \mu\text{m}$, $n = 15$), 8-spored, cylindrical-clavate, relatively thick-walled; pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores (11.5–)12.3–14.6(–15) $\times$ (1.2–)1.36–1.7(–1.8) μm (av. $13.4 \pm 1.2 \times 1.6 \pm 0.2 \mu\text{m}$, $n = 15$), $Q = (6.7\text{--})7.8\text{--}9.6(10.8)$ (av. 8.7 ± 0.9 , $n = 15$), fusiform, aseptate. Paraphyses straight, narrowly lanceolate to lanceolate, up to 2.5 μm wide, septate, exceeding the asci up to 10 μm .

Culture characteristics. Colony of NBRC 114476/TNS-F-81472 on PDA flat, partially protruding and forming mycelial mass, divided into two sectors. One sector flat, wooly to velvety, white to cream; dark ocher from the reverse. The other sector with wooly context, white and partly yellow; pale ocher from the reverse. Aerial mycelia developed throughout the colony, white, sparse to cottony, not forming mycelium strands. Margin distinct, flat and immersed into the agar. Soluble pigment absent. Asexual morph absent.

Ecology. Saprotrophic on dead leaves of bamboo.

Distribution. JAPAN (Hokkaido; subarctic zone).

Notes. *Erioscyphella otanii* was first collected and documented by Otani (1967) under the misapplied name *Dasyscyphus diminutus* (TNS-F-50482). Based on the description, I concluded that the specimen was the same species as TNS-F-81472. The present species is very similar to *Lachnum diminutum* (Roberge ex Desm.) Rehm in the minute apothecia, ascospore size, and narrow paraphyses; however, *E. otanii* is pure white when fresh and dry (Fig. 17A, in dried state) and occurs on bamboo leaves, while *L. diminutum* is somewhat brown in the exterior parts of apothecia and occurs on sheaths of *Juncus* spp. (Dennis 1949). In the mature state, the apothecia of *E. otanii* become urceolate (Fig. 17A and Fig. 18B), whereas the apothecia of *L. diminutum* are flat (Dennis 1949). The ITS sequence of TNS-F-81472 showed low similarity (< 80%) with that of *L. diminutum* collected in France (GenBank accession number: MH857306).

Erioscyphella otanii also resembles the graminicolous species *Lachnum minutum* W.Y. Zhuang and M. Ye documented in China (Ye and Zhuang 2003), but has smaller asci.

***Erioscyphella papillaris* Tochihara, sp. nov.**

Diagnosis. Characterized by protruding papillate hairs with hyaline, apical amorphous materials.

Holotype. JAPAN, Gunma, Minakami, Yubiso, Mt. Tanigawadake, 36.064014, 141.344653, 710 m, 16 Jul 2017, on both sides of a fallen leaf of bamboo, Y. Tochiwara (TNS-F-81272).

GenBank/UNITE no. ex holotype. UDB0779085 (ITS), LC533161 (LSU), LC533285 (mtSSU), LC533204 (RPB2).

Etymology. Referring to papillate hair apices.

Japanese name. Sasaba-hina-no-chawantake.

Description. Apothecia gregarious, superficial, minute, 0.1–0.3 mm in diameter, short-stipitate, up to 0.25 mm high, externally covered with pure white short hairs densely. Disc concave, white to lemon yellow when fresh and dry. Ectal excipulum *textura prismatica* composed of cuboid cells, $3\text{--}13 \times 2.5\text{--}7\ \mu\text{m}$, hyaline, thin-walled, lacking carotenoid pigments; cell walls smooth. Medullary excipulum *textura intricata* of hyaline hyphae up to $3\ \mu\text{m}$ wide. Hairs straight, cylindrical, $45\text{--}75 \times 3\text{--}5\ \mu\text{m}$, 2–3-septate, hyaline, totally granulate, thin-walled, arising from swollen cells; apical cells rather longer than other cells, $30\text{--}40\ \mu\text{m}$ long, with papillate at the apex, sometimes swelling, lacking crystals and resinous materials, equipped with hyaline and globose apical amorphous materials not dissolved with CB/LA, lacking any crystals or resinous matters. Asci (59–)59.8–66(–69) $\times$ (7.5–)7.6–8.3(–9) μm (av. $63 \pm 2.9 \times 8.0 \pm 0.4\ \mu\text{m}$, $n = 16$), 8-spored, cylindrical-clavate; pore inamyloid with MLZ without 3% KOH pretreatment, faint blue with MLZ with 3% KOH pretreatment, dark blue with IKI with and without KOH pretreatment; vesicle apparatus inverted-v-shaped present near the apices; croziers absent at the basal septa; base sympodially branched. Ascospores (16–)17.5–21.7(–24) $\times$ (2–)2.3–2.8(–3) μm (av. $20 \pm 2.1 \times 2.6 \pm 0.3\ \mu\text{m}$, $n = 20$), $Q = (6.4\text{--})6.8\text{--}8.9(9.8)$ (av. 7.8 ± 1.0 , $n = 20$), fusiform, aseptate, or one-septate (rarely two-septate), filled with hyaline oil drops. Paraphyses straight, cylindrical, up to $3\ \mu\text{m}$ wide, septate, containing small hyaline lipid bodies, equal or scarcely exceeding the asci.

Culture characteristics. Colony of NBRC 113937/TNS-F-81272 on PDA divided into two semicircular zones. The first zone umbonate, pruinose, white, producing white aerial mycelia densely, presenting wooly appearance; margin distinct, entire, flat. Second zone flat, glutinous, white to beige with concentric patterns, producing few aerial mycelia;

margin entire, flat and immersed into agar, irregularly undulate. The reverse uniform unrelated to the zoning position, beige to pale dark brown throughout. Soluble pigment and asexual morph absent throughout the colony.

Ecology. Saprotrophic on dead leaves of bamboo.

Distribution. JAPAN (Mt. Tanigawa). Known only from the type locality.

Notes. This species is similar to *Lachnum sclerotii* var. *microascum* in the dimension and shape of asci and ascospores, habitats, and inconspicuous ascus apex reaction in MLZ (Zhuang 2004). However, *E. papillaris* has ascospores containing conspicuous guttules in any mount (Fig. 19G) and filiform paraphyses rarely exceeding the asci, whereas *L. sclerotii* var. *microascum* has non-guttulate asci and narrowly lanceolate to lanceolate paraphyses exceeding the asci by 15–18 μm (Zhuang 2004). Although DNA sequences of *L. sclerotii* var. *microascum* are not available, I judged the present fungus as different from it, because the presence or absence of guttules in ascospores is a significant taxonomic character at the species level (Baral 2015).

Papillate hairs are also shown in the line drawings of *Lachnum gahniae* Spooner (Spooner 1987), suggesting the relationship of the present fungus to Australasian species. However, *L. gahniae* can be distinguished by having longer hairs, occurring on different substrates (leaves of Cyperaceae) and showing clearly positive MLZ+ asci (Spooner 1987).

Iodine reactions of the ascus apical apparatus have been classified into several types (inamyloid, hemiamyloid [Type RB and RR, and euamyloid Type BB]) (Baral 2009), and the reaction ‘MLZ– without KOH pretreatment and MLZ+ with KOH pretreatment’, observed in *E. papillaris* (Fig. 19E) has been restricted to the type of hemiamyloid. However, the apical apparatus of *E. papillaris* showed a dark blue reaction in IKI without KOH pretreatment (Fig. 19E3), while the hemiamyloid apparatus is usually stained red under these conditions. The hemiamyloid ascal apparatus could show IKI-blue without KOH pretreatment due to long storage in the herbarium (Baral 2009), but this is not applicable to the material of *E. papillaris*, which has been maintained for only two years in herbarium until observed. Therefore, the iodine reaction of *E. papillaris* should be classified in a new category, and color reactions with various solutions of the species should be further examined using new materials, because there are few apothecia in the type specimen.

***Erioscyphella paralushanensis* Tochiohara & Hosoya, sp. nov.**

MycoBank No: MB839618

Figs 21, 22

Diagnosis. Characterized by apothecia furnished by red hairs occurring on bamboo sheaths. Similar to *E. lushanensis* in macro and micromorphology and habitats but has larger asci and ascospores.

Holotype. JAPAN, Shizuoka, Atami, Izusan, 35.128834, 139.051194, 620 m, 8 Jun 2015, on fallen sheaths of *Pleioblastus argenteostriatus*, M. Nakajima (TNS-F-61920).

GenBank/UNITE no. ex holotype. UDB0779075 (ITS), LC533141 (LSU), LC533267 (mtSSU), LC533220 (RPB2).

Etymology. Referring to the similarity with *E. lushanensis*.

Japanese name. Akage-hina-no-chawantake.

Description. Apothecia scattered, superficial, 0.7–1.5 mm in diameter, long-stipitate, up to 2.0 mm high, externally covered with dark-red hairs. Disc concave, cream to pale yellow. Ectal excipulum well-developed *textura prismatica* and partly *t. angularis*, 6–13 × 2.0–2.5 µm, hyaline, relatively thick-walled, with smooth walls. Medullary excipulum *textura intricata* of hyaline hyphae up to 2 µm wide. Hairs straight, cylindrical, up to 160 µm long, 2.0–3.0 µm wide, pale brown but hyaline near the bases, narrowly septated composed cells > 7 µm long, covered by big and amber-colored granules; granules big and dense near the apices and smaller and sparse near the bases, up to 2 µm in diameter near the apices, equipped with amber-colored resinous materials that dissolves in CB/LA at any position of hairs; apices with amber-colored apical amorphous materials, lacking any crystals. Asci (59–)61.4–70.2(–73) × (4.5–)4.7–5.6(–6) µm (av. 65.8 ± 4.4 × 5.2 ± 0.4 µm, n = 15), Q = (11.5–)12–13.6(–14.6) (av. 12.8 ± 0.8, n = 15), 8-spored, cylindrical-clavate; pore faintly blue in MLZ without 3% pretreatment, clear blue in MLZ with 3% KOH pretreatment and IKI without 3% KOH pretreatment. Ascospores (14–)15.8–20.7(–22) × (1.5–)1.7–2.0 µm (av. 18.2 ± 2.5 × 1.8 ± 0.2 µm, n = 15), Q = (7.5–)8.7–11.2(–12.6) (av. 9.9 ± 1.3, n = 15), septate, sometimes bent to U-shaped or S-shaped, containing conspicuous guttules; guttules hyaline but sometimes red. Paraphyses straight, up to 2 µm wide, septate, exceeding the asci 5–10 µm initially cylindrical to clavate, later becoming narrowly lanceolate.

Culture characteristics. Colony of NBRC 114468/TNS-F-61920 on PDA flat, sparse, dendritically spread. Context wooly, ocher to pale buff, dark buff from the reverse. Sectors and zonation absent. Aerial mycelium ocher to pale buff, dense cottony, developed near the center, forming white mycelium strands; margin distinct, flat and partly immersed into the agar. Asexual morph absent. Soluble pigments present, buff, dyeing agar without colony pale buff.

Ecology. Saprotrophic on dead sheathes of bamboo.

Distribution. JAPAN (Shizuoka). Currently known only from the type locality.

Notes. *Erioscyphella paralushanensis* is closely related to *E. lushanensis* in having red hairs (Fig. 21I) and the ectal excipulum composed of well-developed rectangular cells in common (Fig. 21H, Fig. 22C, and Fig. 22F) (Zhuang and Wang 1998b). Compared with *E. lushanensis*, *E. paralushanensis* has slightly larger asci, ascospores and hairs. Red guttles in ascospores were observed only in *E. paralushanensis* (Fig. 21F). In this study, I proposed a new species for the present fungus, because species delimitation analyses based on ITS sequences strongly supported that *E. paralushanensis* is different from *E. lushanensis* (Fig. 3).

***Erioscyphella sasibrevispora* Tochiara & Hosoya, sp. nov.**

MycoBank No: MB835706

Figs. 23, 24

Diagnosis. Characterized by wooly appearance and yellow to orange discs and distinguished from similar species *Lachnum novoguineense* var. *yunnanicum* in having shorter ascospores.

Holotype. JAPAN, Hokkaido, Tomakomai, Utonai, 42.705314, 141.7346, 10 m, 16 Jun 2018, on fallen sheaths of *Sasa nipponica*, Y. Tochiara & T. Hosoya (TNS-F-81401).

GenBank/UNITE no. ex holotype. UDB0779082 (ITS), LC533174 (LSU), LC533269 (mtSSU), LC533217 (RPB2).

Other specimen examined. JAPAN, Gunma, Higashiagatsuma, 36.562253, 138.724139, ca 1330 m, 6 Jun 2017, on fallen sheaths of *Sasa veitchii*, Y. Tochiara & T. Hosoya (TNS-F-80399, in bad condition).

Etymology. “sasi” means bamboo [host plants] and “brevispora” means shorter ascospores compared to *L. novoguineense* var. *yunnanicum*.

Japanese name. Sasa-no-youmou-chawantake.

Description. Apothecia gregarious, superficial, 0.6–1.3 mm in diameter, short-stipitate, up to 0.8 mm high, pure white, externally covered with long white hairs. Disc concave, yellow to pale orange when fresh and dry. Ectal excipulum *textura prismatica* to *t. angularis*, 3–16 × 2–10 µm, hyaline, thin-walled; surface smooth. Medullary excipulum *textura intricata* of hyaline hyphae up to 2 µm wide. Hairs straight, delicate, cylindrical with relatively acute apices, up to 190 × 2–3 µm, hyaline, totally granulate, thin-walled; apical cell a little longer than other cells, lacking any crystals, resinous

materials, or apical amorphous materials. Asci (79–)82.5–90(–95) $\times$ (6–)6.6–8.1(–9) μm (av. $86 \pm 4.0 \times 7.4 \pm 0.8 \mu\text{m}$, $n = 15$), 8-spored, cylindrical-clavate; lateral parts sometimes swelling irregularly; pore blue in MLZ without 3% KOH pretreatment; croziers with perforation present at the basal septa. Ascospores (26–)27.9–36.1(–39) $\times$ (1.5–)1.7–2 μm (av. $32 \pm 4.1 \times 1.8 \pm 0.2 \mu\text{m}$, $n = 17$), $Q = (13–)15–19.7(–21)$ (av. 17.5 ± 2.3 , $n = 17$), long fusiform, usually 3-septate, rarely 0- to 2-septate (only observed in TNS-F-81401 because TNS-F-80399 was immature). Paraphyses straight, lanceolate, 2.5–4 μm wide, densely septate, exceeding the asci up to 15 μm . Note that the description is solely based on the holotype because another examined specimen TNS-F-80399 was in bad condition.

Culture characteristics. Colony of NBRC 114475/TNS-F-81401 on PDA wrinkled. Context cottony and partially funiculose, white, turning ocher at the center; almost ocher except for the white margin from the reverse. Sectors and zonation absent. Aerial mycelium developed throughout the colony, concolous, forming mycelium strands. Margin indistinct, flat and immersed into agar. Soluble pigment absent. Asexual morph absent.

Ecology. Saprotrophic on dead leaves of bamboo. Forming apothecia in June.

Distribution. JAPAN (cool-temperate zone, subarctic zone).

Notes. *Erioscyphella sasibrevispora* is closely related to *L. novoguineensis* var. *yunnanicum* (TNS-F-16442, 16642) (Fig. 2) and occurs in the same habitats (bamboo sheaths) but has shorter asci and ascospores. The ascal bases of the two species are very characteristic, in that they have croziers with perforations (Fig. 23G and Fig. 24E). In Lachnaceae, this type of crozier has only been reported in *Lachnellula* (Baral 1984). Additionally, both species exceptionally lack any hair materials in *Erioscyphella*.

The tropical species *E. bambusina* and *Lachnum albidum* var. *americanum* (Dennis) W.Y. Zhuang also occur on bamboo sheaths. However, compared with the present fungus, the former has smaller ascospores and filiform paraphyses (Dennis 1954), and the latter has extremely large asci and ascospores (Dennis 1960). In cool-temperate to subarctic zones, *L. asiaticum* and *Lachnum sasae* Raitv. occur on bamboo sheaths (Otani 1967; Raitviir 1985), but their ascospores are much shorter than those of the present fungus.

The woolly appearance and yellow disc of this species (Fig. 23A) resemble those of *Capitotricha rubi* (Bres.) Baral; however, microscopic observations easily distinguish the two species.

***Erioscyphella sinensis* (Z.H. Yu & W.Y. Zhuang) Sasagawa, Tochihara & Hosoya,**

comb. et stat. nov. [new to Japan]

MycoBank No: MB835709

Figs. 25, 26

Lachnum mapirianum var. *sinense* Z.H. Yu and W.Y. Zhuang, Nova Hedwigia 74(3–4): 422. 2002.

Diagnosis. Occurring on fallen leaves of *Quercus* spp. or *Castanopsis* spp. in early summer and having needle-like ascospores much longer than those of *E. hainanensis*.

Japanese name. Shii-Kashi-hina-no-chawantake-modoki.

Specimen examined. JAPAN, Ibaraki, Tsukuba, Mt. Tsukuba, 36.228539, 140.103504, 870 m, 23 Jun 2007, on fallen leaves of *Castanopsis sieboldii*, R. Sasagawa (TNS-F-16841). JAPAN, Ibaraki, Tsukuba, Amakubo, Tsukuba Botanical Garden, 36.101472, 140.110944, 20 m, 15 Jun 2007, on fallen leaves of *C. sieboldii*, R. Sasagawa (TNS-F-16838). JAPAN, Tottori, Yonago, Yonago Castle, 35.42437, 133.325472, 50 m, 3 Jun 2018, on fallen leaves of *C. sieboldii*, Y. Tochiwara (TNS-F-81383).

Description. Apothecia scattered on the substrate, superficial, initially cup-shaped, completely flat-disc shaped when mature, 0.3–1 mm in diameter when fresh and dry, with long, slender stipes, up to 1 mm high, externally covered with hairs, white to pale cream when fresh and dry. Disc concave, cream to pale yellow when fresh and dry. Ectal excipulum well-developed *textura prismatica*; cells $4\text{--}35 \times 2.5\text{--}8\text{ }\mu\text{m}$, thin- to relatively thick-walled up to $1.5\text{ }\mu\text{m}$ thick, with smooth surfaces. Medullary excipulum *textura intricata* of hyaline hyphae up to $5\text{ }\mu\text{m}$ wide. Hairs straight, cylindrical, up to $100 \times 3\text{--}3.5\text{ }\mu\text{m}$, septate, hyaline, granulated throughout, thin-walled; apex blunt or a little bit acute, lacking any crystals or resinous materials, equipped with hyaline cap-like materials. Asci $(36\text{--})43.7\text{--}51(\text{--}54) \times (4\text{--})4.4\text{--}5.1(\text{--}5.2)\text{ }\mu\text{m}$ (av. $\pm$ s.d. $47.3 \pm 3.6 \times 4.8 \pm 0.3\text{ }\mu\text{m}$, $n = 20$), 8-spored, cylindrical-clavate with slightly protruding apices; apical pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores $(30\text{--})32.3\text{--}39.2(\text{--}45) \times 1\text{--}1.2(\text{--}1.3)\text{ }\mu\text{m}$ (av. $\pm$ s.d. $35.8 \pm 3.5 \times 1.1 \pm 0.1\text{ }\mu\text{m}$, $n = 20$), $Q = (23.8\text{--})28\text{--}36.3(\text{--}40)$ (av. $\pm$ s.d. 32.2 ± 4.2 , $n = 20$), long ellipsoid to longy and narrowly fusiform, straight or bending (U-shaped), aseptate or 1-septate, containing some small lipid bodies. Paraphyses straight, cylindrical, narrowly lanceolate, or clavate, sometimes asymmetrically widen toward the apex, anastomosed with other paraphyses both in the bases and near the apices, septate, up to $3\text{ }\mu\text{m}$ wide, exceeding the asci by $5\text{--}10\text{ }\mu\text{m}$.

Ecology. Saprotrophic on dead leaves of broad-leaved trees. Forming apothecia

in June.

Distribution. CHINA (Hainan, Yunnan; Yu and Zhuang 2003), JAPAN (warm-temperate zone).

Notes. The present fungus was treated as *Lachnum* sp. 13 by Hosoya et al. (2010). This fungus occurs in the same habitats as *E. hainanensis*, but it is easily distinguished in having longer and needle-like ascospores. The present fungus resembles *L. mapirianum* in the shape of ascospores, but the two species are different in that *L. mapirianum* has long slender apothecial stipes, larger asci, longer ascospores, and wider paraphyses.

In the present study, I transferred this fungus to *Erioscyphella* and raise it from variety to species level, because this fungus is not phylogenetically related to '*L. mapirianum*' (Fig. 2). The presence of apical amorphous materials of hairs were confirmed in this study (Fig. 25H).

***Incrucipulum aurantiacum* Tochihara, sp. nov.**

MycoBank No: Undesignated.

Figs. 27, 28

Diagnosis. Characterized by ebullicolous apothecia and conspicuous orange oil drops of carotenoids in hairs, paraphyses, and the ectal excipulum. Very similar to *I. virmbergensis* but differing in the presence of croziers.

Holotype. JAPAN, Gunma, Agatsuma, Nakanojo, Shibutoge Pass, 42.573644, 138.538792, 2110 m, 9 Oct 2020, on dead stems of *Aruncus dioicus* (Walter) Fernald var. *kamtschaticus*, Y. Tochihara (TNS-F-81851).

Other specimen examined. JAPAN, Gunma, Agatsuma, Nakanojo, Damashidaira, 36.664058, 138.545272 (nearby the holotype locality), 2040 m, 15 Sep 2021, on dead stems of *A. dioicus* var. *kamtschaticus*, Y. Tochihara (TNS-F-81904).

Etymology. Referring to the conspicuously orange-colored apothecia.

Description. Apothecia scattered on the substrate, superficial, minute, cup-shaped, 0.2–0.8 mm in diameter when fresh and dry, with white to cream stipes, up to 1 mm high, with white to orange excipulums, externally covered with tightened white and capitate hairs; stipes cream to yellow with brown bases. Disc concave, orange, usually invisible from the outside due to encompassing external hairs. Ectal excipulum *textura prismatica* composed of hyaline cubic cells arranged in parallel rows; cells 4–35 × 3–20 µm, with granulated surface, containing small to large oil drops of carotenoids; cytoplasm often almost fulfilled by the oil drops, thick-walled; wall up to 2.5 µm thick.

Medullary excipulum *textura intricata* of hyaline hyphae up to 4 µm wide. Hairs straight, cylindrical, up to 270 × 6–9 µm, densely septate, hyaline, completely granulated, elongating percurrently and forming secondary hairs with granulate surfaces, containing orange oil drops of carotenoids, very-thick-walled; wall up to 3.5 µm thick; apex blunt or a rapidly becoming thinner, equipped with crystal masses; crystals irregular-shaped, easily detached by squash mounts, dissolvable in LA mounts. Asci 8-spored, cylindrical-clavate, (60–)66.5–80.9(–93) × (5.5–)6.0–7.2(–8) µm (av. ± s.d. 73.7 ± 7.2 × 6.6 ± 0.6 µm, n = 20) µm, relatively thick-walled; apical pore no-coloration in MLZ and IKI with or without 3% KOH pretreatment; croziers present at the basal septa. Ascospores (9.5–)9.6–11.4(–13) × (2.7–)2.8–3.4(–3.7) µm (av. ± s.d. 10.5 ± 0.9 × 3.1 ± 0.3 µm, n = 20), Q = (2.6–)3–3.7(–4.3) (av. ± s.d. 3.4 ± 0.3, n = 20), ellipsoid, aseptate, containing conspicuous lipid bodies. Paraphyses straight, cylindrical to narrowly lanceolate, septate, up to 3.5 µm wide, anastomosed with other paraphyses both in the bases and apical parts, containing conspicuous orange oil drops of carotenoids, rarely exceeding the asci.

Ecology. Saprotrophic on dead stems of herbaceous plants (only discovered on *A. dioicus* var. *kamtschaticus* at this point). Forming apothecia in autumn.

Distribution. JAPAN (Gunma; subalpine zone in Nakanojo-machi).

Notes. In *Incrucipulum*, only *I. sinegoricum* has been reported as occurring on dead stems of herbaceous plants. The present fungus is the second report of ebuliculous member of *Incrucipulum*. *Incrucipulum aurantiacum* apparently differs from *I. sinegoricum* morphologically (Raitviir 1977) in larger asci and much longer hairs. Having orange oil drops within hairs, paraphyses, and ectal excipulums also characterize *I. aurantiacum*, because Raitviir (1977) did not describe such character for *I. sinegoricum*.

***Incrucipulum brevistipitatum* Tochiara, sp. nov.**

Mycobank No: Undesignated

Figs. 29, 30

Diagnosis. Characterized by granulated ectal excipulums like stone pavings, lanceolate paraphyses, and host selectivity to grasses.

Holotype. JAPAN, Hokkaido, Kamikawa, Mt. Niseikaushuppe, 43.775367, 142.960842, 1500 m, 19 Aug 2020, on dead leaves of *Calamagrostis sachalinensis* F. Schmidt., Y. Tochiara (TNS-F-81832).

GenBank/UNITE no. ex holotype. Undesignated.

Etymology. Referring to the short stipes.

Description. Apothecia scattered on the substrate, superficial, cup-shaped, 0.2–0.7 mm in diameter when fresh and dry, having short and bold stipes, up to 0.5 mm high, externally covered with hairs, white to pale cream when fresh and dry. Disc concave, pale orange when fresh, becoming darker when dry. Ectal excipulum *textura prismatica* composed of hyaline cubic cells arranged in parallel rows; cells $3\text{--}15 \times 3\text{--}12 \mu\text{m}$, containing large yellow to orange drops of carotenoids, thick-walled; wall $1\text{--}2 \mu\text{m}$ thick; surfaces granulated, but sometimes completely lacking granules. Medullary excipulum *textura intricata* of hyaline hyphae up to $4 \mu\text{m}$ wide. Hairs straight, cylindrical, up to $180 \times 4\text{--}8 \mu\text{m}$, sometimes becoming thinner toward the base, densely to coarsely septate, hyaline, completely granulated in the young state, coarsely granulated or no-granulated except for the basal cells in the mature state, containing yellow to orange lipid bodies of carotenoids especially in the young state, thick-walled up to $1.8 \mu\text{m}$ thick; walls sometimes becoming thicker in the apical cells; apex blunt or a little bit swollen, equipped with crystals; crystals irregular-shaped, dissolvable in LA mount, easily detached by squash mounts. Asci $(46\text{--})48.9\text{--}55.7(59) \times (4.5\text{--})4.9\text{--}5.7(6)$ (av. $\pm$ s.d. $52.3 \pm 3.4 \times 5.3 \pm 0.4 \mu\text{m}$, $n = 20$) μm , 8-spored, cylindrical-clavate with slightly protruding apices; apical pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores $(9.5\text{--})10.3\text{--}12.8(14.4) \times (1.9\text{--})2\text{--}2.3(2.5) \mu\text{m}$ (av. $\pm$ s.d. $11.6 \pm 1.3 \times 2.1 \pm 0.2 \mu\text{m}$, $n = 20$), $Q = (4.5\text{--})4.9\text{--}6.1(6.9)$ (av. $\pm$ s.d. 5.5 ± 0.6 , $n = 20$) μm , fusiform, aseptate, containing small lipid bodies. Paraphyses straight, long-slender, lanceolate, septate, up to $4.5 \mu\text{m}$ wide, exceeding the asci by up to $30 \mu\text{m}$.

Ecology. Saprotrophic on dead leaves of Poaceae spp.

Distribution. JAPAN (Hokkaido). Known only from type locality.

Notes. *Incrucipulum brevistipitatum* resembles *I. densiseptatum* and *I. saccardoii* in that having lanceolate paraphyses and occurring on the similar habitats (dead leaves of grasses). However, *I. brevistipitatum* has larger asci than the two species (Raitviir and Sacconi 1991; Raitviir 2006). Additionally, *I. brevistipitatum* differs from *I. densiseptatum* in lacking croziers at the basal septa of asci, and lacking lipid bodies of carotenoids in paraphyses.

***Incrucipulum capitatum* (Peck) Baral, Beih. Z. Mykol. 6: 72. 1985. [new to Japan]**
MycoBank No: MB104378

Figs. 31, 32

Peziza capitata Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 30: 60. 1878 [1877].

Trichopeziza capitata (Peck) Sacc., Syll. fung. (Abellini) 8: 417. 1889.
Dasyscyphus capitatus (Peck) Le Gal, Revue Mycol., Paris 4: 29. 1939.
Lachnella capitata (Peck) Seaver, North American Cup-fungi (Inoperculates) (New York): 254. 1951.
Lachnum capitatum (Peck) Svrček, Česká Mykol. 39(4): 214. 1985.
Atractobolus scintillans (Masse) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 446. 1898.
Dasyscyphus scintillans Masse [as '*Dasyscypha*'], Brit. Fung.-Fl. (London) 4: 328. 1895.

Japanese name. Hime-futoge-hina-no-chawantake (designated here).

Description. Apothecia scattered, superficial, very minute, at first spherical and later urceolate, 0.1–0.2 mm in diameter, subsessile to sessile, up to 0.2 mm high, externally covered with short, white, stiff, capitate hairs. Disc concave, almost enclosed by an incurving margin when fresh and dry. Ectal excipulum *textura prismatica* like stone pavings with granulated surface; cells 3–10 × 5–13 µm, hyaline, thick-walled up to 2 µm wide, arranged in parallel rows. Medullary excipulum *textura intricata* of hyaline hyphae up to 2 µm wide. Hairs straight, cylindrical with rounded apices, 55–80 × 5.5–7.5 µm, 2–3-septate, hyaline, totally granulate, thin to slightly thick-walled towards the base, very thick-walled up to 3 µm wide in one or two cells at the apices; apex crowned by crystal masses easily detached in squash mounts. Asci (25–)26.8–31.9 (–32) × (2.8–)2.9–3.5 (–3.7) µm (av. ± s.d. 29 ± 2.5 × 3.2 ± 0.3 µm, n = 20), 8-spored, cylindrical-clavate; pore blue in MLZ without 3 % KOH pretreatment; croziers absent at the basal septa. Ascospores (5.3–)6.2–8.8 (–9.5) × 1–1.3 µm (av. ± s.d. 7.5 ± 1.3 × 1.1 ± 0.1 µm, n = 20), Q = (4.1–)5.4–7.8 (–8.6) (av. ± s.d. 6.6 ± 1.2, n = 20), slightly long fusiform, aseptate, containing some small lipid bodies. Paraphyses straight, lanceolate, up to 4 (–5) µm wide, aseptate, exceeding the asci by 20 µm.

Specimen examined. JAPAN, Hokkaido, Sapporo, Jozankei, 42.95624, 141.153753, 310 m, 17 Jun 2018, on dead leaves of *Quercus crispula*, T. Hosoya and Y. Tochiara (TNS-F-81420).

Ecology. Saprophytic on dead leaves of *Quercus* spp.

Distribution. ARMENIA*, BELGIUM*, ESTONIA***, FRANCE*, GERMANY*, JAPAN (this study), LITHUANIA*, NETHERLANDS*, UK***, USA***, VENEZUELA* (Peck 1878; Dennis 1949; Seaver 1951).

Notes. *Incrucipulum capitatum* is characterized by having lanceolate paraphyses, very-thick-walled and short hairs, and occurring on *Quercus* leaves. Morphology and the host plant agree with descriptions by Peck (1878), Dennis (1949), and Seaver

(1951). *Incrucipulum capitatum* is newly documented from Asia (including the Far East) in this study. In Japan, *Incrucipulum ciliare* (Schrader) Baral is present covering a wide area on *Quercus* leaves, while *I. capitatum* is rare. Since the host *Quercus* spp. are distributed in temperate zones, *I. capitatum* should be widely distributed in the temperate zones.

***Incrucipulum ciliare* (Schrader) Baral**, Beih. Z. Mykol. 6: 72. 1985.

MycoBank No: MB104379

Figs. 33, 34

Peziza ciliaris Schrad., J. Bot. (Schrader) 2 (1): 63. 1799.

Peziza ciliaris subsp. *ciliaris* Schrad., J. Bot. (Schrader) 2(1): 63. 1799.

Peziza ciliaris var. *ciliaris* Schrad., J. Bot. (Schrader) 2(1): 63. 1799.

Peziza ciliaris subsp. *globulifera* Pers., Mycol. eur. (Erlanga) 1: 253. 1822.

Peziza ciliaris var. *globulifera* Pers., Mycol. eur. (Erlanga) 1: 253. 1822.

Hyalopeziza ciliaris (Schrad.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 298. 1870.
[1869–70].

Trichopeziza ciliaris (Schrad.) Rehm, Ascomyceten: no. 258. 1875.

Lachnea ciliaris (Schrad.) Gillet, Champignons de France, Discom. 3: 68. 1880
[1879].

Erinella ciliaris (Schrad.) Quél., Enchir. fung. (Paris): 304. 1886.

Lachnella ciliaris (Schrad.) W. Phillips, Man. Brit. Discomyc. (London): 251.
1887.

Dasyscyphus ciliaris (Schrad.) Sacc., Syll. fung. (Abellini) 8: 443. 1889.

Dasyscyphus ciliaris var. *ciliaris* (Schrad.) Sacc., Syll. fung. (Abellini) 8: 443.
1889.

Lachnum ciliare (Schrad.) Rehm, Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.3(lief. 41):
877. 1896 [1893].

Lachnum ciliare var. *ciliare* (Schrad.) Rehm, Rabenh. Krypt.-Fl., Edn 2
(Leipzig) 1.3(lief. 41): 877. 1896 [1893].

Lachnum ciliare var. *carpineum* Mouton, Bull. Soc. R. Bot. Belg. 36 (no. 2): 20.
1897.

Atractobolus ciliaris (Schrad.) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 445. 1898.

Dasyscyphus ciliaris var. *carpineus* (Mouton) Sacc. [as '*carpineus*'], Syll.
fung. (Abellini) 14 (2): 779. 1899.

Description. Apothecia scattered on the substrate, superficial, cup-shaped, 0.2–0.4 mm in diameter when fresh and dry, having well-developed stipes, up to 0.4 mm high, externally covered with white, stiff, capitate hairs. Disc concave, white to cream-colored when fresh and concolorous when dry. Ectal exipulum *textra prismatica* composed of cubic cells like stone pavings with well-granulated surface, $5\text{--}10 \times 3\text{--}10 \mu\text{m}$, hyaline, very-thick-walled; thick wall up to $2 \mu\text{m}$; cells arranged in parallel rows. Medullary exipulum *textra intricata* of hyaline hyphae up to $2 \mu\text{m}$ wide. Hairs straight, cylindrical, up to $120 \times 4.5\text{--}7 \mu\text{m}$, 4–7-septate, hyaline, totally granulated, thin-walled; wall up to $1 \mu\text{m}$ thick; apex crowned by crystal masses or rarely tetrahedral crystals easily detached in squash mounts. Asci $47\text{--}67 \times 5\text{--}6.5 \mu\text{m}$, 8-spored, cylindrical-clavate; pore blue in MLZ without 3% KOH pretreatment, usually branched at the base; croziers absent at the basal septa. Ascospores $16\text{--}22.5 \times 2\text{--}3 \mu\text{m}$, narrow fusiform, aseptate or 1-septate, containing large lipid bodies. Paraphyses straight, cylindrical to narrowly lanceolate, up to $3 \mu\text{m}$ wide, exceeding the asci up to $7 \mu\text{m}$.

Ecology. Saprophytic on dead leaves of deciduous trees, mainly on fagaceous leaves.

Specimen examined. JAPAN, Hokkaido, Kamikawa, Kamikawa, Mt. Niseikaushuppe, on dead leaves of *Betula ermanii* Cham., 43.775367, 142.960842, 1500 m, 1 Aug 2017, Y. Tochihara (TNS-F-81307). JAPAN, Saitama, Chichibu, Ogano, Mt. Futagoyama, on dead leaves of *Quercus crispula*, 36.068214, 138.867697, 1030 m, 12 Aug 2018, Y. Tochihara, (TNS-F-81514). JAPAN, Saitama, Chichibu, Ogano, Mt. Futagoyama, on dead leaves of *Castanea crenata*, 36.070367, 138.867111, 940 m, 12 Aug 2018, Y. Tochihara, (TNS-F-81516). JAPAN, Shizuoka, Shizuoka, Aoi, Umegashima, on dead leaves of *Q. crispula*, 35.308545, 138.338374, 910 m, 18 Aug 2018, Y. Tochihara, (TNS-F-81520).

Distribution. GBIF (<https://www.gbif.org>, accessed: 20 September 2021) registered 550 occurrences collected in the entire area of Europe, East Coast of USA and Japan. Probably distributed all over the world.

Notes. The present fungus was recorded from Japan by Otani (1967) for the first time. Three specimens examined in this study matched well with preceding descriptions (Saccardo 1889; Dennis 1949; Otani 1967). ITS sequences of the three examined specimens showed high similarity (> 99%) with that of a German material (H.B. 9922).

Incrucipulum foliicola Tochihara, sp. nov.

MycoBank No: MB829358

Diagnosis. Forming yellow minute apothecia on dead leaves of *Myrica gale* subsp. *tomentosa* (C.DC.) E. Murray and has hairs with cross-shaped crystals.

Holotype. JAPAN, Hokkaido, Yamakoshi, Oshamanbe, Shizukari Mire, 42.573644, 140.430907, 5 m, 26 Jun 2018, on dead leaves of *M. gale* subsp. *tomentosa*, Y. Tochiara (TNS-F-81526).

GenBank no. ex holotype. LC438569 (ITS), LC438586 (LSU), LC438599 (RPB2).

Paratype. JAPAN, Hokkaido, Nemuro, Cape Ochiishi Mire, 43.166311, 145.512608, 50 m, 20 Jul 2018, on dead leaves of *M. gale* subsp. *tomentosa*, Y. Tochiara (TNS-F-81508).

Etymology. Referring to occurring on the leaves of the host plants.

Description. Apothecia scattered on the substrate, superficial, cup-shaped, 0.1–0.4 mm in diameter when fresh and dry, having long and slender stipes, up to 0.6 mm high, externally covered with hairs, lemon yellow throughout when fresh and dry. Disc concave, concolourous with external receptacle. Ectal excipulum *textura prismatica* composed of hyaline cubic cells with granulated surface arranged in parallel rows, thick-walled; wall up to 2 µm thick. Medullary excipulum *textura intricata* of hyaline hyphae up to 2 µm wide. Hairs straight, cylindrical, up to 90 × 3–5 µm, densely septate, hyaline, granulated throughout, thick-walled; wall up to 2 µm thick; individual cells 2.5–10 µm long; apex blunt or a little bit swollen, equipped with one cross-shaped crystal like “cross shuriken” ca. 10 µm across or one tetrahedral crystal detached easily in squash mounts. Asci 27–37 × 2.5–4 µm, 8-spored, cylindrical-clavate with slightly protruding apices; apical pore blue in MLZ without 3% KOH pretreatment; croziers present at the basal septa. Ascospores 5–8 × 1.2–1.5 µm, long ellipsoid to narrowly fusiform, aseptate, containing some small lipid bodies. Paraphyses straight, lanceolate, septate, up to 4 µm wide, exceeding the asci up to 25 µm.

Ecology. Saprotrophic on dead leaves of *M. gale* subsp. *tomentosa*.

Distribution. JAPAN (Hokkaido).

Notes. *Incrucipulum foliicola*, *I. pseudosulphurellum*, and *I. sulphurellum* occur on *Myrica gale* L. (including *M. gale* subsp. *tomentosa*) and share tetrahedral or cross-shaped crystals of hairs (Figs. 35F, 36C) as a common character. *Incrucipulum foliicola* much resembles *I. sulphurellum* which differs in the absence of croziers and parts of host, based on the original description (Peck 1878) and redescription of Haines (1989). *Incrucipulum foliicola* differs from *I. pseudosulphurellum* in shorter stipes, the

presence of croziers, hyaline ectal excipular cells and parts of host.

***Incrucipulum hakonechloae-macrae* Tochiara, sp. nov.**

MycoBank No: MB829308

Figs. 37, 38

Diagnosis. Characterized by filiiform paraphyses and occurring on grass leaves.

Holotype. JAPAN, Saitama, Chichibu, Ogano, Mt. Futagoyama, on dead leaves and culms of *H. macra*, 36.068342, 138.867364, 1010 m, 12 Aug 2018, Y. Tochiara (TNS-F-81512).

GenBank no. ex holotype. LC438564 (ITS), LC438581 (LSU), LC438594 (RPB2).

Another specimen examined. The same locality as holotype, 19 Aug 2017 (TNS-F81318).

Etymology. Referring to the scientific name of host plant.

Description. Apothecia scattered on the substrate, superficial, cup-shaped, 0.2–0.4(–0.5) mm in diameter, externally covered by short, white, capitate hairs bearing drops of dew when moist, stipitate, up to 0.2–0.5 mm high when fresh and dry; stipe white, sometimes brown in the lower part. Disc concave, lemon yellow to orange when fresh and dry. Ectal excipulum *textura prismatica* like stone pavings arranged in a row; cells $2.5\text{--}15 \times 5\text{--}13\text{ }\mu\text{m}$, hyaline, containing yellow oil drops, thick-walled; wall up to $3\text{ }\mu\text{m}$ thick with granulate surface; cells arranged in parallel rows. Medullary excipulum *textura intricata* of hyaline hyphae up to $2\text{ }\mu\text{m}$ wide. Hairs straight, cylindrical, sometimes with rounded apices, $38\text{--}90 \times 3\text{--}8\text{ }\mu\text{m}$, 2–5-septate, hyaline, completely covered by granules, thin to relatively thick-walled; wall up to $1.5\text{ }\mu\text{m}$ thick; apices blunt or a little bit swelled, bearing amber crystal caps detached easily in squash mounts. Asci $46\text{--}63(\text{--}75) \times 6\text{--}10\text{ }\mu\text{m}$, 8-spored, cylindrical-clavate; apical pore blue in MLZ without 3% KOH pretreatment; croziers present at the basal septa. Ascospores $(9\text{--})10.3\text{--}12.9(\text{--}14.7) \times (1.6\text{--})2.1\text{--}2.6(\text{--}2.8)\text{ }\mu\text{m}$ (av. $\pm$ s.d. $11.6 \pm 1.3 \times 2.4 \pm 0.3\text{ }\mu\text{m}$, $n = 20$), $Q = (3.5\text{--})4.3\text{--}5.8(\text{--}7.0)$ (av. $\pm$ s.d. 5 ± 0.7 , $n = 20$) μm , fusiform, aseptate or rarely 1-septate, usually packed by gelatinous sheath, containing large lipid bodies conspicuous in the CBLA mount. Paraphyses straight, cylindrical to narrowly lanceolate, rarely swollen at the apices, sometimes branched near the bases, septate, up to $3\text{ }\mu\text{m}$ wide, containing hyaline to orange oil drops of carotenoids, exceeding the asci up to $7.5\text{ }\mu\text{m}$.

Ecology. Saprotrophic on fallen and damp leaves, culms and other leaf-like

parts of *Hakonechloa macra* (Munro) Honda stuck to the ground.

Distribution. JAPAN (Saitama; Chichibu).

Notes. *Incrucipulum hakonechloae-macrae* is distinguishable from other species of *Incrucipulum* in yellow to orange disc (Fig. 37B) and ascospores containing very large oil drops and packed by gel sheath (Figs. 37G, H). Ascospores did not germinate on PDA at all. Some spores germinated on water agar or corn meal agar, but no further extension of hyphae was observed.

Incrucipulum hakonechloae-macrae only occurs on dead leaves of *H. macra*, an endemic grass in the central Honshu, JAPAN (Kato and Ebihara 2011). *Incrucipulum hakonechloae-macrae* is possibly also endemic to the Honshu Island corresponding to the host distribution because most of lachnaceous species have strong host selectivity.

***Incrucipulum lespedezae* (Raitv.) Tochiara, comb. nov.**

MycoBank No: Undesignated.

Dasyscyphus lespedezae Raitv., Kew Bull. 31(3): 691. 1977.

Ecology. Saprotrophic on fallen leaves of *Lespedeza bicolor* Turcz.

Distribution. RUSSIA (Bogataya Griva)**. Known only from the type locality.

Notes. The present fungus was initially described by Raitviir (1977) based on a specimen collected in the Province of Primorskaya in 1970 (TAA 61030) and has not been reported since then. Raitviir (1977) discussed the species differed from the related species *I. capitatum* (referred as ‘*D. capitatus*’) in fusoid ascospores (vs cylindric-clavate), larger asci, and extremely longer hairs. I additionally point out one more important difference that the present fungus has cylindrical paraphyses, while *I. capitatum* has articulate lanceolate paraphyses.

Based on the description and line drawings by Raitviir (1977), the present fungus has characteristic features in *Incrucipulum*, such as thick-walled crystallized hairs and stone-paving-like ectal excipulums composed of prismatic cells. Thus, I am convinced that the fungus is a member of *Incrucipulum*, while I did not examine any specimen of the species, and herein transfer the species into *Incrucipulum*.

For this species, Raitviir (1986) tried to propose a recombined name ‘*Lachnum lespedezae* (Raitv.) Raitv.’ However, as he accidentally cited an incorrect basynonym ‘*Dasyscyphus narynicus* Raitv., Scripta Mycologica 1: 87 (1970)’, and ‘*L. lespedezae*’ is

not a valid synonym of *D. lespedezae*. *Dasyscyphus narynicus* was originally described by Raitviir (1970) based on a specimen collected in 1967 (TAA 44591) and is apparently different from *I. lespedezae*.

***Incrucipulum pseudosulphurellum* Tochihara, sp. nov.**

MycoBank No: MB829362

Figs. 39, 40

Diagnosis. Characterized by wooly and shortly stipitate apothecia on fallen twigs of *M. gale* subsp. *tomentosa*.

Holotype. JAPAN, Hokkaido, Yamakoshi, Oshamanbe, Shizukari Mire, 42.573644, 140.430907, 5 m, 26 Jun 2018, on dead twigs of *M. gale* subsp. *tomentosa*, Y. Tochihara (TNS-F-81441).

GenBank no. ex holotype. LC438570, LC438563 (ITS), LC438587, LC438588 (LSU), LC438600, LC438601 (RPB2).

Other specimens examined. JAPAN, Aomori, Tashiro tai Wetland, 40.694692, 140.917911, 570 m, 9 Jul 2019, on dead twigs of *M. gale* subsp. *tomentosa*, Y. Tochihara (TNS-F-81672, Paratype). JAPAN, Hokkaido, Bibai, Bibai Mire, 43.322636, 141.813347, 15 m, 23 Jul 2019, on dead twigs of *M. gale* subsp. *tomentosa*, Y. Tochihara (TNS-F-81699).

Etymology. Referring to the similarity to *I. sulphurellum*.

Description. Apothecia scattered, superficial, 0.4–0.8 mm in diameter, having short and dark brown stipes, up to 0.5 mm high, white to somewhat dull yellow on the upper part and thin to dark brown in the lower part, densely covered by white, capitate hairs. Disc concave, pure white to orange when fresh and deep orange when dry. Ectal excipulum *textura prismatica* to *t. angularis*, 4–21 × 3–15 µm, hyaline in the upper part and subhyaline to brown in the lower part, slightly thick-walled; wall up to 2 µm thick; surface smooth or rarely equipped with sparse granules. Medullary excipulum *textura intricata* of hyaline hyphae up to 2 µm wide. Hairs straight, cylindrical with slightly rounded apices, up to 130 × 4.5–7.5 µm, completely granulate, multiseptate, arising from rounded basal cells; hair cells 5–25 µm long, thin to slightly thick-walled; wall up to 1.5 µm thick; apical cells thick-walled; wall up to 2 µm thick; apices bearing various shapes of crystals easily detached by squash mounts, i.e. tetrahedral ones, cross-shaped ones like ‘cross shuriken’ or amber crystal masses. Asci 33–44(–52) × 3.5–7.5 µm, 8-spored, cylindrical-clavate; pore blue in MLZ without 3% KOH pretreatment; croziers absent, but base sometimes irregularly branched and forming ‘fake-croziers’.

Ascospores (7–)7.4–9.4(–10.6) × (1.5–)1.7–2.1(–2.3) µm (av. ± s.d. 8.4 ± 1.0 × 1.9 ± 0.2 µm, n = 20), Q = (3.8–)4.0–4.8(–5.1) (av. ± s.d. 4.3 ± 0.4, n = 20) µm, oblong to narrowly fusiform, aseptate. Paraphyses straight, lanceolate, up to 5.5 µm wide, exceeding the asci by 25 µm.

Ecology. Saprotrophic on dead twigs of *M. gale* subsp. *tomentosa*. Occurring on the same habitat as that of *I. sulphurellum*.

Distribution. JAPAN (Hokkaido).

Notes. *Incrucipulum pseudosulphurellum* resembles *I. foliicola* and *I. sulphurellum* but differs in short and dark brown stipes (Figs. 39A–D), brownish ectal excipulum rarely equipped with granules (Figs. 39L–M). Little granules on ectal excipular cells is an atypical character of *Incrucipulum*, but other characters agree with generic definition.

Wooly appearance and short stipes of this species are macroscopically similar to *Capitotricha bicolor* (Bulliard) Baral but differs in smaller asci and lacking orange oil drops in paraphyses.

***Incrucipulum sulphurellum* (Peck) Baral**, in Baral & Kriegelsteiner, Beih. Z. Mykol. 6: 72. 1985. [new to Japan]
MycoBank No: MB104380

Figs. 41, 42

Peziza sulphurella Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 30: 59. 1878 [1877].

Dasyscyphus sulphurellus (Peck) Sacc. [as '*Dasyscypha*'], Syll. fung. (Abellini) 8: 459. 1889.

Atractobolus sulfurellus (Peck) Kuntze, Revis. gen. pl. (Leipzig) 3: 446. 1898.

Lachnum sulphurellum (Peck) Raitv., Folia cryptog. Estonica 20: 2. 1986.

Belonidium sulphurellum (Peck) Raitv. & Leenurm, Folia cryptog. Estonica 37: 88. 2001 [2000].

Peziza crucifera W. Phillips, Gard. Chron., N.S. 10: 397. 1878.

Dasyscyphus cruciferus (W. Phillips) Sacc. [as '*Dasyscypha crucifera*'], Gard. Chron., N.S. 10: 397. 1878.

Lachnella crucifera (W. Phillips) W. Phillips, Man. Brit. Discomyc. (London): 250. 1887.

Atractobolus crucifer (W. Phillips) Kuntze, Revis. gen. pl. (Leipzig) 3: 445. 1898.

Lachnum cruciferum (W. Phillips) Nannf., Trans. Br. mycol. Soc. 20: 194. 1936.

Description. Apothecia gregarious, superficial, cup-shaped, 0.3–0.7 mm in diam, stipitate, up to 0.5 mm high, cream to yellow when fresh and dry, externally covered with capitate hairs. Disc concave, white, cream to pale yellow when fresh and dry. Ectal excipulum *textura globosa*, *t. prismatica* to *t. angularis* composed of thick-walled cells resembling stone paving; cells $4\text{--}18 \times 4\text{--}10 \mu\text{m}$, hyaline, with granulated surfaces. Medullary excipulum *textura intricata* of hyaline hyphae up to $1.5 \mu\text{m}$ wide. Hairs straight, cylindrical with slightly rounded apices, arising from swollen basal cells, up to $120 \times 4\text{--}6 \mu\text{m}$, hyaline, totally granulate, thin- to relatively thick-walled (up to $1 \mu\text{m}$ thick), densely septate, equipped with crystals easily detached and broken in squash mounts; crystals $7.5\text{--}15\text{--}(25) \mu\text{m}$ across in diam, cross-shaped like a shuriken (Japanese traditional weapon), regular octahedron or irregularly shaped, easily dissolved in LA mounts. Asci 8-spored, cylindrical-clavate, $(31\text{--})32\text{--}35.8\text{--}(37) \times 3\text{--}3.6\text{--}(4) \mu\text{m}$ (av. $\pm$ s.d. $33.9 \pm 1.9 \times 3.3 \pm 0.3 \mu\text{m}$, $n = 20$); apical pores blue with MLZ without 3% (w/v) KOH pretreatment; croziers absent at the basal septa. Ascospores $(5\text{--})5.8\text{--}8.5\text{--}(9.5) \times (0.8\text{--})0.9\text{--}1.1\text{--}(1.2) \mu\text{m}$ (av. $\pm$ s.d. $7.2 \pm 1.4 \times 1.0 \pm 0.1 \mu\text{m}$, $n = 21$), $Q = (5\text{--})5.6\text{--}9.2\text{--}(11.9)$ (av. $\pm$ s.d. 7.4 ± 1.8 , $n = 21$), long ellipsoid to fusiform, aseptate or rarely one-septate. Paraphyses straight, lanceolate, up to $5 \mu\text{m}$ wide, septate, exceeding the asci by $12\text{--}20 \mu\text{m}$.

Specimens examined. All materials on dead twigs of *Myrica gale* L. subsp. *tomentosa* C. DC., Y. Tochiara. JAPAN, Hokkaido, Nemuro, Cape Ochiishi Mire (high moor), 43.167403, 145.510625, 30 m, 6 Jun 2019 (TNS-F-81577); Hokkaido, Akkeshi, Hamanaka, Biwase District of the Kiritappu Wetland (mire, low moor), 43.064764, 145.082622, 3 m, 20 Jun 2019 (TNS-F-81621); Hokkaido, Noboribetsu, Kiushito Wetland (mire, low moor), 42.389017, 141.07945, 2 m, 20 Jul 2019 (TNS-F-81685); Hokkaido, Yamakoshi, Oshamambe, Shizukari Mire (high moor), 42.571086, 140.430603, 2 m, 21 Jul 2019 (TNS-F-81690); Hokkaido, Soya, Sarufutsu, Sarufutsu-River Mire (high moor), 45.172867, 142.135554, 20 m, 22 Jul 2019 (TNS-F-81695); Hokkaido, Bibai, Bibai Mire (high moor), 43.322636, 141.813347, 15 m, 23 Jul 2019 (TNS-F-81698); Hokkaido, Sapporo, Shinoro-Fukui Wetland (mire, low moor), 43.152586, 141.41815, 10 m, 23 Jul 2019 (TNS-F-81700); Hokkaido, Shari, Koshimizu, Hama-Koshimizu, east side of Lake Tofutsu (marsh), 43.924533, 144.432769, 1 m, 7 Aug 2019 (TNS-F-81725); Aomori, Kamikita, Rokkasho, anonymous wetland (marsh, sunny place probably without peat), 40.901989, 141.371325, 9 m, 8 Jul 2019 (TNS-F-81663); Aomori, Aomori, Tashirotai Wetland (mire, high moor), 40.694692, 140.917911, 570 m, 9 Jul 2019 (TNS-F-81671); Gunma, Tone, Katashina, Ozegahara Mire (mire, high moor), 36.921469, 139.207589, 1390 m, 16 Aug 2019 (TNS-F-81735).

Ecology. Saprotrophic on dead twigs of *Myrica gale* subsp. *tomentosa* occurring on mires (low to high moor) or marsh.

Distribution. CANADA*, ESTONIA*, GERMANY*, Northern Europe (DENMARK*, the NETHERLANDS*, NORWAY*, SWEDEN***), RUSSIA (Botchinsky Nature Reserve**, Sakhalin Island**), USA*** (Nannfeldt 1936; Dennis 1949; Raitviir 2006; Bogacheva 2017).

Notes. In Japan, *I. sulphurellum* was collected from 10 localities of cool-temperate or subarctic wetlands. Apothecial morphology of *I. sulphurellum* from Japanese materials matched well with the descriptions of Peck (1878), Dennis (1949), and Raitviir (2006). *Incrucipulum sulphurellum* resembles *I. foliicola* and *I. pseudosulphurellum* occurring on the same substrate (residues of *M. gale* var. *tomentosa*) and sometimes coexists with them. However, *I. sulphurellum* differs from *I. foliicola* in lacking croziers at the ascal base and occurring on fallen twigs (not on leaves as in *I. foliicola*). *Incrucipulum sulphurellum* also differs from *I. pseudosulphurellum* in stipitate and hyaline ectal excipular cells.

Raitviir (2006) reported that all the fresh apothecia he observed were pure white. He concluded that sulfur-colored apothecia in the original (Peck 1878) or later descriptions (Dennis 1949) were possibly observed in the dried and tarnished state and advocated that the epithet “sulphurellum” was misleading. However, in Japanese specimens, I observed the co-existence of both pure white and sulfur-colored apothecia in the fresh state. I therefore presumed that the colors of fresh apothecia of *I. sulphurellum* are originally variable among individuals, and the observations of Raitviir (2006) was biased by sulfur-colored apothecia.

***Incrucipulum virtembergense* (Matheis) Baral**, in Baral & Kriegelsteiner, Beih. Z.

Mykol. 6: 73. 1985. [new to Japan]

MycoBank No: 104381.

Figs. 43, 44

Dasyscyphus virtembergensis Matheis, Sydowia 29(1–6): 240. 1977 [1976–77].

Lachnum virtembergense (Matheis) Raitv. [as '*virtembergensis*'], Folia cryptog.

Estonica 17: 4. 1985.

Belonidium virtembergense (Matheis) Raitv. & Leenurm, Folia cryptog. Estonica 37: 89. 2001 [2000].

Description. Apothecia scattered on the substrate, superficial, initially spherical, later

becoming cup-shaped, ultimately flat-disc-shaped, 0.2–1 mm in diameter when fresh and dry, having slender white to dark brown stipes, up to 0.8 mm high, externally covered by long-white-capitate hairs, white to yellow. Disc concave, white to yellow when fresh and dry. Ectal excipulum *textura prismatica* composed of well-developed square cells arranged in parallel rows; cells 3–25 × 2–14 µm, hyaline, with granulate surfaces, containing yellow oil drops of carotenoids; oil drops sometimes enlarging and fulfilling the whole volume of the cytoplasm; wall very thick up to 3 µm thick. Medullary excipulum *textura intricata* of hyaline hyphae up to 4 µm wide, containing yellow oil drops of carotenoids. Hairs straight, cylindrical, up to 300 × (4.5–)6–9 µm, septate, hyaline, granulated throughout, elongating percurrently and forming secondary hairs with granulate surfaces, having faint to remarkable inflation at arbitrary positions as traces of percurrent, scarcely containing small yellow oil drops of carotenoids, extremely-thick-walled; wall up to 3.5 µm thick; secondary hair sometimes much thinner compared to the primary hair; width not constant within one hair; apex blunt or a little bit swollen, never becoming acute, equipped with crystal masses easily detached in squash mounts and dissolved in the LA mount. Asci (46–)48.1–57.5 (–60) × (6–)6.2–7.5(–8) µm (av. ± s.d. 52.8 ± 4.7 × 6.9 ± 0.6 µm, n = 20), 8-spored, cylindrical-clavate, relatively thick-walled especially in the apex; apical pore no-coloration in MLZ without 3% KOH pretreatment, faintly blue in MLZ with 3% KOH treatment; croziers absent at the basal septa. Ascospores (10–)11.1–13.2 (–14) × (2.2–)2.4–3.9(–3) µm (av. ± s.d. 12.2 ± 1.1 × 2.6 ± 0.3 µm, n = 20), Q = (3.7–)4.1–5.2(–5.8) (av. ± s.d. 4.7 ± 0.6, n = 20), fusiform, aseptate, containing conspicuous lipid bodies. Paraphyses straight, cylindrical, septate, up to 2 µm wide, containing yellow oil drops of carotenoids, exceeding the asci up to 12 µm.

Specimen examined. JAPAN, Gunma, Agatsuma, Nakanojo, Yoshigadaira Wetlands, on dead leaves of *Vaccinium uliginosum* L. scattered on sphagnum mosses, 36.65629166, 138.54836944, 1840 m, 15 Sep 2021, Y. Tochihara (TNS-F-80905).

Ecology. Saprotrophic on dead leaves of *Vaccinium* spp. (only recorded on *V. myrtillus* L. and *V. uliginosum*) between sphagnum mosses. Forming apothecia in September (Matheis 1977; Baral and Krieglsteiner 1985; Raitviir 1991).

Distribution. ESTONIA*, FINLAND*, JAPAN (this study), NORWAY*, RUSSIA (Kamchatka)***, SWITZERLAND*, UK*, USA*.

Notes. *Incrucipulum virtembergense* resembles *I. longispineum* occurring on dead leaves of Ericaceae plants (Tanaka and Hosoya 2001). However, the former occurs only on *Vaccinium* spp., while the latter occurs only on *Lyonia ovalifolia* (Wall.) Drude var. *elliptica* (Siebold et Zucc.) Hand.-Mazz.

***Lachnellula pini* (Brunch.) Dennis**, Persoonia 2(2): 184. 1962.

MycoBank No: MB283118

Figs. 45, 46

Lachnella pini Brunch., Z. ParasitKde: 242. 1894.

Dasyscyphus pini (Brunch.) G.G. Hahn & Ayers [as '*Dasyscypha*'], Mycologia 26(6): 487. 1934.

Specimen examined. JAPAN, Hokkaido, Kamikawa, Kamikawa, Mt. Niseikaushuppe, 43.77527, 142.984378, 1800 m, on living twigs of *Pinus pumila* (Pall.) Regel. 19 Aug 2020, Y. Tochihara (TNS-F-81833).

Description. Apothecia scattered on the substrate, breaking out from the bark, superficial, cup-shaped with the extremely undulated margin, 1.2–4.3 mm in diameter when fresh and dry, substipitate to shortly stipitate, up to 1.5 mm high; the external part dark brown concolous with the host twigs, like leather or bark, densely covered by external hairs. Disc concave, bright orange when fresh and dry, somewhat cracked when dry. Ectal excipulum *textura angularis* composed of cuboid cells; cells $3\text{--}17 \times 2.5\text{--}9\text{ }\mu\text{m}$, hyaline near the medullary excipulum, becoming brown toward the lower part, with smooth surfaces, conspicuously thick-walled; wall up to $3\text{ }\mu\text{m}$ thick. Medullary excipulum *textura intricata* of hyaline hyphae up to $3.5\text{ }\mu\text{m}$ wide, containing orange lipid bodies of carotenoids. Hairs densely occurring from the ectal excipulum, irregularly bent, cylindrical with obtuse or acute apices, up to $280 \times 2.5\text{--}5.5\text{ }\mu\text{m}$, septate, branched, hyaline to dark brown, completely equipped with yellow to brown rough granules on surfaces, but sometimes less granules distributed near the apices compared to the basal parts, thick-walled, lacking any crystals or resinous materials. Asci $(115\text{--})117\text{--}126(\text{--}130) \times (9.5\text{--})9.7\text{--}10.7(\text{--}11.3)$ (av. $\pm$ s.d. $121.6 \pm 4.6 \times 10.2 \pm 0.5$, $n = 20$) μm , 8-spored, cylindrical-clavate, latively thick-walled; apical pore no-coloration in MLZ or IKI with or without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores $(15.2\text{--})16.8\text{--}21.1(\text{--}22.5) \times (4.7\text{--})5.3\text{--}6(\text{--}6.8)\text{ }\mu\text{m}$ (av. $\pm$ s.d. $19 \pm 2.2 \times 5.6 \pm 0.4\text{ }\mu\text{m}$, $n = 20$), $Q = (2.7\text{--})2.9\text{--}3.8(\text{--}4.1)$ (av. $\pm$ s.d. 3.4 ± 0.4 , $n = 20$) μm , ellipsoid, often acute toward the ends, aseptate, not containing conspicuous lipid bodies, packed by gel-sheathes not stained by CB mount. Paraphyses straight, filiform, septate, often branched like fingers, containing conspicuous orange lipid bodies of carotenoids, up to $3.5\text{ }\mu\text{m}$ wide, exceeding the asci up to $25\text{ }\mu\text{m}$.

Ecology. Parasitic on living twigs of Pinaceae.

Distribution. CANADA**, FINLAND*, GEORGIA**, JAPAN**, NORWAY*, RUSSIA***, SWEDEN*, SWITZERLAND*, USA*.

Notes. From Japan, the present fungus was reported by Saho and Takahashi (1972) and Oguchi (1981) from Hokkaido. Since specimens collected by them are lost, the present study is the first report with a specimen deposited in the public herbarium. ITS sequence of TNS-F-81833 showed high similarities (> 99.5%) with North American sequences of *L. pini* (MH855623 from USA, KX668534–KX668543 from Canada).

‘*Lachnum*’ novoguineense var. yunnanicum W.Y. Zhuang, Nova Hedwigia 78(3–4): 426. 2004. [new to Japan]

MycoBank No: MB487912; MB528296 [as ‘*L. novoguineense* var. *yunnanicum*’]

Figs. 47, 48

Diagnosis. Characterized by stiff and thick apothecia and dark orange disc occurring on dead bamboo sheathes, and longer ascospores compared to *E. sasibrevispora*.

Description. Apothecia gregarious, superficial, 1–2.5 mm in diameter, subsessile to short- and thick-stipitate, up to 1.5 mm high, pure white, externally covered with long white hairs. Disc concave, pale yellow to pale orange when fresh, turned darker when dry. Ectal excipulum *textura angularis* composed of cubic cells $3\text{--}15 \times 2.5\text{--}8 \mu\text{m}$, hyaline, thin-walled; surface smooth. Medullary excipulum very thick, composed of hyaline hyphae forming *textura intricata*; hyphae $5 \mu\text{m}$ wide. Hairs straight, delicate, cylindrical, over $200 \times 3.0 \mu\text{m}$, hyaline, totally granulate, thin-walled, branched, lacking any crystals, resinous matters or cap-like materials. Asci (91–)94–103.7 (–110) $\times$ (7–)7.2–8(–8.3) μm (av. $\pm$ s.d. $98.9 \pm 4.9 \times 7.6 \pm 0.4 \mu\text{m}$, $n = 20$) μm , 8-spored, cylindrical-clavate; pore blue in MLZ without 3% KOH pretreatment; croziers with perforation present at the basal septa. Ascospores $45\text{--}67 \times 1.5\text{--}2.0 \mu\text{m}$, fusiform to long filiform, aseptate to six-septate (usually three- or four-septate). Paraphyses straight, narrowly lanceolate, $3\text{--}3.5 \mu\text{m}$ wide, septate, exceeding the asci $10\text{--}17 \mu\text{m}$.

Specimen examined. JAPAN, Nagano, Ueda, Sugadaira Research Station, Mountain Science Center, University of Tsukuba, on fallen sheathes of *Sasa* sp. 36.541781, 138.355796, 1350 m, 18 May 2005, R. Sasagawa (TNS-F-16442). JAPAN, Ibaraki, Tsukuba, Mt. Tsukuba, on fallen sheathes of *Sasa* sp., 36.2247, 140.1083, 750 m, 2 May 2006, M. Fujisaki (TNS-F-16642).

Ecology. Saprotrophic on dead sheathes of bamboo.

Distribution. JAPAN (Kanto). China (Yunnan).

Notes. This fungus (TNS-F-16442, 16642) was referred in Hosoya et al. (2010) as

unidentified species '*Lachnum* sp. 1' and positioned in the clade of long-spored *Lachnum* (= *Erioscyphella*) in the phylogenetic analyses. In the present study, I re-examined the two specimens of this fungus and identified them as *Lachnum novoguineëns* var. *yunnanicum*, because the microscopic morphology and the habitat (= dead bamboo culms) matched well with the original description by Zhuang (2004). However, since this fungus has densely septate ascospores while *Lachnum novoguineëns* var. *yunnanicum* has aseptate ones and DNA sequences of Chinese materials are not accessible, I hesitated to conclude that the two are the same species.

'*Lachnum*' *palmae* (Kanouse) Spooner [sensu lato]

MycoBank: MB130961

Dasyscyphella palmae Kanouse, Mycologia 33(5): 464. 1941.

Dasyscyphus palmae (Kanouse) Dennis, Persoonia 2(2): 180. 1962.

Lachnum palmae (Kanouse) Spooner, Bibl. Mycol. 116: 484. 1987.

Ecology. Saprotrophic on fallen petioles of palm trees.

Distribution. BELIZE [type locality]***, BRAZIL*, CHINA***, COSTA RICA*, GUYANA*, JAPAN (Southern part)***, NEW ZEALAND*, PANAMA*. Probably widely distributed in accordance with the distribution of palm trees (Kanouse 1941; Fröhlich 1997; Tanaka and Hosoya 2001; Zhao et al. 2018).

Notes. *Lachnum palmae* [sensu lato] was originally described from Belize by Kanouse (1941). From Japan, Tanaka and Hosoya (2001) reported this species from 4 localities (Bonin Islands, Tokunoshima Island, Minami-daitojima Island, Hiuga Cape; DNA sequences not available) with a detailed description.

As mentioned in Chapter I, this species was suggested as a species complex. Two candidates of independent species belonging to the complex were described in detail below.

'*Lachnum*' *palmae* TNS-F-24600

Figs. 49, 50

Description. Apothecia gregarious, superficial, 0.3–1.0 mm in diameter, with stout stipes, up to 0.7 mm high, pure white when young, pale brown when matured, externally covered with long hairs. Disc concave, dark buff when dry (not observed in fresh state). Ectal excipulum up to 50 µm thick, hyaline, with smooth surfaces,

composed of two layers; outer layer of *textura prismatica* or *textura angularis* composed of long-elongated cells forming agglutinated appearance, $3\text{--}25 \times 2\text{--}10\text{ }\mu\text{m}$; inner layer of *textura porrecta* consisting of hyaline hyphae up to $5\text{ }\mu\text{m}$ wide. Medullary excipulum up to $80\text{ }\mu\text{m}$ thick, composed of hyaline hyphae forming *textura intricata*; hyphae up to $3\text{ }\mu\text{m}$ wide. Hairs straight, cylindrical, up to $150 \times 2.0\text{--}3.0\text{ }\mu\text{m}$, hyaline, totally granulate, thin-walled; apex sometimes equipped with large masses of resinous materials dissolved with CB/LA at any part of hair, also equipped with apical amorphous materials. Asci $(95\text{--})98.4\text{--}111(\text{--}125) \times (7.25\text{--})7.3\text{--}8(\text{--}8.8)$ (av. $\pm$ s.d. $104.7 \pm 6.3 \times 7.7 \pm 0.3$, $n = 20$) μm , 8-spored, cylindrical-clavate with sometimes acute apices, thick-walled, containing irregular-shaped (usually needle-like shaped) britning materials; pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores $(62\text{--})66.1\text{--}75.3(\text{--}78) \times (1.3\text{--})1.6\text{--}1.9(\text{--}2)$ μm (av. $\pm$ s.d. $70.7 \pm 4.6 \times 1.7 \pm 0.2$ μm , $n = 20$), $Q = (32.5\text{--})34.9\text{--}47.2(\text{--}57.7)$ (av. $\pm$ s.d. 41.1 ± 6.2 , $n = 20$), filiform and sometimes U- or S-shaped due to bent of both ends, 0- to 3-septate, vertically stored in upper two thirds parts of asci. Paraphyses straight, narrowly lanceolate, up to $3\text{ }\mu\text{m}$ wide, septate, exceeding the asci up to $15\text{ }\mu\text{m}$, containing irregular-shaped (usually needle-like shaped) britning materials.

Specimen examined. JAPAN, Kagoshima, Amami-Oshima Island, Sumiyou, on fallen leaves of *Livistona chinensis* var. *subglobosa*, 28.252417, 129.404722, 5 m, 25 Feb 2009, T. Hosoya (TNS-F-24600).

Notes. This Japanese material formed a single SH in all methods of species delimitation analyses (Fig. 3), indicating this fungus is an independing species. TNS-F-24600 had relatively longer asci than other Japanese materials of the species complex examined in this study and by Tanaka and Hosoya (2001). However, currently not enough distinguishable features are at hand to propose this fungus as a new species.

***'Lachnum' palmae* TNS-F-17567**

Figs. 51, 52

Description. Apothecia gregarious, superficial, $0.7\text{--}1.4\text{ mm}$ in diameter, shortly to longly stipitate, up to 2 mm high, externally dull cream, densely covered by short white hairs. Disc concave, dull orange when dry (fresh state not examined). Ectal excipulum composed of two layers; outer layer of *textura prismatica* or *textura angularis* composed of long-elongated cells forming agglutinated appearance; cells $4\text{--}25 \times 1.5\text{--}7\text{ }\mu\text{m}$; inner layer of *textura porrecta* composed of interwoven, undulating, parallel somewhat agglutinated hyphae; hyphae up to $4.5\text{ }\mu\text{m}$ wide. Medullary excipulum

composed of hyaline hyphae forming *textura intricata*; hyphae up to 4 μm wide. Hairs straight, cylindrical, sometimes with swelling apices, sometimes branched near the bases, up to 70 $\times$ 3–4 μm , hyaline, totally granulate, thin-walled; apex equipped with needle-like crystal masses and hyaline cap-like materials. Asci (107.5–)111–124.3 (–130) $\times$ (6.3–)7.3–9.2–9.5) (av. $\pm$ s.d. $117.7 \pm 6.6 \times 8.2 \pm 0.9 \mu\text{m}$, $n = 15$) μm , 8-spored, cylindrical-clavate, thick-walled, containing small irregular-shaped (usually needle-like shaped) britning materials especially in the young state; pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores (64–)67.4–73.1(–75) $\times$ (1.5–)1.7–2.1(–2.2) μm (av. $\pm$ s.d. $70.3 \pm 2.9 \times 1.9 \pm 0.2 \mu\text{m}$, $n = 20$), Q = (32–)33.3–40.9(–45.3) (av. $\pm$ s.d. 37.1 ± 3.8 , $n = 20$), filiform, straight or slightly curved, aseptate or rarely one-septate, containing numerous guttles. Paraphyses straight, cylindrical to narrowly lanceolate, up to 3.5 μm wide, aseptate except for basal parts, containing irregular-shaped (usually needle-like shaped) britning materials, exceeding the asci up to 15 μm .

Specimens examined. NEW ZEALAND, Waitakere Ranges, Cascades Track, on fallen leaves of palms, 28 May 2005, T. Hosoya & P. Johnston (TNS-F-17564, 17567).

Notes. This fungus morphologically much resembles TNS-F-24600 but has relatively larger asci. Based on species delimitation analyses, this fungus formed a single SH together with MH921862 at majority of 5 methods (Fig. 3). Since both TNS-F-17567 (and 17564) and MH921862 were from New Zealand, they may be characteristic species to New Zealand.

***Neolachnum* Tochihara & Hosoya, gen. nov.**

MycoBank No: Undesignated

Diagnosis. Characterized by crystallized hairs, but not apparently distinguished from *Lachnum* sensu stricto based only on morphology. DNA-based work is desirable to recognize members of this genus.

Type. *Neolachnum rhytismatis* (W. Phillips) Tochihara and Hosoya, comb nov.

Etymology. Referring to the similar morphology to *Lachnum* sensu stricto.

Description. Apothecia gregarious, solitary or fascicled, superficial, flat-disc-shaped with long stipes, externally covered with short and capitate hairs. Disc thin, concave, yellow to orange when fresh and dry. Ectal excipulum well-developed *textra prismatica*, hyaline, thick-walled, with smooth surfaces. Medullary excipulum *textra intricata* of hyaline hyphae. Hairs straight, cylindrical, hyaline, completely granulate, thin-walled, crowned by crystal masses; crystals dissolvable in LA mount, easily

detached by squash mounts. Asci 8-spored, cylindrical-clavate; pore blue in MLZ without 3% KOH pretreatment; croziers present or absent at the basal septa. Ascospores ellipsoid with sometimes acute apices, aseptate. Paraphyses straight, lanceolate, septate, long exceeding the asci.

***Neolachnum rhytismatis* (W. Phillips) Tochihara & Hosoya, comb nov.**

MycoBank No: Undesignated

Figs. 53, 54

Peziza rhytismatis W. Phillips [as '*rhytismae*'], Grevillea 8(no. 47): 101. 1880.

Lachnella rhytismatis (W. Phillips) W. Phillips, Man. Brit. Discomyc. (London): 250. 1887.

Dasyscyphus rhytismatis (W. Phillips) Sacc., Syll. fung. (Abellini) 8: 453. 1889.

Atractobolus rhytismatis (W. Phillips) Kuntze, Revis. gen. pl. (Leipzig) 3(3): 446. 1898.

Lachnum rhytismatis (W. Phillips) Nannf., Trans. Br. mycol. Soc. 23(3): 242. 1939.

Peziza minutissima P. Crouan & H. Crouan, Florule Finistère (Paris): 52. 1867.

Erinella minutissima Sacc., Syll. fung. (Abellini) 8: 508. 1889.

Dasyscyphus minutissimus (Sacc.) Le Gal [as '*Dasyscypha minutissima*'], Revue Mycol., Paris 18(1): 95. 1953.

Lachnum minutissimum (Sacc.) Baral, in Baral & Krieglsteiner, Beih. Z. Mykol. 6: 79. 1985.

Peziza echinulata Auersw., Hedwigia 7: 136. 1868.

Lachnella echinulata (Auersw.) W. Phillips, Man. Brit. Discomyc. (London): 249. 1887.

Hyalopeziza echinulata (Auersw.) Lambotte, Mém. Soc. roy. Sci. Liège, Série 2 14: 293 [prepr.]. 1888 [1887].

Description. Apothecia scattered on the substrate, minute, superficial, cup-shaped, 0.2–0.7 mm in diameter when dry (fresh state not examined), stipitate, up to 0.4 mm high, externally covered with short capitate hairs, white to pale cream when dry. Disc concave, pale orange when dry (fresh state not examined). Ectal excipulum well-developed *textura prismatica*; cells 3–25 × 2–8 µm, thin-walled, with smooth surfaces. Medullary excipulum *textura intricata* of hyaline hyphae up to 4.5 µm wide. Hairs straight, cylindrical, up to 85 × 3–4.5 µm, usually 2- to 4-septate, hyaline, granulated throughout, thin-walled; apex blunt, never swollen, equipped with globose crystal

masses easily detached in squash mounts and dissolvable in the LA mount. Asci (21–)24.6–31(–33) $\times$ (3.5–)3.6–4.3(–4.6) μm (av. $\pm$ s.d. $27.8 \pm 3.2 \times 4 \pm 3.7 \mu\text{m}$, $n = 20$), 8-spored, cylindrical-clavate; apical pore blue in MLZ without 3% KOH pretreatment; croziers with perforation present at the basal septa. Ascospores (4.6–)5.1–6.1(–7) $\times$ (1.2–)1.4–1.6(–1.7) μm (av. $\pm$ s.d. $5.6 \pm 0.5 \times 1.5 \pm 0.1 \mu\text{m}$, $n = 20$), $Q = (3.1–)3.3–4.3(–4.8)$ (av. $\pm$ s.d. 3.8 ± 0.5 , $n = 20$), drop-shaped (becoming narrower toward one end), aseptate, not containing lipid bodies. Paraphyses straight, lanceolate, septate, up to 4 μm wide, exceeding the asci up to 20 μm .

Specimens examined. SWITZERLAND, Lajoux, Sâces, on dead leaves of *Acer pseudoplatanoides*, 47.288569, 7.129244, 960 m, 4 Jun 2016, T. Hosoya (TNS-F-65645, 65655).

Ecology. Saprotrophic on dead leaves of broad-leaved trees, especially on dead leaves of *A. pseudoplatanus* or *Quercus* spp. (Dennis 1949).

Distribution. AUSTRIA*, CANADA*, COLOMBIA*, CZECH*, DENMARK*, ESTONIA*, FINLAND*, FRANCE*, GERMANY*, ITALY*, LËTZEBUERG*, the NEDERLAND*, NORWAY*, RUSSIA*, SLOVENIA*, SPAIN*, SWEDEN*, SWITZERLAND*, UK*, USA*.

Notes. The present fungus was originally described by Phillips and Plowright (1880) from Scotland in 1878 on leaves of *A. pseudoplatanus* (Phillips and Plowright 1880; Dennis 1949) and has widely been reported from the whole area of Europe. Specimens examined in this study well matched in macro and micromorphology with the original description (Phillips and Plowright 1880) and redescrptions by Dennis (1949) based on parts of original specimens. It occurred on the same host plants as the the original report (Phillips and Plowright 1880).

Hosoya et al. (2010) included this species (TNS-F-16545) in their phylogenetic analyses, but the specimens were misidentified *Capitotricha fagicola* (Yas. Ono & Hosoya) Tochiara & Hosoya (Tochiara 2019). This study confirmed the proper taxonomy based on the phylogenetic analysis.

***Neolachnum tenuipileum* Tochiara & Hosoya, sp. nov.** ($\leftarrow$ *Lachnum* sp. 1)

MycoBank No: undesignated

Figs 55, 56

Diagnosis. Characterized by thin-layered apothecia occurring on dead herbaceous plants.

Holotype. JAPAN, Hokkaido, Chitose, on fallen stems of *Fallopia sachalinensis*, 42.735397, 141.370056, 280 m, 19 Jun 2018, Y. Tochiara and T. Hosoya (TNS-F-81438).

GenBank/UNITE no. ex holotype. undesigned (ITS), undesigned (LSU), undesigned (mtSSU), undesigned (RPB2).

Etymology. Referring to the thin-layered apothecia.

Japanese name. Usu-hina-no-chawantake (designated here).

Description. Apothecia gregarious, solitary or fascicled, superficial, flat-disc-shaped with long stipes, 0.4–1.0 mm in diameter, up to 1.0 mm high, externally covered with short and capitate hairs. Disc thin, concave, yellow to orange when fresh and dry, sometimes denting like belly buttons. Ectal excipulum well-developed *textra prismatica*, 3–19 × 2–6 µm, hyaline, thick-walled up to 2 µm thick, with smooth surfaces. Medullary excipulum *textra intricata* of hyaline hyphae up to 4 µm wide. Hairs straight, cylindrical, up to 100 × 2–4.5 µm, hyaline, completely granulate, thin-walled, arising from swollen cells of ectal excipulums, crowned by crystals; crystals located on upper parts of hairs, irregular-shaped, dissolvable in LA mount, easily detached by squash mounts. Asci 8-spored, cylindrical-clavate, (32–)34.4–39.9(–42) × (2.5–)2.8–3.6(–4) µm (av. ± s.d. 37.1 ± 2.8 × 3.2 ± 0.4 µm, n = 20); pore blue in MLZ without 3% KOH pretreatment; croziers absent at the basal septa. Ascospores (4.8–)5.1–6.4(–7.2) × (1–)1.1–1.5(–1.6) µm (av. ± s.d. 5.7 ± 0.7 × 1.3 ± 0.2 µm, n = 20), Q = (3.2–)3.8–5(–5.4) (av. ± s.d. 4.4 ± 0.6, n = 20), ellipsoid, aseptate. Paraphyses straight, lanceolate, up to 4.5 µm wide, septate, exceeding the asci up to 25 µm.

Ecology. Saprotrophic on dead stems of tall herbaceous plants.

Distribution. JAPAN (Hokkaido, known only from type locality).

Notes. *Neolachnum tenuipileum* is easily distinguishable from *N. rhytismatis* in having larger asci and longer and slenderer stipes and occurring on different habitats.

***Trichopezizella japonica* (Syd. & P. Syd.) Tochihara & Hosoya, comb. nov.**

[**Solenopezziaceae**]

MycoBank no: MB832700

Fig. 57

Lachnum japonicum Syd. & P. Syd., Annls mycol. 10(4): 409. 1912.

Lectotype (examined). JAPAN, Gifu, Nakatsugawa, Kawaue, 23 Jan 1912, on dead stems of *Boehmeria nivea* (var. *nippononivea*), K. Hara (TNS-F-209717).

Isolectotype (not examined). The same as lectotype (S-Fungi F6259).

Japanese name. Kuro-hina-no-chawan-take (Hara 1954).

Description. Apothecia gregarious on the substrate, at first spherical, later flat-

disc-shaped, 0.5–1.2 mm in diameter, sessile, up to 0.5 mm high. Dried disc concave, white to pale yellow, margin incurving, densely covered with dark brown hairs. Hairs cylindrical, straight or slightly curved, up to $375 \times 2.8\text{--}4.0\text{ }\mu\text{m}$, multiseptate, pale to dark brown, completely smooth, equipped with no crystals or resinous matters, relatively thick walled; wall up to $1.5\text{ }\mu\text{m}$ thick; apex blunt and sometimes swelled or protruding, pale brown to hyaline. Ectal excipulum *textura globulosa* lacking intercellular spaces and partially *textura prismatica* composed of cubic cells; cells $2.5\text{--}11\text{ }\mu\text{m}$ in diameter; wall dark brown in the lower part and light brown to hyaline near medullary excipulum, up to $2.5\text{ }\mu\text{m}$ thick. Medullary excipulum of *textura intricata* composed of hyaline hyphae. Asci $100\text{--}115 \times 5\text{--}7.5\text{ }\mu\text{m}$, cylindrical-clavate, 8-spored, arising from croziers; apex conical or hemispherical; pore not reaction against MLZ with or without 3% KOH pretreatment or IKI. Ascospores $(12.4\text{--})13.5\text{--}15.4(\text{--}16.5) \times (2.5\text{--})2.9\text{--}3.5(\text{--}3.8)\text{ }\mu\text{m}$ (av. $\pm$ s.d. $14.4 \pm 0.95 \times 3.2 \pm 0.3\text{ }\mu\text{m}$, $n = 20$), $Q = (3.6\text{--})3.9\text{--}5.2(\text{--}6)$ (av. $\pm$ s.d. 4.5 ± 0.6 , $n = 20$), cylindric-ellipsoid, non-septate, containing no guttules. Paraphyses narrowly lanceolate to lanceolate, septate, up to $6\text{ }\mu\text{m}$ at the widest point, exceeding the asci up to $15\text{ }\mu\text{m}$, encrusted by amorphous, nonrefractive matters conspicuous in the upper half; amorphous matters easily detached in the squash mounts; apex acute and sometimes protruded.

Ecology. Saprotrophic on dead stems of tall herbaceous plants.

Distribution. JAPAN (Gifu). Known only from the type locality.

Notes. The present fungus was originally described by Sydow and Sydow (1912) based on specimens collected by K. Hara in 1912 on dead stems of *Boehmeria nivea*. *Lachnum japonicum* was mentioned a few times only by Hara himself (Hara 1912, 1936, 1954) citing an original description. He regarded *L. japonicum* as a plant pathogen (Hara 1936). In the original description, Sydow and Sydow (1912) pointed out *L. japonicum* had ‘sordide flavis vel flavo-brunneis’ (= dull yellow to yellow-brown) and ‘lenissime asperulis’ (= mildly rough) hairs, indicating the presence of granules on hair surface. In the present concept, brown and granulate hairs are characteristic not of *Lachnum* but of *Brunnipila* (Baral and Krieglsteiner 1985; Hosoya et al. 2010). Since the original description was not clear especially in hair morphology, type study had been required to determine the generic position of *L. japonicum*. However, no type specimens were designated in the original description (Sydow and Sydow 1912) and specimens used in it have long been missing.

In the present study, I fortunately discovered a specimen housed in TNS, which was found to be an ‘isosyntype’ specimen (TNS-F-209717). Since DNA extraction from the specimen was in vein, it was examined only morphologically. Most of the

morphological characters matched with the original description except for the presence of granules on the surface of hairs. Hairs of TNS-F-209717 were apparently smooth and covered with no granules unlike the account of ‘lenissime asperulis’ in Sydow and Sydow (1912).

Morphological examination revealed that the proper genus to dispose this fungus was *Trichopezizella*, because of its smooth, brown hairs. Comparison with other *Trichopezizella* spp. descriptions (Raitviir 1970; Haines 1974) elucidated *L. japonicum* was very similar to *Trichopezizella relicina* (Fries) Raitviir especially in having paraphyses encrusted by amorphous matters. Haines (1974) observed number of specimens of *T. relicina* including the type and some of them were conserved in the New York Botanical Garden (NYS). In this study, I borrowed seven specimens of *T. relicina* (NYS-D-23, 374, 471, 3365, 4082, 4105, 4258) collected and cited by Haines (1974) to compare the morphology with *L. japonicum* and following differences were noticed: *L. japonicum* was sessile while *T. relicina* was stipitate, *L. japonicum* has ectal excipulum of *textura globulosa* to *prismatica* not arranged in a row while *T. relicina* only of *textura prismatica* composed of cubic cells arranged in a row and *L. japonicum* had larger asci than *T. relicina*. Based on these characteristics, *L. japonicum* was proved to be different from *T. relicina* (and other known species of *Trichopezizella*).

General discussion

Genus-level taxonomy of Lachnaceae

The present study comprehensively studied the genus-level taxonomy of Lachnaceae, to four remained genera with unsolved taxonomy (*Erioscyphella*, *Incrucipulum*, *Lachnum*, *Lachnopsis*), in the light of molecular phylogenetic analyses in combination with morphological and ecological features (Fig. 58).

Erioscyphella was redefined morphologically focusing on an ascospored length and overlooked hair morphology (= presence of apical amorphous materias), and monophyly of *Incrucipulum* was verified based on wider size of taxon sampling. A new genus *Neolachnum* was proposed focusing on hairs with crystals increasing comprehension of the generic taxonomy. In lachnaceous genera, since generic delimitation and/or morphological definition of some *Lachnum*-like members (= Clade D in the present study) and *Dasyscyphella* is still unresolved, continuous collecting specimens from all over the world and genus-level refinement for taxonomy are desired. However, in most genera of Lachnaceae, redefinition based on morphology/ecology/phylogeny were completed in the present study (Fig. 58), and now each genus was turned to become identifiable based mainly on morphological/ecological traits (Table 4).

Species recognition and species diversity in Lachnaceae

In Lachnaceae, traditional species-level taxonomy based on morphology/ecology/phylogeny is not easy to find species boundaries because species boundaries are somewhat continuous. In the present study, species-level taxonomy using species delimitation analyses of DNA barcode was attempted in *Erioscyphella*, for more objective species recognition (Fig. 58). As a result, species delimitation analyses not only validated the species boundaries, but also detected some speices as taxonomically problematic, such as species which should be synonymized of other known species, or species which may form species complex. Hereafter, ITS-based species recognition is desirable to be applied to other lachnaceous genera for species-level taxonomy.

In the present study, I proposed 12 new species and 5 new combinations in Lachnaceae based on detailed morphological examinations of apothecia, ecology, phylogeny, and ITS-based species delimitation analyses. Such steady works accompanying vouchers and/or isolates are inevitable to do species-level taxonomic treatments for undescribed/taxonomically problematic members of Lachnaceae, and to grasp species-level diversity. On the other hand, as can be seen from the large number of

new species reported in this study from Japan, there are still too numerous undescribed species in Lachnaceae. Therefore, ‘true species-level diversity’ of Lachnaceae is hard to grasp for the present based only on the occurrence data of apothecia. Instead, there are enough unidentified/undescribed ITS-sequences of Lachnaceae available on public DNA databases (e.g. INSDC, UNITE), which were obtained from various fungal states (e.g. apothecia, asexual states, endophytes, ectomycorrhizal fungi) and collected from various environments (e.g. environmental DNA from soil, air, or water). Such sequences enabled us to roughly grasp where or how lachnaceous members exist and function by exclustering such ITS sequences into species-level using species delimitation methods mentioned in Chapter I.

Hereafter, I will try to grasp lachnaceous species-level diversity by comprehensively analyzing numerous ITS-sequences on public DNA databases. Since most of such sequences are not accurately identified at species-level, I will also do steady taxonomic studies mainly based on features of apothecia/isolates continuously as a taxonomist, and to submit a lot of ITS-sequences, which are accurately identified at species-level, into public DNA databases.

Evolutional tendency of morphological or ecological characters

To reveal evolutionary tendency of morphological/ecological traits of Lachnaceae, robust phylogenetic trees, whose many branches are strongly supported, were desired. In the present study, while most of infragenus-level phylogenetic relationships were resolved by multigene phylogenetic analyses, most of intergeneric phylogenetic relationships were remained unsolved by multigene phylogenetic analyses. Based mainly on infragenus-level phylogeny and morphology/ecology, both morphological and ecological (= host selectivity) characters were turned out to partly reflect the phylogenetic relationships, but to also be changed flexibly. Therefore, lachnaceous species are presumed to have been diversified by sometimes flexibly changing their morphology or ecology.

Hereafter, if intergeneric phylogenetic relationships are resolved by phylogenetic analyses using more informative datasets, such as genome-wide analyses (e.g. Johnston et al. (2019)), more concrete evolutionary tendency of Lachnaceae may be revealed. Moreover, since apothecia of Lachnaceae are characterized by stipitate and hairy apothecia, I’m also considering comparing their evolutionary tendency of morphology to that of other helotialean members with similar morphology (e.g. the family Helotiaceae Rehm having apothecial stipes, and the genus *Hyaloscypha* Boud. having apothecial hairs), to reveal the evolutionary roles of apothecial stipes or hairs.

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References

- Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M, Kurina O, Ostonen I, Jõgeva J, Halapuu S, Põldmaa K, Toots M, Truu J, Larsson K-H, Kõljalg U (2010) PlutoF—a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics* 6: 189–196.
<https://doi.org/10.4137/EBO.S6271>
- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19(6): 716–723.
<https://doi.org/10.1109/TAC.1974.1100705>
- Alexopoulos CJ, Mims CW, Blackwell M (1996) *Introductory Mycology*, 4th Edition, John Wiley & Sons Inc., New York, 880 pp.
- Baral HO (1984) Taxonomische und ökologische Studien über die Koniferen bewohnenden europäischen Arten der Gattung *Lachnellula* Karsten. *Beiträge zur Kenntnis der Pilze Mitteleuropas* 1: 143–156 [in German].
- Baral HO, Krieglsteiner GJ (1985) Bausteine zu einer Askomyzeten-Flora der Bundesrepublik Deutschland. In: *Süddeutschland gefundene inoperculte Diskomyceten mit taxonomischen, ökologischen, chorologischen Hinweisen und einer Farbtafel. Beiheften zur Zeitschrift für Mykologie* 6: 1–160 [in German].
- Baral HO (2009) Iodine reaction in Ascomycetes: why is Lugol's solution superior to Melzer's reagent? <http://www.gbif-mycology.de/HostedSites/Baral/IodineReaction.htm> [Accessed on: 2021-03-15]
- Baral HO (2015) *Hymenoscyphus menthae*, *H. macroguttatus* and *H. scutula*, a comparative taxonomic study emphasizing the value of spore guttulation and croziers. *Ascomycete.org* 7(6): 255–287. <https://doi.org/10.25664/art-0147>
- Baral HO (2016) Inoperculate discomycetes. In: Jaklitsch W, Baral HO, Lücking R, Lumbsch T (eds) *Syllabus of Plant Families: Adolf Engler's Syllabus der Pflanzenfamilien. Part 1/2 Ascomycota* (13th ed. by Frey W). Borntraeger Science Publishers, Stuttgart, pp. 157–205.
- Begerow D, Nilsson H, Unterseher M, Maier W (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* 87: 99–108. <https://doi.org/10.1007/s00253-010-2585-4>
- Bogacheva AV (2017) Additional data on discomycetes of the Botchinsky State Nature Reserve (Khabarovsk region). *Mikologiya i Fitopatologiya* 51: 19–25.

- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolk T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Priesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hoof JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37: 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, Maio ND, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu C-H, Xie D, Zhang C, Stadler T, Drummond AJ (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* 15(4): e1006650. <https://doi.org/10.1371/journal.pcbi.1006650>
- Cantrell SA, Hanlin RT (1997) Phylogenetic relationships in the family Hyaloscyphaceae inferred from sequences of ITS regions, 5.8S ribosomal DNA and morphological characters. *Mycologia* 89(5): 745–755. <https://doi.org/10.1080/00275514.1997.12026841>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25(15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Crous PW, Wingfield MJ, Schumacher RK, Summerell BA, Giraldo A, Gené J, Guarro J, Wanasinghe DN, Hyde KD, Camporesi E, Garethjones EB, Thambugala KM,

- Malysheva EF, Malysheva VF, Acharya K, Álvarez J, Alvarado P, Assefa A, Barnes CW, Bartlett JS, Blanchette RA, Burgess TI, Carlavilla JR, Coetzee MPA, Damm U, Decock CA, Denbreeÿen A, Devries B, Dutta AK, Holdom DG, Rooney-Latham S, Manjón JL, Marincowitz S, Mirabolfathy M, Moreno G, Nakashima C, Papizadeh M, Shahzadehfazeli SA, Amoozegar MA, Romberg MK, Shivas RG, Stalpers JA, Stielow B, Stukely MJC, Swart WJ, Tan YP, Vanderbank M, Wood AR, Zhang Y, Groenewald JZ (2014) Fungal Planet description sheets: 281–319. *Persoonia* 33: 212–289.
<http://dx.doi.org/10.3767/003158514X685680>
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2019) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37(1): 291–294.
<https://doi.org/10.1093/molbev/msz189>
- Dennis RWG (1949) A revision of the British Hyaloscyphaceae with notes on related European species. *Mycological Papers* 32: 1–97.
- Dennis RWG (1954) Some inoperculate discomycetes of tropical America. *Kew Bulletin* 9(2): 289–348. <https://doi.org/10.2307/4114399>
- Dennis RWG (1960) Fungi venezuelani: III. *Kew Bulletin* 14(3): 418–458.
<https://doi.org/10.2307/4114758>
- Dennis RWG (1962) A reassessment of *Belonidium* Mont. & Dur. *Persoonia* 2(2): 171–191.
- Dennis RWG (1972) The type species of *Dasyscyphella* Tranzschel. *Kew Bulletin* 27(2): 273–274.
- Dennis RWG (1981) British ascomycetes. J. Cramer, Vaduz Liechtenstein, 485 pp.
- Dharne CG (1965) Taxonomic investigations on the discomycetous genus *Lachnellula* Karst. *Phytopathologische Zeitschrift* 53(2): 101–144.
<https://doi.org/10.1111/j.1439-0434.1965.tb02194.x>
- Ekanayaka AH, Hyde KD, Gentekaki E, McKenzie EHC, Zhao Q, Bulgakov TS, Camporesi E (2019) Preliminary classification of Leotiomycetes. *Mycosphere* 10: 310–489. <https://doi.org/10.5943/mycosphere/10/1/7>
- Felsenstein J (1984) Distance methods for inferring phylogenies: a justification. *Evolution* 38: 16–24. <https://doi.org/10.1111/j.1558-5646.1984.tb00255.x>
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. <https://doi.org/10.2307/2408678>
- Fröhlich J (1997) Biodiversity of microfungi associated with palms in the tropics [Ph D Thesis]. The University of Hong Kong, 645 pp.

- Fujisawa T, Barraclough TG (2013) Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* 62(5): 707–724.
<https://doi.org/10.1093/sysbio/syt033>
- Fujita H (2017) A natural history of wetlands and plants in Hokkaido. University of Tokyo Press, Tokyo, 256 pp. [in Japanese]
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2): 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- GBIF (2018) Adding sequence-based identifiers to backbone taxonomy reveals 'dark taxa' fungi. <https://www.gbif.org/news/2LrgV5t3ZuGeU2WlYmSEuk/adding-sequence-based-identifiers-to-backbonetaxonomy-reveals-dark-taxa-fungi> [Accessed on: 2020-02-10]
- GBIF (2021) Available from: <https://www.gbif.org> [Accessed on: 2021-10-27]
- Gelardi M, Vizzini A, Ercole E, Horak E, Ming Z, Li TH (2015) Circumscription and taxonomic arrangement of *Nigroboletus roseonigrescens* Gen. Et Sp. Nov., a new member of Boletaceae from tropical South–Eastern China. *PloS One* 10: e0134295. <https://doi.org/10.1371/journal.pone.0134295>
- Guatimosim E, Schwartzburd PB, Barreto RW, Crous PW (2016) Novel fungi from an ancient niche: cercosporoid and related sexual morphs on ferns. *Persoonia* 37: 106–141. <https://doi.org/10.3767/003158516X690934>
- Haines JH (1974) Notes on the genus *Trichopezizella* with descriptions of new taxa. *Mycologia* 66(2): 213–241. <https://doi.org/10.1080/00275514.1974.12019598>
- Haines JH (1980) Studies in the Hyaloscyphaceae. I: Some species of *Dasyscyphus* on tropical ferns. *Mycotaxon* 11(1): 189–216.
- Haines JH, Dumont KP (1983) Studies in the Hyaloscyphaceae II: *Proliferodiscus*, A new genus of Arachnopezizoideae. *Mycologia* 75(3): 535–543.
<https://doi.org/10.1080/00275514.1983.12023717>
- Haines JH, Dumont KP (1984) Studies in the Hyaloscyphaceae III: The long-spored, lignicolous species of *Lachnum*. *Mycotaxon* 19: 1–39.
- Haines JH, Kaneko S (1984) A new foliicolous *Lachnum* from Japan. *Transactions of the Mycological Society of Japan* 25: 237–242.
- Haines (1989) Studies in the Hyaloscyphaceae V: species described by C. H. Peck. *Mycotaxon* 35(2): 317–352.
- Haines JH (1992) Studies in the Hyaloscyphaceae. VI: The genus *Lachnum* (ascomycetes) of the Guayana Highlands. *Nova Hedwigia* 54: 97–112.

- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Han JG, Hosoya T, Sung GH, Shin HD (2014) Phylogenetic reassessment of Hyaloscyphaceae sensu lato (Helotiales, Leotiomycetes) based on multigene analyses. Fungal Biology 118: 150–167.
<https://doi.org/10.1016/j.funbio.2013.11.004>
- Hansen K, LoBuglio KF, Pfister DH (2005) Evolutionary relationships of the cup-fungus genus *Peziza* and Pezizaceae inferred from multiple nuclear genes: RPB2, β -tubulin, and LSU rDNA. Molecular Phylogenetics and Evolution 36(1): 1–23. <https://doi.org/10.1016/j.ympev.2005.03.010>
- Hara K (1912) Some notes of fungi 1. Journal of Plant Research 26: 207–208 [in Japanese].
- Hara K (1936) Studies on harmful fungi in Japan, Yokendo, Tokyo, 358 pp. [in Japanese]. <https://doi.org/10.11501/1256833>
- Hara K (1954) A list of Japanese fungi hitherto known, fourth edition, Nippon-Mycological-Society, Gifu, 447 pp. [in Japanese]
- Hibbett DS (1996) Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Molecular Biology and Evolution 13(7): 903–917.
<https://doi.org/10.1093/oxfordjournals.molbev.a025658>
- Hosaka K, Castellano MA (2008) Molecular phylogenetics of Geastrales with special emphasis on the position of Sclerogaster. Bulletin of the National Science Museum. Series B, Botany 34(4): 161–173.
https://www.kahaku.go.jp/research/publication/botany/download/34_4/BNMNS_B340403.pdf [Accessed on: 2021-03-21]
- Hosoya T, Sasagawa R, Hosaka K, Gi-Ho S, Hirayama Y, Yamaguchi K, Toyama K, Kakishima M (2010) Molecular phylogenetic studies of *Lachnum* and its allies based on the Japanese material. Mycoscience 51(3): 170–180.
<https://doi.org/10.1007/S10267-009-0023-1>
- Hosoya T, Saito Y, Sasagawa R (2013) Enumeration of remarkable Japanese discomycetes (7): Notes on one operculate discomycete and one inoperculate discomycete. Bulletin of the National Science Museum. Series B, Botany 39(4): 151–158.
https://www.kahaku.go.jp/research/publication/botany/download/39_4/BNMNS_B39-4_151-158.pdf [Accessed on: 2020-03-12]

- Hosoya T (2021) Systematics, ecology, and application of Helotiales: Recent progress and future perspectives for research with special emphasis on activities within Japan. *Mycoscience* 62(1): 1–9. <https://doi.org/10.47371/mycosci.2020.05.002>
- Index Fungorum (2021) Index Fungorum.
<http://www.indexfungorum.org/names/Names.asp> [Accessed on: 2021-12-15]
- Johnston PR, Quijada L, Smith CA, Baral HO, Hosoya T, Baschien C, Pärtel K, Zhuang WY, Haelewaters D, Park D, Carl S, López-Giráldez F, Wang Z, Townsend JP (2019) A multigene phylogeny toward a new phylogenetic classification of Leotiomyces. *IMA Fungus* 10: 1. <https://doi.org/10.1186/s43008-019-0002-x>
- Johnston PR, Baschien C (2020) Tricliadiaceae fam. nov. (Helotiales, Leotiomyces). *Fungal Systematics and Evolution* 6: 233–242.
<https://doi.org/10.3114/fuse.2020.06.10>
- Kanouse BB (1941) New and unusual species of discomycetes. *Mycologia* 33: 461–467.
<https://doi.org/10.1080/00275514.1941.12020841>
- Katoh M, Ebihara A (2011) Endemic plants of Japan. Tokai University Press, Hadano, 528 pp. [in Japanese]
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kirschstein W (1938) Über neue, seltene und kritische Ascomyceten und Fungi imperfecti. I. *Annales Mycologici* 36(5–6): 367–400.
- Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saar I, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22: 5271–5277.
<https://doi.org/10.1111/mec.12481>
- Kõljalg U, Tedersoo L, Nilsson RH, Abarenkov K (2016) Digital identifiers for fungal species. *Science* 352(6290): 1182–1183. <https://doi.org/10.1126/science.aaf7115>
- Kõljalg U, Nilsson HR, Schigel D, Tedersoo L, Larsson K-H, May TW, Taylor AFS, Jeppesen TS, Frøslev TG, Lindahl BD, Põldmaa K, Saar I, Suija A, Savchenko A, Yatsiuk I, Adojaan K, Ivanov F, Piirmann T, Pöhönen R, Zirk A, Abarenkov K (2020) The taxon hypothesis paradigm—On the unambiguous detection and

- communication of taxa *Microorganisms* 8(12): 1910.
<https://doi.org/10.3390/microorganisms8121910>
- Korf RP (1973) Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) *The fungi*, vol. IV A. Academic Press, New York, pp. 249–319.
- Korf RP (1978) Nomenclatural and taxonomic notes on *Lasiobelonium*, *Erioscypha* and *Erioscyphella*. *Mycotaxon* 7(2): 399–406.
- Koukol O, Delgado G (2021) Why morphology matters: the negative consequences of hasty descriptions of putative novelties in asexual ascomycetes. *IMA Fungus* 12(1): 26. <https://doi.org/10.1186/s43008-021-00073-z>
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35(21): 4453–4455.
<https://doi.org/10.1093/bioinformatics/btz305>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Le Gal M (1939) Florule mycologique des Bois de la Grange et de l'Etoile, Discomycetes. *Revue de Mycologie* 4: 25–63.
- Lemoine F, Entfellner JBD, Wilkinson E, Correia D, Felipe MD, Oliveira TD, Gascuel O (2018) Renewing Felsenstein's phylogenetic bootstrap in the era of big data. *Nature* 556: 452–456. <https://doi.org/10.1038/s41586-018-0043-0>
- Liu YL, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808. <https://doi.org/10.1093/oxfordjournals.molbev.a026092>
- Marincowitz S, Gryzenhout M, Wingfield MJ (2010) New and rare coelomycetes with appendage-bearing conidia from Pondoland, South Africa. *Mycotaxon* 111: 309–322. <https://doi.org/10.5248/111.309>
- Matheis W (1977) Über einige *Dasyscyphus*-Arten auf Blättern von *Vaccinium*. *Sydowia* 29(1–6): 237–244.
- Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe, Agaricales). *Molecular Phylogenetics and Evolution* 35: 1–20. <https://doi.org/10.1016/j.ympev.2004.11.014>
- Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge ZW, Kerrigan RW, Slot JC, Yang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS (2007)

- Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* 43(2): 430–451. <https://doi.org/10.1016/j.ympev.2006.08.024>
- Miyoshi T, Ono Y, Shimizu S (2007) Occurrence of concave stem canker of citrus in Ehime prefecture [Japan] and detection of the pathogenic fungus *Lachnum abnorme* by PCR. *Japanese Journal of Phytopathology* 73(1): 9–14 [in Japanese]. <https://doi.org/10.3186/jjphytopath.73.9>
- Nannfeldt JA (1932) Morphologie und systematik der nicht lichenisierten inoperculaten discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* 8: 5–368.
- Nannfeldt JA (1936) Contributions to the mycoflora of Sweden. *Svensk Botanisk Tidskrift* 30(3): 295–306.
- Nilsson RH, Abarenkov K, Veldre V, Nylinder S, De Wit P, Brosché S, Alfredsson JF, Ryberg M, Kristiansson E. (2010). An open source chimera checker for the fungal ITS region. *Molecular Ecology Resources* 10(6): 1076–1081. <https://doi.org/10.1111/j.1755-0998.2010.02850.x>
- Nilsson RH, Tedersoo L, Ryberg M, Kristiansson E, Hartmann M, Unterseher M, Porter TM, Bengtsson-Palme J, Walker DM, de Sousa F, Gamper HA, Larsson E, Larsson KH, Kõljalg U, Edgar RC, Abarenkov K (2015) A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. *Microbes and Environments* 30(2): 145–150. <https://doi.org/10.1264/jsme2.ME14121>
- Oguchi T (1981) Morphological and cultural studies on *Lachnellula* spp. in Hokkaido I. Species on *Abies*. *Transactions of the Mycological Society of Japan* 21(4): 435–447.
- Otani Y (1967) Notes on some cup fungi of the Hyaloscyphaceae collected in Hokkaido, Japan. *Transactions of the Mycological Society of Japan* 8: 33–42.
- Peck CH (1878) Report of the botanist (1877). *Annual Report on the New York State Museum of Natural History* 31: 19–60.
- Perić B, Baral HO (2014) *Erioscyphella curvispora* spec. nov. from Montenegro. *Mycologia Montenegrina* 17: 89–104. <https://www.etis.ee/File/DownloadPublic/ab9f7b44-b094-4148-8508-a48479be0ba9?> [Accessed on: 2020-02-15]
- Phillips W, Plowright C (1880) New and rare British fungi. *Grevillea* 8(no. 47): 97–109.
- Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55(4): 595–609.

- <https://doi.org/10.1080/10635150600852011>
- Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* 21(2): 609–620.
<https://doi.org/10.1111/1755-0998.13281>
- Quandt C, Haelewaters D (2021) Phylogenetic advances in Leotiomycetes, an understudied clade of taxonomically and ecologically diverse fungi. *Encyclopedia of Mycology* 1: 284–294. <https://doi.org/10.1016/B978-0-12-819990-9.00052-4>
- Raitviir A (1970) Synopsis of the Hyaloscyphaceae. *Scripta Mycologica* 1: 1–115.
- Raitviir A (1977) Some new species of *Dasyscyphus*. *Kew Bulletin* 31(3): 691–693.
<https://doi.org/10.2307/4119419>
- Raitviir A (1978) The genus *Lasiobelonium*. *Scripta mycologica* 9: 99–132.
- Raitviir A (1985) Species Hyaloscyphacearum in *Sasa* spp. inventae. *Novosti Sistematiki Nizshikh Rastenii* 22: 157–162.
- Raitviir A (1986) Some new combinations to the genus *Lachnum* Retz. *Folia Cryptogamica Estonica* 20: 1–11.
- Raitviir A (1987) System of hyaloscyphic fungi. *Mikologiya i Fitopatologiya* 21(3): 200–206 [in Russian].
- Raitviir A (1991) The order Helotiales Nannf. *Nizshie Rasteniya, Griby i Mokhoobraznye Sovetskogo Dal'nego Vostoka, Griby*. 2: 254–363.
- Raitviir A, Sacconi S (1991) Some interesting Hyaloscyphaceae from north Italy. 2. *Mycologia Helvetica* 4(2): 161–168.
- Raitviir A (2002) A revision of the genus *Dasyscyphella* (Hyaloscyphaceae, Helotiales). *Polish Botanical Journal* 47(2): 227–241.
- Raitviir A (2004) Revised synopsis of the Hyaloscyphaceae. *Scripta Mycologica* 20: 1–133.
- Raitviir A (2006) Rare or noteworthy Helotiales. *Fungi Non Delineati* 31: 1–55.
- Rambaut A (2018a) Tracer. Molecular evolution, phylogenetics and epidemiology, Edinburgh. <http://beast.community/tracer> [Accessed on: 2021-03-15]
- Rambaut A (2018b) FigTree. Molecular evolution, phylogenetics and epidemiology, Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/> [Accessed on: 2020-03-15]
- Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584. <https://doi.org/10.7717/peerj.2584>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic*

- Biology 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Saccardo PA (1889) Sylloge Fungorum 8: ix–xvii, Padua, Saccardo PA, 1143 pp.
- Saho H, Takahashi I (1972) *Scleroderris lagerbergii* GREMMEN, *Lachnellula fuscanguinea* (REHM) DENNIS and *Lachnellula suecica* (de BY.ex FR) NANNF. on coniferous trees. Forest Pests 21: 209–211 [in Japanese].
- Schmitt I, Crespo A, Divakar PK, Fankhauser JD, Herman-Sackett E, Kalb K, Nelsen MP, Nelson NA, Rivas-Plata E, Shimp AD, Widhalm T, Lumbsch HT (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23: 35–40. <https://doi.org/10.3767/003158509X470602>
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. PNAS 109(16): 6241–6246. <https://doi.org/10.1073/pnas.1117018109>
- Seaver FJ (1951) The North American cup-fungi (Inoperculates). Published by the author, New York, 428 pp. <https://doi.org/10.5962/bhl.title.5657>
- Spooner BM (1987) Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. Bibliotheca Mycologica 116: 1–711.
- Stiller JW, Hall BD (1997) The origin of red algae: Implications for plastid evolution. PNAS 94(9): 4520–4525. <https://doi.org/10.1073/pnas.94.9.4520>
- Suková M (2005) A revision of selected material of lignicolous species of *Brunnipila*, *Capitotricha*, *Dasyscyphella* and *Neodasyscypha* from the Czech Republic. Czech Mycology 57(1–2): 139–172. <https://doi.org/10.33585/cmy.57108>
- Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4.0 b10. Sinauer Associates, Sunderland, MA.
- Sydow H, Sydow P (1912) Novae fungorum species -VIII). Annales Mycologici 10: 405–411.
- Tanaka I, Hosoya T (2001) Hyaloscyphaceae in Japan (4): New records of the genus *Lachnum*. Mycoscience 42(6): 597–609. <https://doi.org/10.1007/BF02460959>
- Tello S, Baral HO (2016) *Erioscyphella lunata* (Lachnaceae), a rare discomycete collected in Spain. Ascomycete.org 8(4): 157–162. <https://doi.org/10.25664/ART-0183>
- Tochihara Y (2019) Taxonomic assessment of the three genera of Lachnaceae (Helotiales, Ascomycota) [Master's thesis]. Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 58 pp. [in Japanese].
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of

- Bacteriology 172: 4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- White T, Bruns TD, Lee A, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Ye M, Zhuang WY (2003) New taxa of *Lachnum* (Helotiales, Hyaloscyphaceae) from temperate China. Nova Hedwigia 76(3–4): 443–450. <https://doi.org/10.1127/0029-5035/2003/0076-0443>
- Yu Z-H, Zhuang WY (2003) Species diversity of the fungal genus *Lachnum* (Hyaloscyphaceae, Ascomycotina) from tropical China. Biodiversity Science 11(2): 341–346. <https://doi.org/10.17520/biods.2003019>
- Zhao P, Zhuang WY (2011) Evaluation of ITS region as a possible DNA barcode for the genus *Lachnum* (Helotiales). Mycosystema 30(6): 932–937.
- Zhao YJ, Hosoya T, Baral HO, Hosaka K, Kakishima M (2012) *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. Mycotaxon 122: 25–41. <https://doi.org/10.5248/122.25>
- Zhao M, Yuan LY, Guo DL, Ye Y, Da-Wa ZM, Wang XL, Ma FW, Chen L, Gu YC, Ding LS, Zhou Y (2018) Bioactive halogenated dihydroisocoumarins produced by the endophytic fungus *Lachnum palmae* isolated from *Przewalskia tangutica*. Phytochemistry 148: 97–103. <https://doi.org/10.1016/j.phytochem.2018.01.018>
- Zhuang WY, Wang Z (1998a) Discomycetes of tropical China. I. Collections from Hainan Island. Mycotaxon 67: 21–31.
- Zhuang WY, Wang Z (1998b) Some new species and new records of discomycetes in China. VIII. Mycotaxon 66: 429–438.
- Zhuang WY (2004) New taxa of *Lachnum* (Ascomycetes, Helotiales) on bamboo and a key to the bambusicolous species of the genus. Nova Hedwigia 78(3–4): 425–433.
- Zhuang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 15(29): 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499>
- Zoller S, Scheidegger C, Sperisen C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. The Lichenologist 31: 511e516. <https://doi.org/10.1006/lich.1999.0220>

Tables

Table 1. Taxa analyzed in the phylogenetic analyses.

Specimen no. (TNS-F-)	Taxon	Collection site	Collected Date	Host plants and parts	Strain no. (# NBRC)	# UNITE/GenBank accession no.			
						ITS	LSU	mtSSU	RPB2
†16740	<i>Albotricha acutipila</i> (P. Karst.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2006-06-17	culm of unidentified bamboo	104380	AB481234	LC438571	LC431751	AB481354
†16497	<i>Albotricha albotestacea</i> (Desm.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of <i>Miscanthus sinensis</i>	101346	AB481235	LC424943	LC431747	AB481340
†16635	<i>Brunnipila fuscescens</i> (Pers.) Baral	JAPAN, Gunma, Higashi-Agatsuma	2006-04-27	leaf of unidentified tree	104365	AB481255	LC424945	LC431750	AB481348
†16690	<i>Brunnipila pseudocannabinata</i> (Raitv.) Tochiwara, Sasagawa & Hosoya	JAPAN, Akita, Kosaka	2006-05-26	stem of unidentified herb	104374	AB481272	LC533520	LC533522	LC533521
†65670	<i>Capitotricha bicolor</i> (Bull.) Baral	SWITZERLAND, Filisur	2016-06-06	twig of <i>Prunus spinosa</i>	(FC-6101)	LC424834	LC424942	LC533244	LC425011
†65752	<i>Capitotricha rubi</i> (Bres.) Baral	SWITZERLAND, Saicourt	2016-06-04	twig of <i>Rubus idaeus</i>	(FC-6075)	LC438560	LC438573	LC533243	LC440395
†16439	<i>Dasyscyphella longistipitata</i> Hosoya	JAPAN, Kanagawa, Yamakita	2005-04-17	cupule of <i>Fagus crenata</i>	101335	AB481239	LC424947	LC533228	AB481331
†16527	<i>Dasyscyphella montana</i> Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	wood of unidentified tree	102336	AB481242	LC438577	LC533241	AB481336
‡16556	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Oita, Kokonoe	2005-05	wood of unidentified tree	114449	UDB0779051	LC533153	LC533257	LC533198
‡16582	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yamakita	2005-07-02	wood of unidentified tree	104360	AB481249	LC533176	LC533233	LC533199
‡16606	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yamakita	2005-07-03	wood of unidentified tree	114450	UDB0779053	LC533154	LC533258	LC533200
‡16609	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yamakita	2005-07-03	wood of <i>Cephalotaxus harringtonia</i>	101350	††AB705234	LC533175	LC533256	LC533184
‡16639	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Ibaraki, Tsukuba Botanical Garden	2006-05-01	twig of unidentified tree	114451	UDB0779054	LC533155	LC533259	LC533201
‡25579	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Tokyo, Hongo	2009-05-25	twig of unidentified tree	(FC-1887)	UDB0779057	LC533146	LC533250	LC533191
‡32163	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Odawara	2010-05-14	twig of unidentified tree	114456	UDB0779062	LC533158	LC533260	LC533203
‡38452	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Gunma, Naganohara	2013-06-27	wood of unidentified tree	114463	††UDB0779069	LC533171	LC533262	LC533210
‡46416	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	TAIWAN, Taipei	2012-04-15	wood of unidentified tree	(FC-2906)	UDB0779067	LC533132	LC533277	LC549671
‡46841	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Gifu, Gujo	2012-05-28	wood of unidentified tree	114462	UDB0779086	LC533170	LC533279	LC533209
‡61773	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yokohama	2015-04-01	twig of unidentified tree	114464	††UDB0779074	LC533137	LC533264	LC533211
‡61931	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Zushi	2015-04-16	wood of unidentified tree	114466	UDB0779072	LC533139	LC533266	LC533213
‡80478	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Shizuoka, Oyama	2017-06-26	twig of unidentified tree	113934	LC424837	LC424949	LC533283	LC425009
‡26520	<i>Erioscyphella boninensis</i> Tochiwara & Hosoya	JAPAN, Tokyo, Chichijima Island	2009-06-28	trunk of unidentified tree	114447	UDB0779049	LC533151	LC533254	LC533196
‡46419	<i>Erioscyphella brasiliensis</i> (Mont.) Baral, Šandová & B. Perić	TAIWAN, Taipei	2012-04-20	wood of unidentified tree	(FC-2910)	UDB0779068	LC533133	LC533278	LC549672
‡35049	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Niigata, Minamiuonuma	2010-05-14	leaf of <i>Quercus glauca</i>	114457	UDB0779064	LC533168	LC533274	LC533205
‡35056	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Niigata, Minamiuonuma	2010-05-14	leaf of <i>Quercus serrata</i>	114458	UDB0779065	LC533169	LC533275	LC533206
‡61775	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Kanagawa, Hiratsuka	2015-04-12	leaf of <i>Quercus myrsinifolia</i>	114465	UDB0779071	LC533138	LC533265	LC533212
‡61941	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Kanagawa, Kamakura	2015-04-24	leaf of <i>Quercus glauca</i>	112569	UDB0779073	LC533140	LC533280	LC533214
‡65722	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Gunma, Midori	2016-04-24	leaf of <i>Quercus serrata</i> subsp. <i>mongolicoides</i>	114469	UDB0779076	LC533142	LC533281	LC533215
‡80356	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Kanagawa, Hiratsuka	2017-05-18	leaf of <i>Quercus glauca</i>	114470	UDB0779077	LC533172	LC533282	LC533186
‡80371	<i>Erioscyphella hainanensis</i> (W.Y. Zhuang and Zheng Wang) Hosoya and Tochiwara (← <i>Lachnum hainanense</i> W.Y. Zhuang and Zheng Wang)	JAPAN, Kanagawa, Hiratsuka	2017-05-18	leaf of <i>Castanopsis sieboldii</i>	114472	UDB0779078	LC533135	LC533246	LC533188
‡26500	<i>Erioscyphella insulae</i> Tochiwara & Hosoya	JAPAN, Tokyo, Hahajima Island	2009-06-24	wood of unidentified tree	114445	UDB0779060	LC533149	LC533252	LC533194
‡39720	<i>Erioscyphella insulae</i> Tochiwara & Hosoya	JAPAN, Okinawa, Iriomote Island	2011-06-12	bark of unidentified tree	114459	UDB0779063	LC533177	LC533261	LC533207
‡61920	<i>Erioscyphella paralushanensis</i> Tochiwara & Hosoya	JAPAN, Shizuoka, Atami	2015-06-08	culm of <i>Pleioblastus argenteostriatus</i>	114468	††UDB0779075	LC533141	LC533267	LC533220
†81472	<i>Erioscyphella otanii</i> Tochiwara	JAPAN, Hokkaido, Horonobe, Teshio Experimental Forest, Hokkaido University	2018-07-11	leaf of <i>Sasa senanensis</i>	114476	UDB0779085	LC533179	LC533286	LC533226
‡81272	<i>Erioscyphella papillaris</i> Tochiwara	JAPAN, Gunma, Minakami	2017-07-16	leaf of unidentified bamboo	113937	UDB0779081	LC533161	LC533285	LC533204
‡80399	<i>Erioscyphella sasibrevipora</i> Tochiwara & Hosoya	JAPAN, Gunma, Higashi-Agatsuma	2017-06-06	sheath of <i>Sasa veitchii</i>	—	UDB0779082	LC533173	LC533268	LC533216
‡81401	<i>Erioscyphella sasibrevipora</i> Tochiwara & Hosoya	JAPAN, Hokkaido, Tomakomai	2018-06-16	culm of <i>Sasa nipponica</i>	114475	UDB0779084	LC533174	LC533269	LC533217
‡26492	<i>Erioscyphella sclerotii</i> (A.L. Sm.) Baral, Šandová & B. Perić	JAPAN, Tokyo, Hahajima Island	2009-06-24	wood of unidentified tree	114448	UDB0779050	LC533152	LC533255	LC533197
‡38480	<i>Erioscyphella sclerotii</i> (A.L. Sm.) Baral, Šandová & B. Perić	TAIWAN, Wulai	2013-07-12	twig of unidentified tree	(FC-5208)	††UDB0779070	LC533134	LC533263	LC549673
‡16838	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochiwara & Hosoya (← <i>Lachnum mapirianum</i> var. <i>sinense</i> Z.H. Yu and W.Y. Zhuang)	JAPAN, Ibaraki, Tsukuba Botanical Garden	2007-06-15	leaf of unidentified broad-leaved tree	104389	AB481280	LC533164	LC533235	AB481364
‡80354	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochiwara & Hosoya (← <i>Lachnum mapirianum</i> var. <i>sinense</i> Z.H. Yu and W.Y. Zhuang)	JAPAN, Kanagawa, Manazuru	2017-05	leaf of <i>Castanopsis sieboldii</i>	114471	UDB0779083	LC533143	LC533245	LC533187
‡16841	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochiwara & Hosoya (← <i>Lachnum mapirianum</i> var. <i>sinense</i> Z.H. Yu and W.Y. Zhuang)	JAPAN, Ibaraki, Mt. Tsukuba	2007-06-23	leaf of unidentified broad-leaved tree	104390	AB481281	LC533157	LC533236	LC533218
‡32161	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochiwara & Hosoya (← <i>Lachnum mapirianum</i> var. <i>sinense</i> Z.H. Yu and W.Y. Zhuang)	JAPAN, Kanagawa, Odawara	2010-05-14	leaf of <i>Quercus myrsinifolia</i>	113715	UDB0779061	LC533167	LC533273	LC533219
‡16837	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochiwara & Hosoya (← <i>Lachnum mapirianum</i> var. <i>sinense</i> Z.H. Yu and W.Y. Zhuang)	JAPAN, Ibaraki, Tsukuba Botanical Garden	2007-06-15	leaf of unidentified broad-leaved tree	114452	UDB0779055	LC533156	LC533272	LC533202

Specimen no. (TNS-F-)	Taxon	Collection site	Collected Date	Host plants and parts	Strain no. (¶ NBRC)	# UNITE/GenBank accession no.			
						ITS	LSU	mtSSU	RPB2
†81520	<i>Incrucipulum ciliare</i> (Schräd.) Baral	JAPAN, Shizuoka, Shizuoka	2018-08-18	leaf of <i>Quercus mongolica</i> subsp. <i>crispula</i>	113941	LC438566	LC438583	LC533284	LC438596
†17632	<i>Incrucipulum longispineum</i> Sasagawa & Hosoya	JAPAN, Miyagi, Sendai	2006-07-29	leaf of <i>Lyonia ovalifolia</i>	102347	AB481256	LC438579	LC533234	AB481362
†81248	<i>Lachnellula calyciformis</i> (Batsch) Dharne	JAPAN, Hokkaido, Engaru	2017-07-12	twig of <i>Abies sachalinensis</i>	113935	LC438561	LC438574	LC533247	LC438590
†16529	<i>Lachnellula suecica</i> (de Bary ex Fuckel) Nannf.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	twig of <i>Larix kaempferi</i>	101348	AB481248	LC424944	LC533231	AB481341
†16494	<i>Lachnum asiaticum</i> (Y. Otani) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of unidentified bamboo	101341	AB481251	LC533162	LC533229	AB481334
‡17249	<i>Lachnum mapirianum</i> (Pat. & Gaillard) M.P. Sharma	MALAYSIA, Gerik	2004-09-07	leaf of unidentified tree	—	UDB0779088	LC533182	—	LC533223
‡17245	<i>Lachnum mapirianum</i> (Pat. & Gaillard) M.P. Sharma	MALAYSIA, Gerik	2004-09-07	leaf of unidentified tree	—	UDB0779087	LC533181	—	LC533222
‡16442	<i>Lachnum novoguineense</i> var. <i>yunnanicum</i> W.Y. Zhuang	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of unidentified bamboo	102339	AB481270	LC533163	LC533232	AB481342
‡16642	<i>Lachnum novoguineense</i> var. <i>yunnanicum</i> W.Y. Zhuang	JAPAN, Ibaraki, Mt. Tsukuba	2006-05-02	culm of unidentified bamboo	104368	AB481271	LC533165	LC533227	§§LC533225
‡11197	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Shizuoka, Shimoda	2004-07-26	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	106495	UDB0779047	LC533166	LC533248	LC533185
‡13500	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Kagoshima, Yakushima Island	2005-10-19	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	114441	††LC425039/ UDB779046	LC429382	LC533240	‡‡LC431718
‡17567	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	NEW ZEALAND	2005-05-28	leaf of unidentified palm	—	UDB0779089	LC533183	LC533288	—
‡24588	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Kagoshima, Amami-Oshima	2009-02-24	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	114442	UDB0779052	LC533144	LC533270	LC533190
‡24600	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Kagoshima, Amami-Oshima	2009-02-25	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	114443	UDB0779056	LC533145	LC533249	LC533224
‡26161	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Tokyo, Chichijima Island	2009-06-27	leaf of <i>Livistona boninensis</i>	114446	UDB0779048	LC533150	LC533253	LC533195
‡26172	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Tokyo, Kita-Iwojima Island	2009-06-17	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	(FC-1935)	UDB0779058	LC533147	LC533251	LC533192
‡26185	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Tokyo, Kita-Iwojima Island	2009-06-18	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	114444	UDB0779059	LC533148	LC533271	LC533193
‡39729	<i>Lachnum palmae</i> sensu lato (← <i>Lachnum palmae</i> (Kanouse) Spooner)	JAPAN, Okinawa, Iriomote Island	2011-06-13	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	114460	UDB0779066	LC533178	LC533276	LC533208
†16501	<i>Lachnum pudibundum</i> (Qué.) J. Schröt.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	wood of unidentified tree	102335	AB481259	LC533160	LC533230	AB481335
†81229	<i>Lachnum rachidicola</i> J.G. Han, Raitv. & H.D. Shin	JAPAN, Hokkaido, Tomakomai, Tomakomai Experimental Forest	2017-08-09	petiole of <i>Juglans</i> sp.	114473	UDB0779079	LC533136	—	LC533189
†16583	<i>Lachnum virgineum</i> (Batsch) P. Karst.	JAPAN, Kanagawa, Yamakita	2005-07-02	wood of unidentified tree	104358	AB481268	AB926119	LC431748	AB481343
†65625	<i>Neodasyscypha cerina</i> (Pers.) Spooner	SWITZERLAND, Saicourt	2016-06-08	twig of <i>Crataegus</i> sp.	(FC-6068)	LC424836	LC424948	LC533242	LC425013
†17436	<i>Proliferodiscus albobiridis</i> (Sacc.) Spooner	JAPAN, Ibaraki, Tsukuba Botanical Garden	2006-07-08	wood of unidentified tree	108594	LC438558	LC533159	LC533239	LC425014
§17909	<i>Hyaloscypha spiralis</i> (Velen.) J.G. Han, Hosoya & H.D. Shin	JAPAN, Kumamoto, Kikuchi	2005-10-10	wood of unidentified tree	108585	††LC438602	LC438604	LC533237	LC438606
§16472	<i>Hymenoscyphus varicosporoides</i> Tubaki	JAPAN, Ibaraki, Kasumigaura	2005-05-05	wood of unidentified tree	104355	AB926052	LC424952	LC431746	AB481329
§18014	<i>Urceolella carestiana</i> (Rabenh.) Dennis	JAPAN, Iwate, Hanamaki	2006-05-23	stem of <i>Parathelypteris nipponica</i>	108588	††LC438603	LC438605	LC533238	LC438607

† Lachnaceae except for Erioscyphella and its potential species tentatively identified based on morphology

‡ *Erioscyphella* or its potential species tentatively identified based on morphology

§ Outgroup

| Original taxon name labeled on the specimen is shown enclosed by "(←)" and is only shown when it is different from a name determined in this study.

¶ Cultures not donated in NBRC is beginning with "FC-", local suffix in TNS. '—' represents no culture exist and DNA was extracted from apothecia.

UNITE accession no. is beginning with 'UDB'. GenBank accession no. is beginning with 'AB' or 'LC'.

†† Primer pair ITS1 and ITS4 was used. In ITS sequences without notes (††), primer pair ITS1F and ITS4 was used.

‡‡ Primer pair rRPB2-5F and RPB2-P7R was used.

§§ Primer pair RPB2-P6Fa and bRPB2-7.1R2 was used.

|| Primer pair RPB2-P6Fa and RPB2-P7R was used. In RPB2 sequences without any notes (‡‡, §§, ||), primer pair RPB2-P6F and RPB2-P7R was used.

Table 2. ITS sequences analyzed by the species delimitation analyses.

ITS sequence GenBank/UNITE accession no.	TNS-F specimen no.	Reference (initial appearance)	Taxon name ultimately allocated in this study	UNITE taxon name	INSDC taxon name	Country	Host plants and parts	UNITE SH code (DOI) at 3% threshold	UNITE SH code (DOI) at 1.5% threshold	VSEARCH SH at 3% threshold	VSEARCH SH at 1.5% threshold
AB267634		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Ehime	twig of <i>Citrus junos</i>	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
AB267636 (duplicate; AB267635)		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Ehime	twig of <i>Citrus junos</i>	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
AB267641 (duplicate; AB267639, AB267640)		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Tokushima	twig of <i>Citrus junos</i>	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
AB267642		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Tokushima	twig of <i>Citrus junos</i>	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
JF937578		Zhao and Zhuang (2011)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	CHINA	(unspecified)	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
JN033395		Han et al. (2014)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	KOREA	wood	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
UDB0779067	46416	this study	<i>E. abnormis</i>	-	-	TAIWAN, Taipei	wood of unidentified tree	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
UDB0779074	61773	this study	<i>E. abnormis</i>	-	-	JAPAN, Kanagawa, Yokohama	twig of unidentified tree	SH1155612.08FU	SH1522994.08FU	VSH97_1	VSH985_2
MK584950		Ekanayaka et al. (2019)	<i>E. abnormis</i>	<i>E. abnormis</i>	<i>E. abnormis</i>	CHINA, Yunnan	(unspecified)	SH1155612.08FU	†SH1522994.08FU	VSH97_1	VSH985_2
AB267637		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Nara	twig	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_1
AB267638		Miyoshi et al. (2007)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Shizuoka	twig	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_1
AB481249	16582	Hosoya et al. (2010)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Kanagawa, Yamakita	wood of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
AB705234	16609	Zhao et al. (2012)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Kanagawa, Yamakita	wood of <i>Cephalotaxus harringtonia</i>	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
LC424837	80478	this study	<i>E. abnormis</i>	-	-	JAPAN, Shizuoka, Oyama	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
MG712307		unpublished	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	CHINA	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
MK282241		unpublished	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	(unspecified)	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_1
MK584957		Ekanayaka et al. (2019)	<i>E. abnormis</i>	<i>E. aseptata</i>	<i>E. aseptata</i>	THAILAND, Chiang Rai	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_1
MN082536		unpublished	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	(unspecified)	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
MT995055		unpublished	<i>E. abnormis</i> (misregistered?)	<i>Chapsa patens</i>	<i>Chapsa patens</i>	(unspecified)	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
MW007918		unpublished	<i>E. abnormis</i> (misregistered?)	<i>Chapsa patens</i>	<i>Chapsa patens</i>	(unspecified)	(unspecified)	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779051	16556	this study	<i>E. abnormis</i>	-	-	JAPAN, Oita, Kokonoe	wood of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_1
UDB0779053	16606	this study	<i>E. abnormis</i>	-	-	JAPAN, Kanagawa, Yamakita	wood of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779054	16639	this study	<i>E. abnormis</i>	-	-	JAPAN, Ibaraki, Tsukuba Botanical Garden	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779057	25579	this study	<i>E. abnormis</i>	-	-	JAPAN, Tokyo, Hongo	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779062	32163	this study	<i>E. abnormis</i>	-	-	JAPAN, Kanagawa, Odawara	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779069	38452	this study	<i>E. abnormis</i>	-	-	JAPAN, Gunma, Naganohara	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
UDB0779072	61931	this study	<i>E. abnormis</i>	-	-	JAPAN, Kanagawa, Zushi	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_2	VSH985_3
UDB0779086	46841	this study	<i>E. abnormis</i>	-	-	JAPAN, Gifu, Gujo	twig of unidentified tree	SH1155612.08FU	SH1523013.08FU	VSH97_1	VSH985_1
AB481250	16617	Hosoya et al. (2010)	<i>E. abnormis</i>	<i>Lachnum abnorme</i>	<i>Lachnum abnorme</i>	JAPAN, Kanagawa, Yamakita	twig of unidentified tree	‡SH1155612.08FU	‡SH1523013.08FU	VSH97_1	VSH985_1
UDB0779055	16837	this study	<i>E. sinensis</i> (← <i>Lachnum mapirianum</i> var. <i>sinense</i>)	-	-	JAPAN, Ibaraki, Tsukuba Botanical Garden	leaf of unidentified broad- leaved tree	SH1155682.08FU	SH1523107.08FU	VSH97_4	VSH985_5
AB481280	16838	Hosoya et al. (2010)	<i>E. sinensis</i> (← <i>Lachnum mapirianum</i> var. <i>sinense</i>)	<i>Lachnum</i> sp.	<i>Lachnum</i> (<i>Lachnum</i> sp. FC-2355)	JAPAN, Ibaraki, Tsukuba Botanical Garden	leaf of unidentified broad- leaved tree	SH1155682.08FU	SH1523107.08FU	VSH97_4	VSH985_5
AB481281	16841	Hosoya et al. (2010)	<i>E. sinensis</i> (← <i>Lachnum mapirianum</i> var. <i>sinense</i>)	<i>Lachnum</i> sp.	<i>Lachnum</i> (<i>Lachnum</i> sp. FC-2358)	JAPAN, Ibaraki, Mt. Tsukuba	leaf of unidentified broad- leaved tree	SH1155682.08FU	SH1523107.08FU	VSH97_4	VSH985_5

ITS sequence GenBank/UNITE accession no.	TNS-F specimen no.	Reference (initial appearance)	Taxon name ultimately allocated in this study	UNITE taxon name	INSDC taxon name	Country	Host plants and parts	UNITE SH code (DOI) at 3% threshold	UNITE SH code (DOI) at 1.5% threshold	VSEARCH SH at 3% threshold	VSEARCH SH at 1.5% threshold
UDB0779061	32161	this study	<i>E. sinensis</i> (← <i>Lachnum mapirianum</i> var. <i>sinense</i>)	-	-	JAPAN, Kanagawa, Odawara	leaf of <i>Quercus myrsinifolia</i>	SH1155682.08FU	SH1523107.08FU	VSH97_4	VSH985_5
UDB0779083	80354	this study	<i>E. sinensis</i> (← <i>Lachnum mapirianum</i> var. <i>sinense</i>)	-	-	JAPAN, Kanagawa, Manazuru	leaf of <i>Castanopsis sieboldii</i>	†SH1155682.08FU	†SH1523107.08FU	VSH97_4	VSH985_5
UDB023346		unpublished	<i>E. curvispora</i>	<i>E. curvispora</i>	-	MONTENEGRO, Žijevu Mountains	needle of <i>Pinus heldreichii</i>	SH1155703.08FU	SH1523136.08FU	VSH97_12	VSH985_14
MH190414		Perić and Baral (2014)	<i>E. curvispora</i>	<i>E. curvispora</i>	<i>E. curvispora</i>	MONTENEGRO, Žijevu Mountains	needle of <i>Pinus heldreichii</i>	†SH1155703.08FU	†SH1523136.08FU	VSH97_12	VSH985_14
JF937580		Zhao and Zhuang (2011)	<i>E. brasiliensis</i>	<i>Lachnum brasiliense</i>	<i>Lachnum brasiliense</i>	CHINA	(unspecified)	SH1155705.08FU	SH1523142.08FU	VSH97_6	VSH985_7
MK584953		Ekanayaka et al. (2019)	<i>E. brasiliensis</i>	<i>E. brasiliensis</i>	<i>E. brasiliensis</i>	(unspecified)	(unspecified)	SH1155705.08FU	SH1523142.08FU	VSH97_6	VSH985_7
MK584967		Ekanayaka et al. (2019)	<i>E. brasiliensis</i>	<i>E. brasiliensis</i>	<i>E. brasiliensis</i>	THAILAND, Chiang Rai	(unspecified)	SH1155705.08FU	SH1523142.08FU	VSH97_6	VSH985_7
UDB0779068	46419	this study	<i>E. brasiliensis</i>	-	-	TAIWAN, Taipei	wood of unidentified tree	SH1155705.08FU	SH1523142.08FU	VSH97_6	VSH985_7
JF937579		Zhao and Zhuang (2011)	<i>E. brasiliensis</i>	<i>Lachnum brasiliense</i>	<i>Lachnum brasiliense</i>	CHINA	(unspecified)	†SH1155705.08FU	†SH1523142.08FU	VSH97_6	VSH985_7
KX501132		Tello and Baral (2016)	<i>E. lunata</i>	<i>E. lunata</i>	<i>E. lunata</i>	SPAIN, Andalucía	needle of <i>Pinus nigra</i> subsp. <i>nigra</i>	†SH1155760.08FU	†SH1523257.08FU	VSH97_18	VSH985_19
JX984680		unpublished	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	Hyaloscyphaeae	Fungi (uncultured fungus)	KOREA, Seoul	(Total suspended particulate matter (TSP) in urban air during non-Asian dust days)	SH1155844.08FU	SH1523423.08FU	VSH97_3	VSH985_4
UDB0779064	35049	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Niigata, Minamiuonuma	leaf of <i>Quercus glauca</i>	SH1155844.08FU	SH1523423.08FU	VSH97_3	VSH985_4
UDB0779065	35056	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Niigata, Minamiuonuma	leaf of <i>Quercus serrata</i>	SH1155844.08FU	SH1523423.08FU	VSH97_3	VSH985_4
UDB0779073	61941	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Kanagawa, Kamakura	leaf of <i>Quercus glauca</i>	SH1155844.08FU	SH1523423.08FU	VSH97_3	VSH985_4
UDB0779076	65722	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Gunma, Midori	leaf of <i>Quercus serrata</i> subsp. <i>mongolicoides</i>	SH1155844.08FU	SH1523423.08FU	VSH97_3	VSH985_4
MK282242		unpublished	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	<i>Lachnum</i> sp.	<i>Lachnum albidulum</i>	KOREA	(unspecified)	SH1155844.08FU	†SH1523423.08FU	VSH97_3	VSH985_4
UDB0779077	80356	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Kanagawa, Hiratsuka	leaf of <i>Quercus glauca</i>	SH1155844.08FU	SH3597461.08FU	VSH97_3	VSH985_9
UDB0779078	80371	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Kanagawa, Hiratsuka	leaf of <i>Castanopsis sieboldii</i>	SH1155844.08FU	SH3597461.08FU	VSH97_3	VSH985_9
UDB0779071	61775	this study	<i>E. hainanensis</i> (← <i>Lachnum hainanense</i>)	-	-	JAPAN, Kanagawa, Hiratsuka	leaf of <i>Quercus myrsinifolia</i>	†SH1155844.08FU	†SH3597461.08FU	VSH97_3	VSH985_9
UDB0779050	26492	this study	<i>E. sclerotii</i>	-	-	JAPAN, Tokyo, Hahajima Island	wood of unidentified tree	SH1155848.08FU	SH1523429.08FU	VSH97_5	VSH985_6
JF937584		Zhao and Zhuang (2011)	<i>E. sclerotii</i>	<i>Lachnum sclerotii</i>	<i>Lachnum sclerotii</i>	CHINA	(unspecified)	SH1155848.08FU	SH1523429.08FU	VSH97_5	VSH985_6
MK584951		Ekanayaka et al. (2019)	<i>E. sclerotii</i>	<i>E. sclerotii</i>	<i>E. sclerotii</i>	THAILAND, Chiang Rai	(unspecified)	SH1155848.08FU	SH1523429.08FU	VSH97_5	VSH985_6
UDB0779070	38480	this study	<i>E. sclerotii</i>	-	-	TAIWAN, Wulai	twig of unidentified tree	SH1155848.08FU	SH1523429.08FU	VSH97_5	VSH985_6
MK584969		Ekanayaka et al. (2019)	<i>E. sclerotii</i>	<i>E. sclerotii</i>	<i>E. sclerotii</i>	THAILAND, Chiang Rai	(unspecified)	†SH1155848.08FU	†SH1523429.08FU	VSH97_5	VSH985_6
AB481271	16642	Hosoya et al. (2010)	<i>Lachnum novoguineense</i> var. <i>yunnanicum</i>	<i>Lachnum</i> sp.	<i>Lachnum</i> sp. (<i>Lachnum</i> sp. FC-2211)	JAPAN, Ibaraki, Mt. Tsukuba	culm of unidentified bamboo	SH1236904.08FU	SH1648536.08FU	VSH97_10	VSH985_12
AB481270	16442	Hosoya et al. (2010)	<i>Lachnum novoguineense</i> var. <i>yunnanicum</i>	<i>Lachnum</i> sp.	<i>Lachnum</i> sp. (<i>Lachnum</i> sp. FC-2117)	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	culm of unidentified bamboo	†SH1236904.08FU	†SH1648536.08FU	VSH97_10	VSH985_12
MK584965		Ekanayaka et al. (2019)	<i>E. alba</i>	<i>E. alba</i>	<i>E. alba</i>	THAILAND, Chiang Mai	(unspecified)	†SH2596405.08FU	†SH2712425.08FU	VSH97_22	VSH985_25
AB267647		Miyoshi et al. (2007)	<i>Lachnum palmae</i> sensu lato	<i>Lachnum palmae</i>	<i>Lachnum palmae</i>	JAPAN, Oita	leaf of <i>Livistona chinensis</i>	SH1149764.08FU	SH1515235.08FU	VSH97_7	VSH985_8
LC425039 (duplicate; UDB0779046)	13500	Johnston et al. (2019)	<i>Lachnum palmae</i> sensu lato	<i>Lachnum palmae</i>	<i>Lachnum palmae</i>	JAPAN, Kagoshima, Yakushima Island	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	SH1149764.08FU	SH1515235.08FU	VSH97_7	VSH985_8
UDB0779066	39729	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Okinawa, Iriomote Island	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	SH1149764.08FU	SH1515235.08FU	VSH97_7	VSH985_8
MG283320		Zhao et al. (2018)	<i>Lachnum palmae</i> sensu lato	<i>Lachnum palmae</i>	<i>Lachnum palmae</i>	CHINA, Linzhou	root of <i>Przewalskia tangutica</i> (endophyte)	†SH1149764.08FU	†SH1515235.08FU	VSH97_7	VSH985_8
UDB0779089	17567	this study	<i>Lachnum palmae</i> sensu lato	-	-	NEW ZEALAND	leaf of unidentified palm	SH2594271.08FU	SH2709065.08FU	VSH97_15	VSH985_16

ITS sequence GenBank/UNITE accession no.	TNS-F specimen no.	Reference (initial appearance)	Taxon name ultimately allocated in this study	UNITE taxon name	INSDC taxon name	Country	Host plants and parts	UNITE SH code (DOI) at 3% threshold	UNITE SH code (DOI) at 1.5% threshold	VSEARCH SH at 3% threshold	VSEARCH SH at 1.5% threshold
MH921862		unpublished	<i>Lachnum palmae</i> sensu lato	<i>Lachnum palmae</i>	<i>Lachnum palmae</i>	NEW ZEALAND	unidentified part of <i>Rhopalostylis sapida</i>	†SH2594271.08FU	†SH2709065.08FU	VSH97_15	VSH985_16
UDB0779052	24588	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Kagoshima, Amami-Oshima	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	SH3569651.08FU	SH3597456.08FU	VSH97_9	VSH985_17
UDB0779047	11197	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Shizuoka, Shimoda	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	†SH3569651.08FU	†SH3597456.08FU	VSH97_9	VSH985_17
UDB0779048	26161	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Tokyo, Chichijima Island	leaf of <i>Livistona boninensis</i>	SH3569651.08FU	SH3597457.08FU	VSH97_9	VSH985_11
UDB0779058	26172	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Tokyo, Kita- Iwojima Island	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	SH3569651.08FU	SH3597457.08FU	VSH97_16	VSH985_11
UDB0779059	26185	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Tokyo, Kita- Iwojima Island	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	SH3569651.08FU	†SH3597457.08FU	VSH97_16	VSH985_11
UDB0779056	24600	this study	<i>Lachnum palmae</i> sensu lato	-	-	JAPAN, Kagoshima, Amami-Oshima	leaf of <i>Livistona chinensis</i> var. <i>subglobosa</i>	†SH3569653.08FU	†SH3597459.08FU	VSH97_25	VSH985_28
U58640		Cantrel and Hanlin (1997)	<i>E. euterpes</i>	<i>Lachnum euterpes</i>	<i>Lachnum euterpes</i>	PUERTO RICO	(unspecified)	†SH1236906.08FU	†SH1648538.08FU	VSH97_21	VSH985_24
KT384413		Ekanayaka et al. (2019)	<i>E. fusiformis</i>	<i>Lachnum fusiforme</i>	<i>Lachnum fusiforme</i>	THAILAND	dead stems	‡SH1236907.08FU	‡SH1648539.08FU	VSH97_11	VSH985_13
MK584948		Ekanayaka et al. (2019)	<i>E. fusiformis</i>	<i>Lachnum fusiforme</i>	<i>Lachnum fusiforme</i>	CHINA	dead stems	SH1236907.08FU	SH1648539.08FU	VSH97_11	VSH985_13
UDB0779049	26520	this study	<i>E. boninensis</i>	-	-	JAPAN, Tokyo, Hahajima Island	wood of unidentified tree	†SH3569652.08FU	†SH3597458.08FU	VSH97_20	VSH985_21
UDB0779060	26500	this study	<i>E. insulae</i>	-	-	JAPAN, Tokyo, Hahajima Island	wood of unidentified tree	SH3569654.08FU	SH3597460.08FU	VSH97_14	VSH985_15
UDB0779063	39720	this study	<i>E. insulae</i>	-	-	JAPAN, Okinawa, Iriomote Island	bark of unidentified tree	†SH3569654.08FU	†SH3597460.08FU	VSH97_14	VSH985_15
UDB0779075	61920	this study	<i>E. paralushanensis</i>	-	-	JAPAN, Shizuoka, Atami	culm of <i>Pleioblastus argenteostriatus</i>	†SH3569655.08FU	†SH3597462.08FU	VSH97_19	VSH985_20
AF505515			<i>E. lushanensis</i>	<i>Lachnum lushanense</i>	<i>Lachnum lushanense</i>	(unspecified)	(unspecified)	†SH1155706.08FU	†SH1523143.08FU	VSH97_8	VSH985_10
JF937582		Zhao and Zhuang (2011)	<i>E. lushanensis</i>	<i>Lachnum lushanense</i>	<i>Lachnum lushanense</i>	CHINA	(unspecified)	SH1155706.08FU	SH1523143.08FU	VSH97_8	VSH985_10
MG434782		unpublished	<i>E. lushanensis</i>	<i>Erioscyphella sp.</i>	<i>E. lushanensis</i>	INDIA, Tangmarg	root tips of <i>Pinus wallichiana</i> (ectomycorrhiza)	(unassigned)	(unassigned)	VSH97_8	VSH985_10
UDB0779081	81272	this study	<i>E. papillaris</i>	-	-	JAPAN, Gunma, Minakami	leaf of unidentified bamboo	†SH3569656.08FU	†SH3597463.08FU	VSH97_23	VSH985_26
UDB0779084	81401	this study	<i>E. sasibrevispora</i>	-	-	JAPAN, Hokkaido, Tomakomai	culm of <i>Sasa nipponica</i>	SH3569657.08FU	SH3597464.08FU	VSH97_13	VSH985_23
UDB0779082	80399	this study	<i>E. sasibrevispora</i>	-	-	JAPAN, Gunma, Higashi-Agatsuma	sheath of <i>Sasa veitchii</i>	†SH3569657.08FU	†SH3597464.08FU	VSH97_13	VSH985_22
UDB0779085	81472	this study	<i>E. otanii</i>	-	-	JAPAN, Hokkaido, Horonobe, Teshio Experimental Forest, Hokkaido University	leaf of <i>Sasa senanensis</i>	†SH3569658.08FU	†SH3597465.08FU	VSH97_24	VSH985_27
UDB0779087	17245	this study	<i>Lachnum mapirianum</i>	-	-	MALAYSIA, Gerik	leaf of unidentified tree	†SH3569659.08FU	†SH3597466.08FU	VSH97_17	VSH985_18
UDB0779088	17249	this study	<i>Lachnum mapirianum</i>	-	-	MALAYSIA, Gerik	leaf of unidentified tree	SH3569659.08FU	SH3597466.08FU	VSH97_17	VSH985_18

† Representative sequence of each SH

‡ Reference sequence of each SH

Table 3. Taxa analyzed in the phylogenetic analyses in Chapter II.

Specimen no. (TNS-F-)	Strain no.	Phylogenetic analyses		Taxon	Collection site	Collected Date	Host plants and parts	§ GenBank/UNITE accession no.			
		ITS+ LSU+ mtSSU +RPB2	ITS+ LSU					ITS	LSU	RPB2	mtSSU
16740	NBRC 104380	○	○	<i>Albotricha acutipila</i> (P. Karst.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2006-06-17	culm of unidentified bamboo	AB481234	LC438571	AB481354	LC431751
16497	NBRC 101346	○	○	<i>Albotricha albotestacea</i> (Desm.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of <i>Miscanthus sinensis</i>	AB481235	LC424943	AB481340	LC431747
16635	NBRC 104365	○	○	<i>Brunnipila fuscescens</i> (Pers.) Baral	JAPAN, Gunma, Higashi- Agatsuma	2006-04-27	leaf of unidentified tree	AB481255	LC424945	AB481348	LC431750
16690	NBRC 104374	○	○	<i>Brunnipila pseudocannabina</i> (Raitv.) Tochihiro, Sasagawa & Hosoya	JAPAN, Akita, Kosaka	2006-05-26	stem of unidentified herb	AB481272	LC533520	LC533521	LC533522
65670	(FC-6101)	○	○	<i>Capitotricha bicolor</i> (Bull.) Baral	SWITZERLAND, Filisur	2016-06-06	twig of <i>Prunus spinosa</i>	LC424834	LC424942	LC425011	LC533244
65752	(FC-6075)	○	○	<i>Capitotricha rubi</i> (Bres.) Baral	SWITZERLAND, Saicourt	2016-06-04	twig of <i>Rubus idaeus</i>	LC438560	LC438573	LC440395	LC533243
—	(FC-6187)	○	○	<i>Crucellisporiopsis marquesiae</i> Crous	ZAMBIA	unidentified	living leaf of <i>Nectaropetalum zuluense</i>	undesigned	undesigned	undesigned	undesigned
—	(FC-6188)	○	○	<i>Crucellisporium umtamvunae</i> Marinc., Gryzenh. & M.J. Wingf.	SOUTH AFRICA	unidentified	twig of <i>Marquesia acuminata</i>	undesigned	undesigned	undesigned	undesigned
81459	(FC-6837)	○	○	<i>Dasyscyphella cassandrae</i> Tranzschel	JAPAN, Hokkaido, Teshio, Horonobe, Sarobetsu Wetland	2018-07-10	dead twigs of <i>Chamaedaphne calyculata</i>	undesigned	undesigned	undesigned	undesigned
16439	NBRC 101335	○	○	<i>Dasyscyphella longistipitata</i> Hosoya	JAPAN, Kanagawa, Yamakita	2005-04-17	cupule of <i>Fagus crenata</i>	AB481239	LC424947	AB481331	LC533228
16527	NBRC 102336	○	○	<i>Dasyscyphella montana</i> Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	wood of unidentified tree	AB481242	LC438577	AB481336	LC533241
16701	(FC-2209)	○	○	<i>Dasyscyphella nivea</i> (R. Hedw.) Raitv.	JAPAN, Aomori, Sannobe, Mayogadai	2006-05-27	wood of unidentified tree	AB481243	undesigned	AB481350	undesigned
81444	(FC-6824)	○	○	<i>Dasyscyphella</i> sp. 1	JAPAN, Tochigi, Nikko, Utsunomiya University Forest	2018-06-29	wood of unidentified tree	undesigned	undesigned	undesigned	undesigned
16617	(FC-2172)	○	○	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yamakita	2005-07-03	wood of unidentified tree	AB481250	undesigned	AB481346	LC431749
16838	(FC-2355)	○	○	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochihiro & Hosoya	JAPAN, Ibaraki, Tsukuba Botanical Garden	2007-06-15	leaf of unidentified broad-leaved tree	AB481280	LC533164	AB481364	LC533235
81904	—	○		<i>Incrucipulum aurantiacum</i> Tochihiro	JAPAN, Gunma, Agatsuma, Nakanojo, Shibutoge	2020-10-09	dead stems of <i>A. dioicus</i> var. <i>kamtschaticus</i>	undesigned	undesigned	undesigned	undesigned
81851	(FC-11139)	○		<i>Incrucipulum aurantiacum</i> Tochihiro	JAPAN, Gunma, Agatsuma, Nakanojo, Damashi-daira	2021-09-15	dead stems of <i>A. dioicus</i> var. <i>kamtschaticus</i>	undesigned	undesigned	undesigned	undesigned
81832	(FC-11128)	○		<i>Incrucipulum brevistipitatum</i> Tochihiro	JAPAN, Hokkaido, Kamikawa, Mt. Niseikaushuppe	2020-08-19	dead leaves of Poaceae	undesigned	undesigned	undesigned	undesigned
81420	NBRC 113938	○		<i>Incrucipulum capitatum</i> (Peck) Baral	JAPAN, Hokkaido, Sapporo, Jozankei	2018-06-17	dead leaves of <i>Quercus crispula</i> subsp. <i>crispula</i>	LC424838	LC424954	LC438592	undesigned
81514	—	○	○	<i>Incrucipulum ciliare</i> (Schr.) Baral	JAPAN, Saitama, Chichibu, Ogano, Mt. Futago	2018-08-12	dead leaves of <i>Quercus crispula</i> subsp. <i>crispula</i>	LC438568	LC438585	LC438598	undesigned
81516	NBRC 113940	○		<i>Incrucipulum ciliare</i> (Schr.) Baral	JAPAN, Saitama, Chichibu, Ogano, Mt. Futago	2018-08-12	dead leaves of <i>Castanea crenata</i>	LC438565	LC438582	LC438595	undesigned
81520	NBRC 113941	○		<i>Incrucipulum ciliare</i> (Schr.) Baral	JAPAN, Shizuoka, Shizuoka	2018-08-18	leaf of <i>Quercus mongolica</i> subsp. <i>crispula</i> (Blume) Menitsky	LC438566	LC438583	LC438596	LC533284
81526	—	○		<i>Incrucipulum foliicola</i> Tochihiro	JAPAN, Hokkaido, Yamakoshi, Oshamambe, Shizukari Mire	2018-06-26	dead leaves of <i>Myrica gale</i> subsp. <i>tomentosa</i>	LC438569	LC438586	LC438599	undesigned

Specimen no. (TNS-F-)	Strain no.	Phylogenetic analyses		Taxon	Collection site	Collected Date	Host plants and parts	§ GenBank/UNITE accession no.			
		ITS+ LSU+ mtSSU +RPB2	ITS+ LSU					ITS	LSU	RPB2	mtSSU
81508	NBRC 113942	○		<i>Incrucipulum foliicola</i> Tochihara	JAPAN, Hokkaido, Nemuro, Cape Ochiishi Mire	2018-07-20	dead leaves of <i>Myrica gale</i> subsp. <i>tomentosa</i>	LC438567	LC438584	LC438597	undesigned
81512	—	○		<i>Incrucipulum hakonechloae-macrae</i> Tochihara	JAPAN, Saitama, Chichibu, Ogano, Mt. Futago	2018-08-12	dead leaves of <i>Hakonechloa macra</i>	LC438564	LC438581	undesigned	undesigned
17632	NBRC 102347	○	○	<i>Incrucipulum longispineum</i> Sasagawa & Hosoya	JAPAN, Miyagi, Sendai	2006-07-29	leaf of <i>Lyonia ovalifolia</i> (Wall.) Drude	AB481256	LC438579	AB481362	LC533234
81441	(FC-6822)	○		<i>Incrucipulum pseudosulphurellum</i> Tochihara	JAPAN, Hokkaido, Yamakoshi, Oshamambe, Shizukari Mire	2018-06-26	dead twigs of <i>Myrica gale</i> subsp. <i>tomentosa</i>	LC438563	LC438588	LC438601	undesigned
81672	(FC-11091)	○		<i>Incrucipulum pseudosulphurellum</i> Tochihara	JAPAN, Aomori, Tashirotai Wetland	2019-07-09	dead twigs of <i>Myrica gale</i> subsp. <i>tomentosa</i>	undesigned	undesigned	undesigned	undesigned
36248	113933	○	○	<i>Incrucipulum radiatum</i> Sasagawa & Hosoya	JAPAN, Niigata, Tokamachi, Atemayama	2010-09-20	dead leaves of <i>Fagus crenata</i>	LC438559	LC438580	LC438593	undesigned
16769	NBRC 104385	○		<i>Incrucipulum radiatum</i> Sasagawa & Hosoya	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2006-09-24	dead leaves of <i>Fagus crenata</i>	AB481261	AB481322	AB481359	undesigned
81621	(FC-6984)	○	○	<i>Incrucipulum sulphurellum</i> (Peck) Baral	JAPAN, Hokkaido, Hamanaka, Akkeshi, Kiritappu Wetland	2019-06-20	dead twigs of <i>Myrica gale</i> subsp. <i>tomentosa</i>	undesigned	undesigned	undesigned	undesigned
81695	(FC-11093)	○		<i>Incrucipulum sulphurellum</i> (Peck) Baral	JAPAN, Hokkaido, Soya, Sarufutsu, Sarufutsu River Mire	2019-07-22	dead twigs of <i>Myrica gale</i> subsp. <i>tomentosa</i>	undesigned	undesigned	undesigned	undesigned
81905	—	○	○	<i>Incrucipulum virmbergense</i> (Matheis) Baral	JAPAN, Gunma, Agatsuma, Nakanajo, Yoshigadaira Wetlands	2021-09-15	dead leaves of <i>Vaccinium uliginosum</i>	undesigned	undesigned	undesigned	undesigned
81248	NBRC 113935	○	○	<i>Lachnellula calyciformis</i> (Batsch) Dharne	JAPAN, Hokkaido, Engaru, Mt. Hirayama	2017-07-12	twig of <i>Abies sachalinensis</i>	LC438561	LC438574	LC438590	LC533247
65594	(FC-6049)	○		<i>Lachnellula subtilissima</i> (Cooke) Dennis	SWITZERLAND	2016-06-02	twig of <i>Abies</i> sp.	undesigned	undesigned	undesigned	undesigned
16529	NBRC 101348	○	○	<i>Lachnellula suecica</i> (de Bary ex Fuckel) Nannf.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	twig of <i>Larix kaempferi</i>	AB481248	LC424944†	AB481341	LC533231
—	— (CPC 24723)		○	<i>Lachnopsis catarinensis</i> Guatim., R.W. Barreto & Crous	BRAZIL	unidentified	<i>Dicksonia sellowiana</i>	KU597793†	KU597760†	—	—
—	— (CPC 24714)		○	<i>Lachnopsis catarinensis</i> Guatim., R.W. Barreto & Crous	BRAZIL	unidentified	<i>Dicksonia sellowiana</i>	KU597795†	KU597762†	—	—
—	— (CPC 24713)		○	<i>Lachnopsis catarinensis</i> Guatim., R.W. Barreto & Crous	BRAZIL	unidentified	<i>Dicksonia sellowiana</i>	KU597794†	KU597761†	—	—
—	— (CPC 24742)		○	<i>Lachnopsis dicksoniae</i> Guatim., R.W. Barreto & Crous	BRAZIL	unidentified	<i>Dicksonia sellowiana</i>	KU597796†	KU597763†	—	—
16494	NBRC 101341	○	○	<i>Lachnum asiaticum</i> (Y. Otani) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of unidentified bamboo	AB481251	LC533162	AB481334	LC533229
61858	(FC-5721)	○	○	<i>Lachnum controversum</i> (Cooke) Rehm	SWITZERLAND, Zürich	2015-04-29	culm of <i>Phragmitis</i> sp.	undesigned	undesigned	undesigned	undesigned
65591	(FC-6047)	○		<i>Lachnum eburneum</i> Kirschst.	SWITZERLAND	2016-06-02	stem of <i>Polygonatum</i> sp.	undesigned	undesigned	undesigned	undesigned
—	MAFFI 244392 (FC-5525)	○	○	<i>Lachnum nipponicum</i> J.H. Haines & S. Kaneko	JAPAN, Tottori	unidentified	dead leaves of <i>Quercus glauca</i>	undesigned	undesigned	undesigned	undesigned
16651	NBRC 104369	○	○	<i>Lachnum nudipes</i> (Fuckel) Nannf.	JAPAN, Aomori, Hirosaki, Mt. Iwaki	2006-05-24	stem of <i>Fallopia</i> sp.	AB481257	undesigned	AB481351	undesigned
16501	NBRC 102335	○	○	<i>Lachnum pudibundum</i> (Quél.) J. Schröt.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	wood of unidentified tree	AB481259	LC533160	AB481335	LC533230

Specimen no. (TNS-F-)	Strain no.	Phylogenetic analyses		Taxon	Collection site	Collected Date	Host plants and parts	§ GenBank/UNITE accession no.			
		ITS+ LSU+ mtSSU +RPB2	ITS+ LSU					ITS	LSU	RPB2	mtSSU
81229	NBRC 114473	○		<i>Lachnum rachidicola</i> J.G. Han, Raitv. & H.D. Shin	JAPAN, Hokkaido, Tomakomai, Tomakomai Experimental Forest	2017-08-09	petiole of <i>Juglans</i> sp.	UDB0779079	LC533136	LC533189	undesigned
16551	NBRC 104361	○	○	<i>Lachnum soppittii</i> (Masee) Raitv.	JAPAN, Ibaraki, Tsukuba, Mt. Tsukuba	2005-05-28	dead leaves	AB481266	LC438578	AB481344	undesigned
17631	NBRC 102332	○	○	<i>Lachnum varians</i> (Rehm) M.P. Sharma	JAPAN, Kagoshima, Yakushima Island	2005-10-23	dead petioles of <i>Pteris wallichiana</i>	AB481267	LC438576	AB481330	undesigned
13495	(FC-1256)	○	○	<i>Lachnum varians</i> (Rehm) M.P. Sharma	JAPAN, Kagoshima, Yakushima Island	2005-10-18	dead petioles of <i>Pteris wallichiana</i>	undesigned	undesigned	undesigned	undesigned
24598	(FC-1854)	○	○	<i>Lachnum varians</i> (Rehm) M.P. Sharma	JAPAN, Kagoshima, Amami Oshima, Mt. Yuwan	2009-02-25	dead petioles of fern	undesigned	undesigned	undesigned	undesigned
81533	(FC-6917)	○	○	<i>Lachnum varians</i> (Rehm) M.P. Sharma	JAPAN, Wakayama, Higashimure, Kozagawa	2019-05-07	dead petioles of <i>Gleichenia japonica</i>	undesigned	undesigned	undesigned	undesigned
16583	NBRC 104358	○	○	<i>Lachnum virgineum</i> (Batsch) P. Karst.	JAPAN, Kanagawa, Yamakita	2005-07-02	wood of unidentified tree	AB481268; AB705235	undesigned	AB481343	LC431748
65625	(FC-6068)	○	○	<i>Neodasyscypha cerina</i> (Pers.) Spooner	SWITZERLAND, Saicourt	2016-06-08	twig of <i>Crataegus</i> sp.	LC424836	LC424948	LC425013	LC533242
81438	(FC-6819)	○	○	<i>Neolachnum tenuipileum</i> Tochihara & Hosoya (← <i>Lachnum</i> sp. 1)	JAPAN, Hokkaido, Chitose	2018-06-18	on fallen stems of <i>Fallopia sachalinensis</i>	undesigned	undesigned	undesigned	undesigned
65645	(FC-6080)	○	○	<i>Neolachnum rhytismatis</i> (W. Phillips) Tochihara & Hosoya (← <i>Lachnum rhytismatis</i>)	SWITZERLAND, Lajoux, Sâces	2016-06-04	dead leaves of <i>Acer pseudoplatanus</i>	LC424835	LC424946	LC425012	undesigned
17436	NBRC 108594	○	○	<i>Proliferodiscus alboviridis</i> (Sacc.) Spooner	JAPAN, Ibaraki, Tsukuba Botanical Garden	2006-07-08	wood of unidentified tree	LC438558	LC533159	LC438589	LC533239
‡17909	NBRC 108585	○	○	<i>Hyaloscypha spiralis</i> (Velen.) J.G. Han, Hosoya & H.D. Shin	JAPAN, Kumamoto, Kikuchi	2005-10-10	wood of unidentified tree	LC438602	LC438604	LC438606	LC533237
‡16472	NBRC 104355	○		<i>Hymenoscyphus varicosporoides</i> Tubaki	JAPAN, Ibaraki, Kasumigaura	2005-05-05	wood of unidentified tree	AB481291	AB926121	AB481329	LC431746
‡18014	NBRC 108588	○	○	<i>Urceolella carestiana</i> (Rabenh.) Dennis	JAPAN, Iwate, Hanamaki	2006-05-23	stem of <i>Thelypteris nipponica</i>	LC438603	LC438605	LC438607	LC533238

† Downloaded from GenBank

‡ Outgroup

§ UNITE accession no. is beginning with 'UDB'. GenBank accession no. is beginning with 'AB' or 'LC'.

Table 4. Morphological and ecological features of sexual states of lachnaceous genera.

Genera	ectal excipulum			hairs						disc color	ascospores	paraphyses	asexual state	substrates	notes	references
	structure (textura-)	color	surface	shape	granulation	crystals	other materials	cell wall	color							
<i>Albotricha</i> (monophyletic)	angularis	hyaline	smooth	tapering	apical >2 cells smooth	absent	sometimes resinous materials	thin	hyaline	white, yellow	fusiform, ellipsoid	lanceolate	not observed	miscellaneous		Hosoya et al. (2010), Tochiara (2019)
<i>Brunnipila</i> (monophyletic)	prismatica to angularis	brown	smooth	cylindrical	completely granulate	present	absent	thin	brown	white	ellipsoid, fusiform	lanceolate	not observed	miscellaneous		Hosoya et al. (2010)
<i>Capitotricha</i> (monophyletic)	thick-walled prismatica	hyaline	smooth	cylindrical with acute apex	apical <3 cells smooth	present (rarely absent)	absent	thick	hyaline	yellow (sometimes white)	ellipsoid, fusiform	lanceolate	not observed	miscellaneous		Baral and Krieglsteiner (1985), Tochiara (2019)
<i>Dasyscyphella</i> (not monophyletic)	well developed prismatica	hyaline	smooth	cylindrical with swollen apex	apical multiple cells smooth	present, absent	rarely resinous materials	thin	hyaline	white, yellow	ellipsoid, fusiform, long cylindrical	filiform, narrowly lanceolate, lanceolate	not observed	miscellaneous		Raitviir (2002), Hosoya et al. (2010), Tochiara (2019)
<i>Erioscyphella</i> (monophyletic)	prismatica to angularis	hyaline (rarely brown)	smooth (rarely granulate)	cylindrical with never swollen apex	completely granulate	absent	apical amorphous materials, resinous materials	thin	hyaline, brown	white, yellow, orange (not discussed in this study)	mainly long fusiform (needle-like shaped)	narrowly lanceolate (rarely lanceolate)	not observed	miscellaneous		Peric and Baral (2014), this study
<i>Incrucipulum</i> (monophyletic)	thick-walled prismatica like stone pavings	hyaline (rarely brown)	granulate	cylindrical	completely granulate	present	absent	thick	hyaline	white, orange	fusiform, ellipsoid	filiform, lanceolate	not observed	miscellaneous		Baral and Krieglsteiner (1985), this study
<i>Lachnellula</i> (monophyletic)	prismatica, intricata, globulosa	hyaline	smooth	cylindrical	completely granulate	absent	absent	thin	hyaline, rarely brown	yellow, orange	globose, ellipsoid to tear-shaped	filiform, narrowly lanceolate	sometimes observed	twig (rarely leaf) of Pinaceae spp.		Hosoya et al. (2010), Baral (2015)
<i>Lachnum</i> sensu stricto (monophyletic)	prismatica to angularis	hyaline	smooth	cylindrical with swollen apex	completely granulate	absent (rarely present)	rarely resinous materials	thin	hyaline	white, pale yellow	fusiform, ellipsoid	lanceolate	not observed	miscellaneous		Hosoya et al. (2010), this study
<i>Neodasyscypha</i> †	irregularly arranged or disposed in rows at a high angle to the surface.	brown	smooth	cylindrical	completely and roughly granulate	absent	absent	thin	brown	yellow to orange	ellipsoid	cylindrical to narrowly lanceolate	not observed	rotten wood		Spooner (1987), Skova (2005)
<i>Neolachnum</i> (monophyletic)	prismatica	hyaline	smooth	cylindrical	completely granulate	present	absent	thin	hyaline	white to orange	ellipsoid, tear-shaped	lanceolate	not observed	miscellaneous		this study
<i>Proliferodiscus</i> †	prismatica to porrecta	hyaline	smooth	irregular shaped (irregularly curved)	completely and coarsely granulate	absent	sometimes resinous materials	thick	hyaline	white, yellow, orange	ellipsoid, clavate-fusoid	filiform	not observed	wood	apothecia proliferating	Haines (1983); Spooner (1987)
Clade D (monophyletic)	prismatica	hyaline	smooth	cylindrical	completely granulate	absent	sometimes resinous materials	thin	hyaline	white, cream, ocher	fusiform	filiform to narrow lanceolate	sometimes observed	miscellaneous		this study

† Monophyly have not been discussed.

Table 5. Taxa analyzed in the phylogenetic analyses in Chapter III.

Specimen no. (TNS-F-)	Strain no.	Taxon	Collection site	Collected Date	Host plants and parts	GenBank accession no.						
						ITS	LSU	RPB1	RPB2	mtSSU	SSU	MCM7
16740	NBRC 104380	<i>Albotricha acutipila</i> (P. Karst.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2006-06-17	culm of unidentified bamboo	AB481234	LC438571	LC431679‡	AB481354	LC431751	LC434582§	undesigned
16497	NBRC 101346	<i>Albotricha albotestacea</i> (Desm.) Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-18	culm of <i>Miscanthus sinensis</i>	AB481235	LC424943	undesigned ‡	AB481340	LC431747	undesigned	undesigned
16635	NBRC 104365	<i>Brunnipila fuscescens</i> (Pers.) Baral	JAPAN, Gunma, Higashi-Agatsuma	2006-04-27	leaf of unidentified tree	AB481255	LC424945	LC431677‡	AB481348	LC431750	LC434580§	undesigned
16690	NBRC 104374	<i>Brunnipila pseudocannabina</i> (Raitv.) Tochihara, Sasagawa & Hosoya	JAPAN, Akita, Kosaka	2006-05-26	stem of unidentified herb	AB481272	LC533520	undesigned	LC533521	LC533522	—	undesigned
65670	(FC-6101)	<i>Capitotricha bicolor</i> (Bull.) Baral	SWITZERLAND, Filisur	2016-06-06	twig of <i>Prunus spinosa</i>	LC424834	LC424942	undesigned ‡	LC425011	LC533244	undesigned	undesigned
65752	(FC-6075)	<i>Capitotricha rubi</i> (Bres.) Baral	SWITZERLAND, Saicourt	2016-06-04	twig of <i>Rubus idaeus</i>	LC438560	LC438573	undesigned	LC440395	LC533243	undesigned §	undesigned
—	(FC-6187)	<i>Crucellisporiopsis marquesiae</i> Crous	ZAMBIA	unidentified	living leaf of <i>Nectaropetalum zuluense</i>	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned
—	(FC-6188)	<i>Crucellisporium umtamvunae</i> Marinc., Gryzenh. & M.J. Wingf.	SOUTH AFRICA	unidentified	twig of <i>Marquesia acuminata</i>	undesigned	undesigned	undesigned	undesigned	—	undesigned	—
81459	(FC-6837)	<i>Dasyscyphella cassandrae</i> Tranzschel	JAPAN, Hokkaido, Teshio, Horonobe, Sarobetsu Wetland	2018-07-10	dead twigs of <i>Chamaedaphne calyculata</i>	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned
16433	NBRC 101335	<i>Dasyscyphella longistipitata</i> Hosoya	JAPAN, Kanagawa, Yamakita	2005-04-17	cupule of <i>Fagus crenata</i>	undesigned	undesigned	—	undesigned	LC431745	—	undesigned
16527	NBRC 102336	<i>Dasyscyphella montana</i> Raitv.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	wood of unidentified tree	AB481242	LC438577	undesigned	AB481336	LC533241	undesigned	undesigned
16701	(FC-2209)	<i>Dasyscyphella nivea</i> (R. Hedw.) Raitv.	JAPAN, Aomori, Sannobe, Mayogadai	2006-05-27	wood of unidentified tree	AB481243	undesigned	undesigned	AB481350	undesigned	undesigned	undesigned
81444	(FC-6824)	<i>Dasyscyphella</i> sp. 1	JAPAN, Tochigi, Nikko, Utsunomiya University Forest	2018-06-29	wood of unidentified tree	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned
16617	(FC-2172)	<i>Erioscyphella abnormis</i> (Mont.) Baral, Šandová & B. Perić	JAPAN, Kanagawa, Yamakita	2005-07-03	wood of unidentified tree	AB481250	undesigned	LC431676‡	AB481346	LC431749	LC434554§	undesigned
16838	(FC-2355)	<i>Erioscyphella sinensis</i> (Z.H. Yu and W.Y. Zhuang) Sasagawa, Tochihara & Hosoya	JAPAN, Ibaraki, Tsukuba Botanical Garden	2007-06-15	leaf of unidentified broad-leaved tree	AB481280	LC533164	undesigned	AB481364	LC533235	undesigned	undesigned
81520	NBRC 113941	<i>Incrucipulum ciliare</i> (Schrad.) Baral	JAPAN, Shizuoka, Shizuoka	2018-08-18	leaf of <i>Quercus mongolica</i> subsp. <i>crispula</i>	LC438566	LC438583	undesigned	LC438596	LC533284	undesigned	undesigned
17632	NBRC 102347	<i>Incrucipulum longispineum</i> Sasagawa & Hosoya	JAPAN, Miyagi, Sendai	2006-07-29	leaf of <i>Lyonia ovalifolia</i>	AB481256	LC438579	undesigned	AB481362	LC533234	undesigned	undesigned
65594	(FC-6049)	<i>Lachnellula subtilissima</i> (Cooke) Dennis	SWITZERLAND	2016-06-02	twig of <i>Abies</i> sp.	undesigned	undesigned	undesigned ‡	undesigned	undesigned	undesigned §	undesigned

Specimen no. (TNS-F-)	Strain no.	Taxon	Collection site	Collected Date	Host plants and parts	GenBank accession no.						
						ITS	LSU	RPB1	RPB2	mtSSU	SSU	MCM7
16529	NBRC 101348	<i>Lachnellula suecica</i> (de Bary ex Fuckel) Nannf.	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2005-05-21	twig of <i>Larix kaempferi</i>	AB481248	LC424944	undesigned ‡	AB481341	LC533231	—	undesigned
61858	(FC-5721)	<i>Lachnum controversum</i> (Cooke) Rehm	SWITZERLAND, Zürich	2015-04-29	culm of <i>Phragmitis</i> sp.	undesigned	undesigned	undesigned ‡	undesigned	undesigned	undesigned §	undesigned
65591	(FC-6047)	<i>Lachnum eburneum</i> Kirschst.	SWITZERLAND	2016-06-02	stem of <i>Polygonatum</i> sp.	undesigned	undesigned	undesigned ‡	undesigned	undesigned	undesigned §	undesigned
—	MAFFI 244392 (FC-5525)	<i>Lachnum nipponicum</i> J.H. Haines & S. Kaneko	JAPAN, Tottori	unidentified	dead leaves of <i>Quercus glauca</i>	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned
17631	NBRC 102332	<i>Lachnum varians</i> (Rehm) M.P. Sharma	JAPAN, Kagoshima, Yakushima Island	2005-10-23	dead petioles of <i>Pteris wallichiana</i>	AB481267	LC438576	undesigned	AB481330	undesigned	undesigned	undesigned
16583	NBRC 104358	<i>Lachnum virgineum</i> (Batsch) P. Karst.	JAPAN, Kanagawa, Yamakita	2005-07-02	wood of unidentified tree	AB481268; AB705235	undesigned	undesigned ‡	AB481343	LC431748	LC434553§	undesigned
65625	(FC-6080)	<i>Neodasyscypha cerina</i> (Pers.) Spooner	SWITZERLAND, Saicourt	2016-06-08	twig of <i>Crataegus</i> sp.	LC424836	LC424948	undesigned ‡	LC425013	LC533242	undesigned §	undesigned
65645	(FC-6080)	<i>Neolachnum tenuipileum</i> Tochiara & Hosoya	JAPAN, Hokkaido, Chitose	2018-06-18	on fallen stems of <i>Fallopia sachalinensis</i>	LC424835	LC424946	undesigned ‡	LC425012	undesigned	undesigned §	undesigned
81438	(FC-6819)	<i>Neolachnum rhytismatis</i> (W. Phillips) Tochiara & Hosoya	SWITZERLAND, Lajoux, Sâces	2016-06-04	dead leaves of <i>Acer pseudoplatanus</i>	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned	undesigned
17436	NBRC 108594	<i>Proliferodiscus alboviridis</i> (Sacc.) Spooner	JAPAN, Ibaraki, Tsukuba Botanical Garden	2006-07-08	wood of unidentified tree	LC438558	LC533159	undesigned	LC438589	LC533239	undesigned §	undesigned
16472	NBRC 104355	<i>Hymenoscyphus varicosporoides</i> Tubaki	JAPAN, Ibaraki, Kasumigaura	2005-05-05	wood of unidentified tree	AB481291	AB926121	LC431678‡	AB481329	LC431746	LC434566	undesigned
16760	NBRC 102342	<i>Trichopeziza mollissima</i> Fuckel	JAPAN, Nagano, Ueda, Sugadaira Montane Research Center	2009-09-22	stem of herbaceous plants	AB481285	undesigned	LC431681‡	AB481360	undesigned	LC434567	undesigned
16579	NBRC 101353	<i>Trichopezizella otanii</i> J.H.Haines	JAPAN, Oita, Kokonoe	2005-06-22	on dead stem of <i>Fallopia</i> sp.	AB481287	LC424951	LC431685‡	AB481345	undesigned	LC434590§	Undesigned

‡ Cultures not donated in NBRC is beginning with "FC-", local suffix in TNS. '—' represents no culture exist and DNA was extracted from apothecia.

‡ Primer pair RPB1-Af and RPB1-Cr was used. In RPB1 sequences without notes, primer pair gRPB1-Af and fRPB1-Cr was used.

§ Primer pair NS1 and NS41 was used. In SSU sequences without notes, primer pair NS1 and NS4 was used.

| Outgroup

Figures

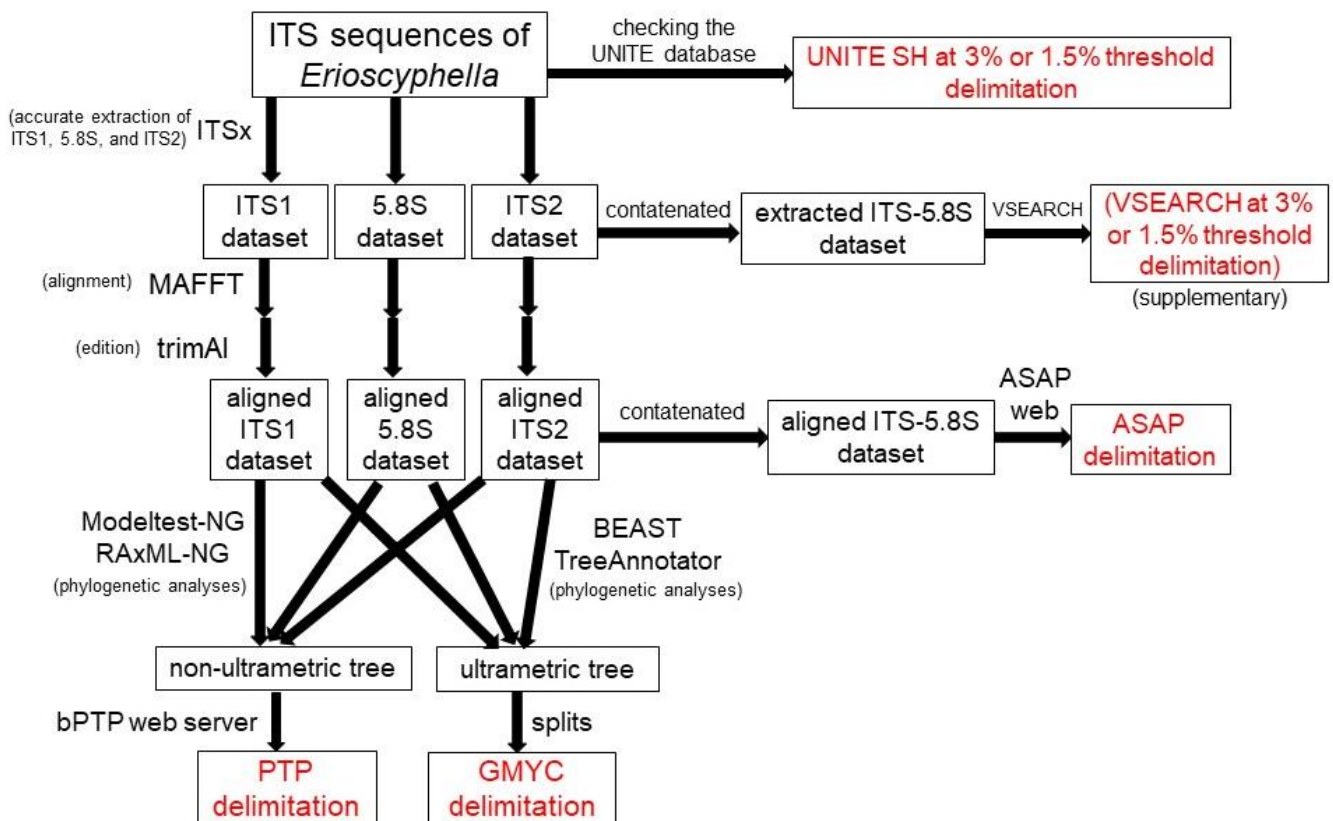


Figure 1. Diagrammatic representation showing the species delimitation analyses using ITS sequences.

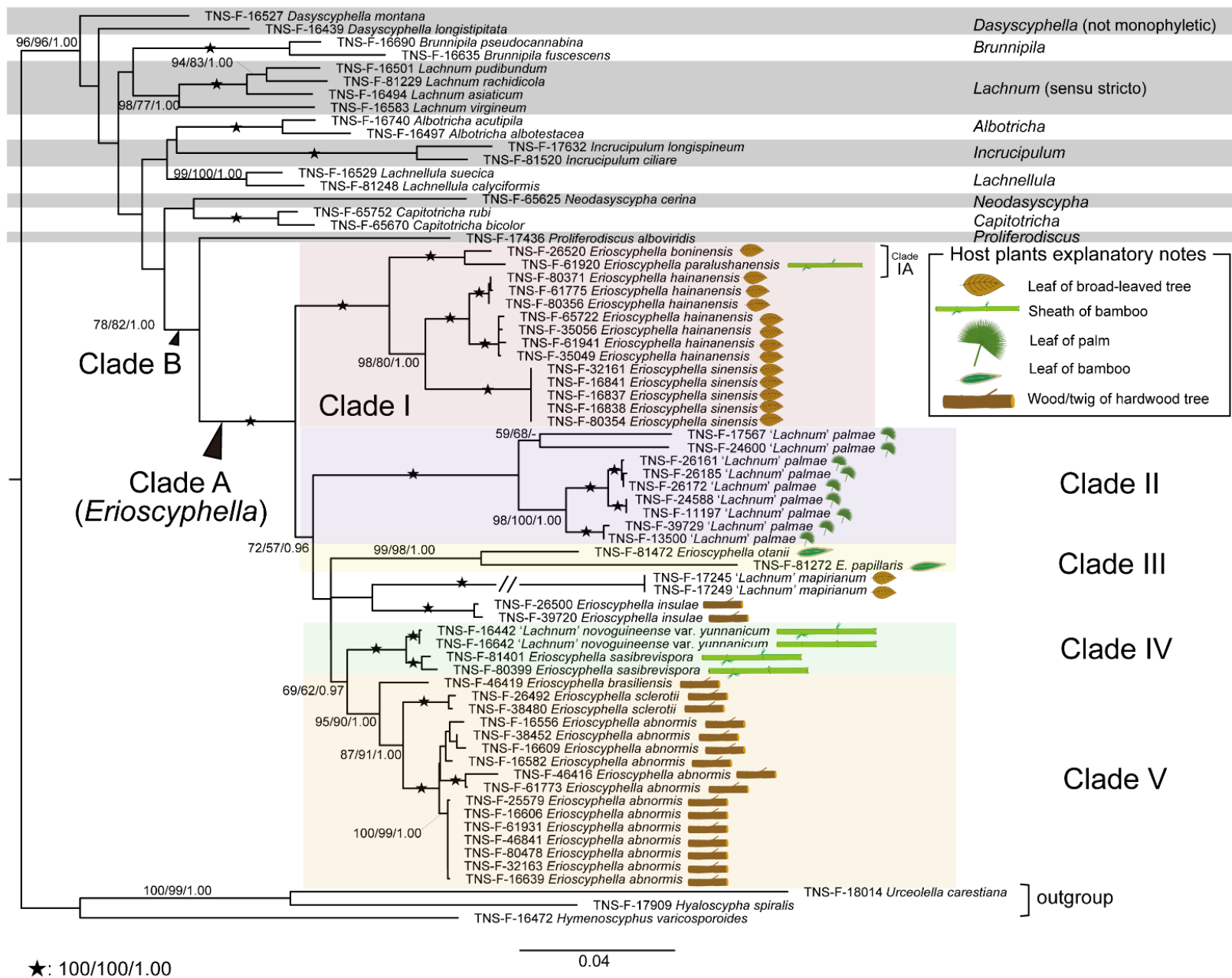


Figure 2. ML best-scored phylogenetic tree based on the concatenated dataset of ITS, LSU, mtSSU, and RPB2 constructed using RAxML-NG. MLBP/MPBP/BPP are represented on branches in this order. In MLBP/MPBP < 50% or BPP < 0.95, a hyphen appears. No evaluation values are shown on branches when MLBP and MPBP < 50% and BPP < 0.95. The branch of a clade TNS-F-17245 + 17249 to its most recent common ancestor is only one-third of the intended length due to space limitation.

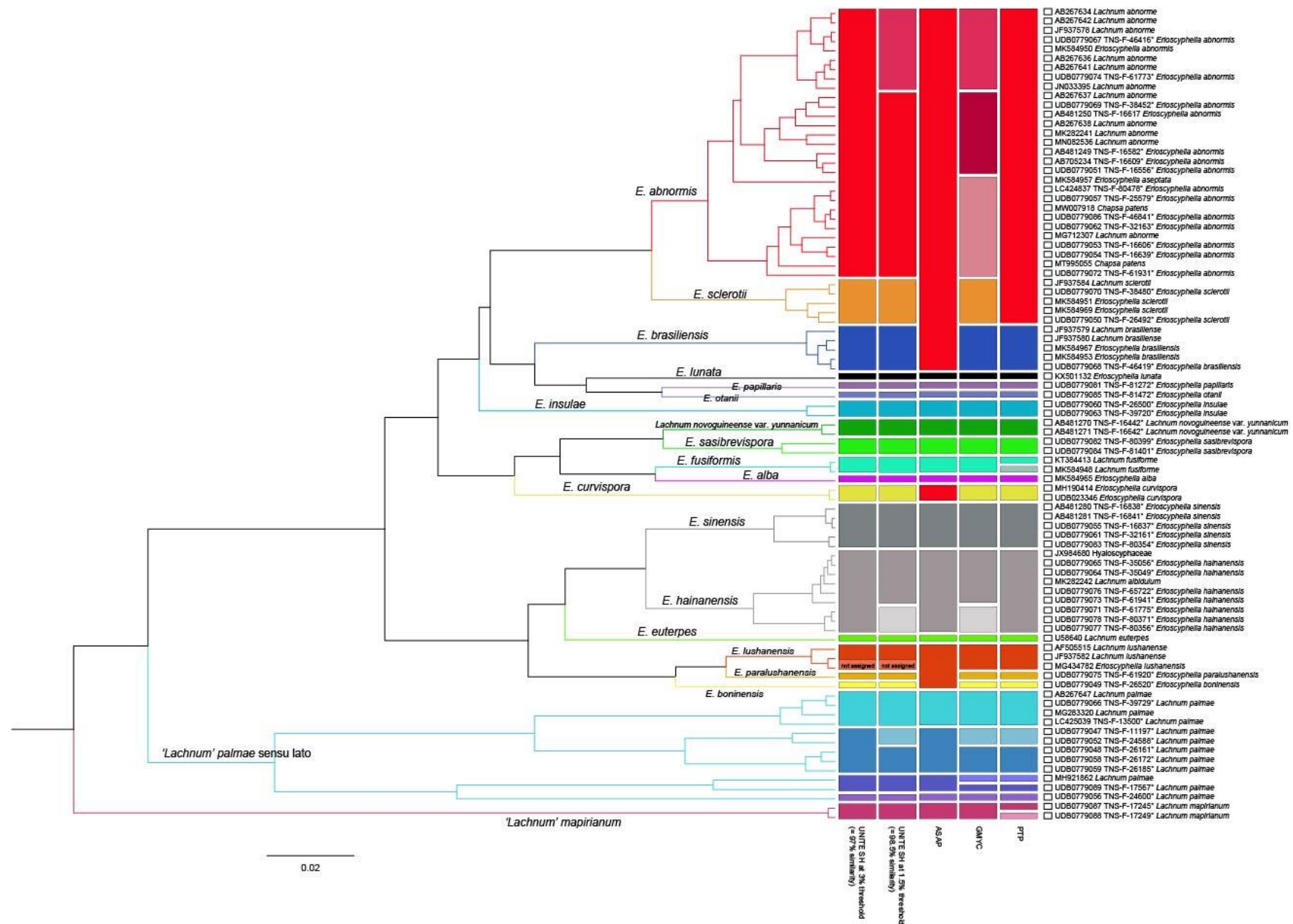


Figure 3. Species delimitation analyses using ITS sequences of *Erioscyphella* and its potential members. Clusters based on UNITE SH at 3% and 1.5% thresholds at UNITE v8.2, ASAP, GMYC, and PTP are displayed. Schematic phylogenetic relationships are shown using the ultrametric tree constructed for the GMYC analysis. The taxon names shown on the tree branches follow the results of the present study. In UNITE SH at 3% and 1.5% thresholds, cluster including MG434782 was shown based on the results of VSEARCH at 3% and 1.5% threshold values (see Appendix 3).

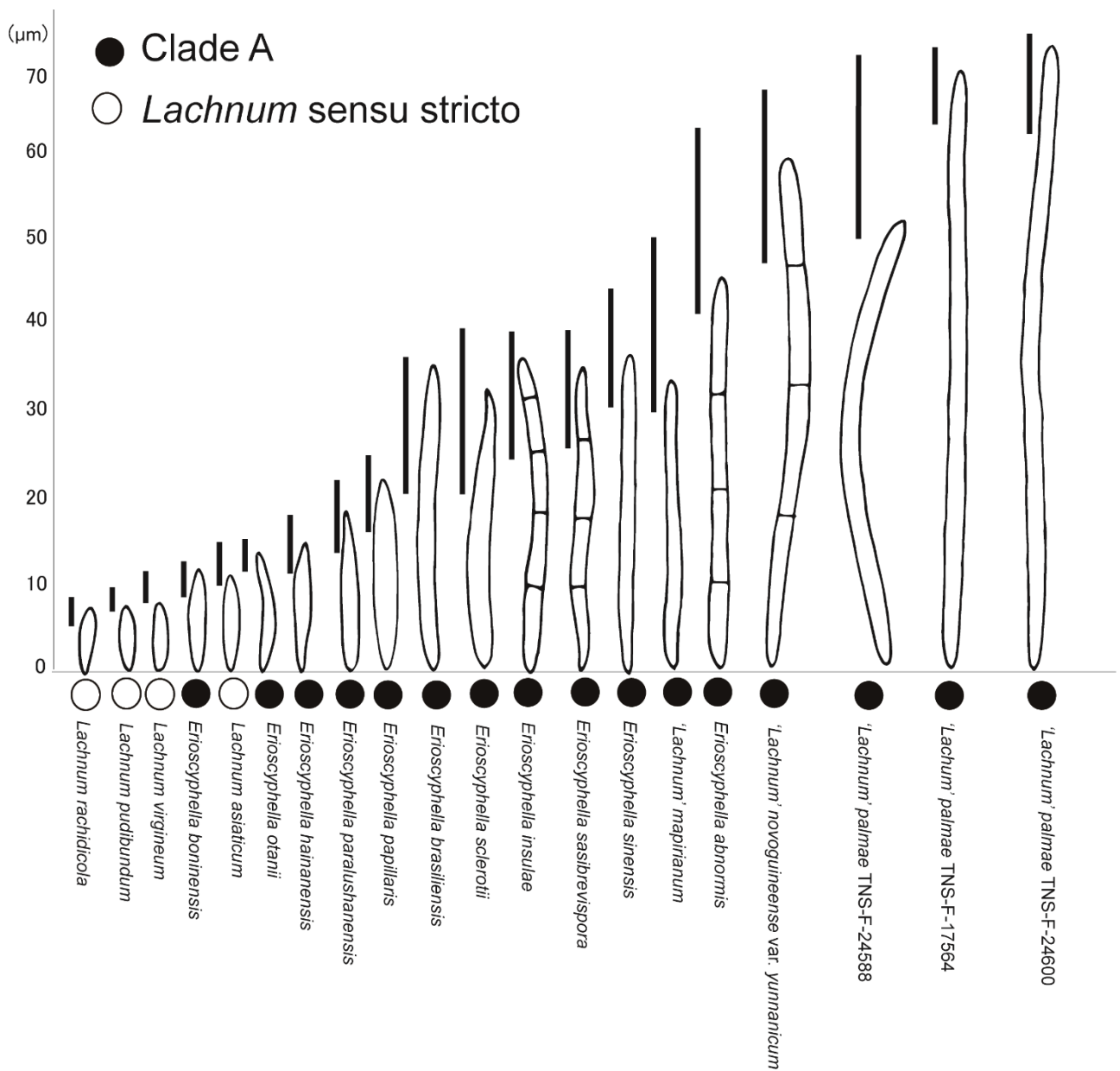


Figure 4. Comparison of ascospores of Clade A (= *Erioscyphella*) and the clade of *Lachnum sensu stricto* in Fig. 2. Subclade numbers for members of Clade A in Fig. 2 are shown in parentheses. Bars show variation of ascospore length within each species.

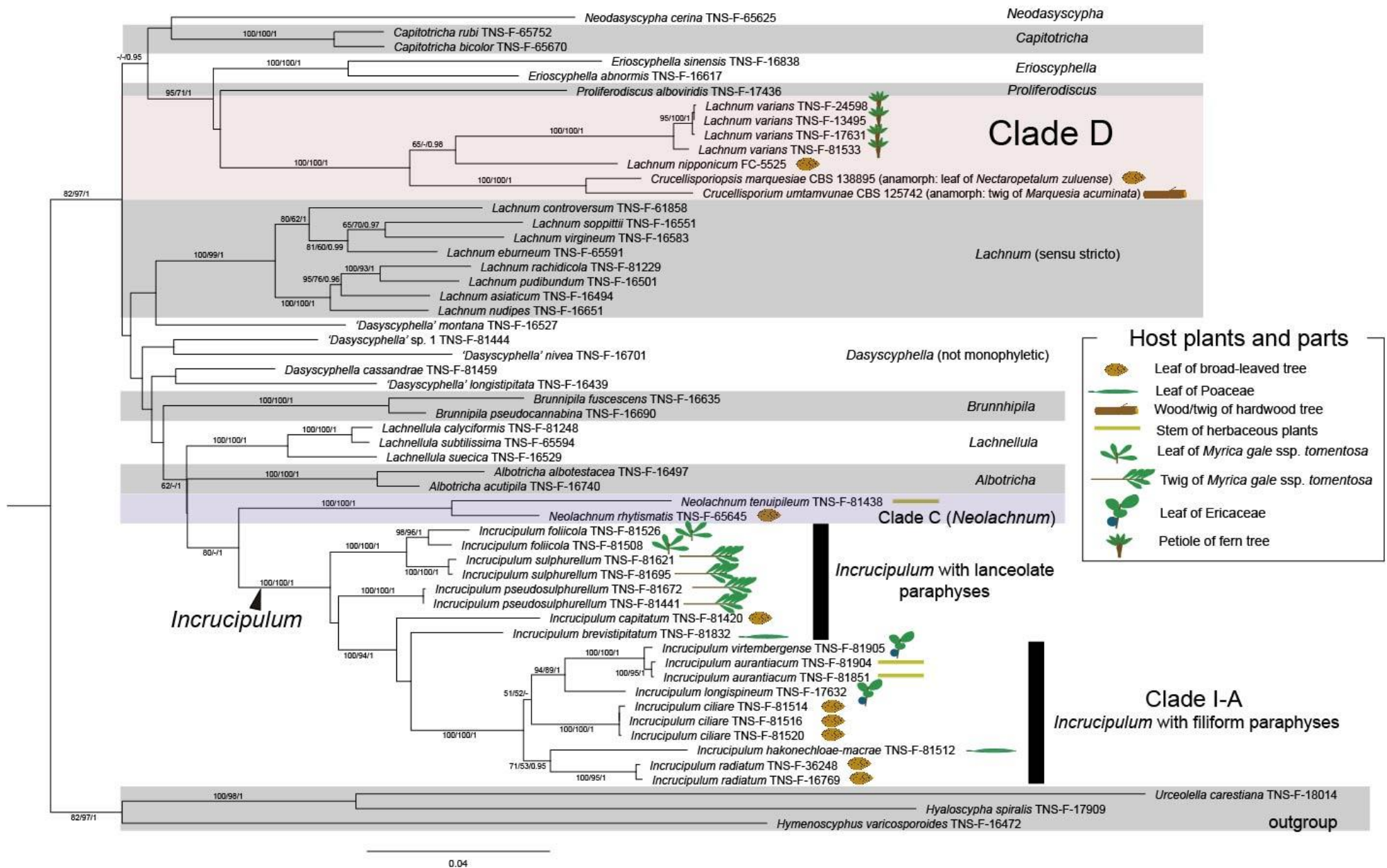


Figure 5. ML best-scored phylogenetic tree based on the concatenated dataset of ITS, LSU, mtSSU, and RPB2 constructed using RAXML-NG. MLBP/MPBP/BPP are represented on branches in this order. Hyphen indicates that MLBP/MPBP < 50% or BPP < 0.95. No evaluation values are shown on branches when MLBP and MPBP < 50% and BPP < 0.95.

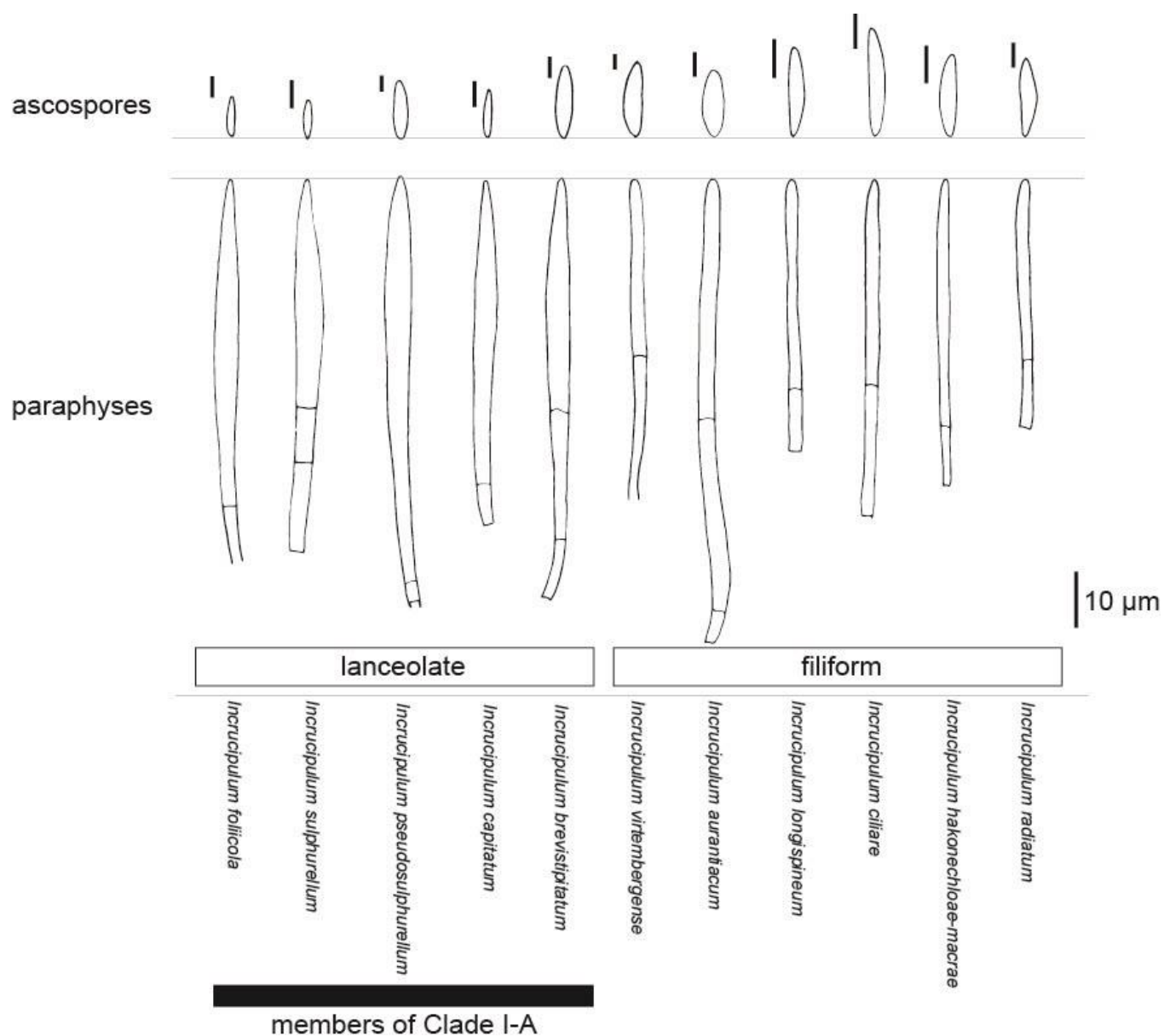


Figure 6. Comparison of shape and size of ascospores and paraphyses within the clade *Incrucipulum*.

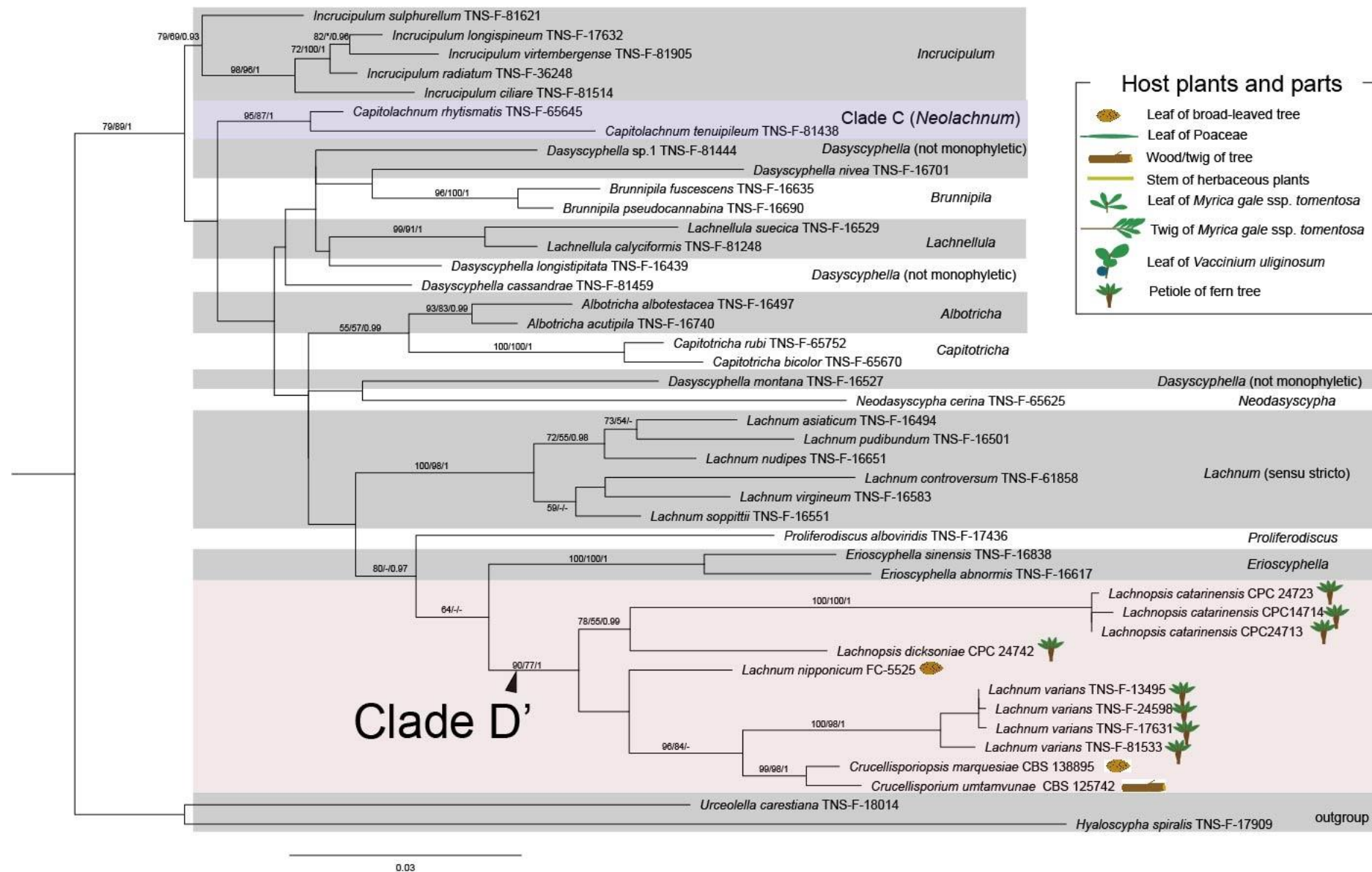


Figure 7. ML best-scored phylogenetic tree based on the concatenated dataset of ITS and LSU constructed by RAxML-NG. MLBP/MPBP/BPP are represented on branches in this order. ‘-’ indicates that MLBP/MPBP < 50% or BPP < 0.95. No evaluation values are shown on branches when MLBP and MPBP < 50% and BPP < 0.95.

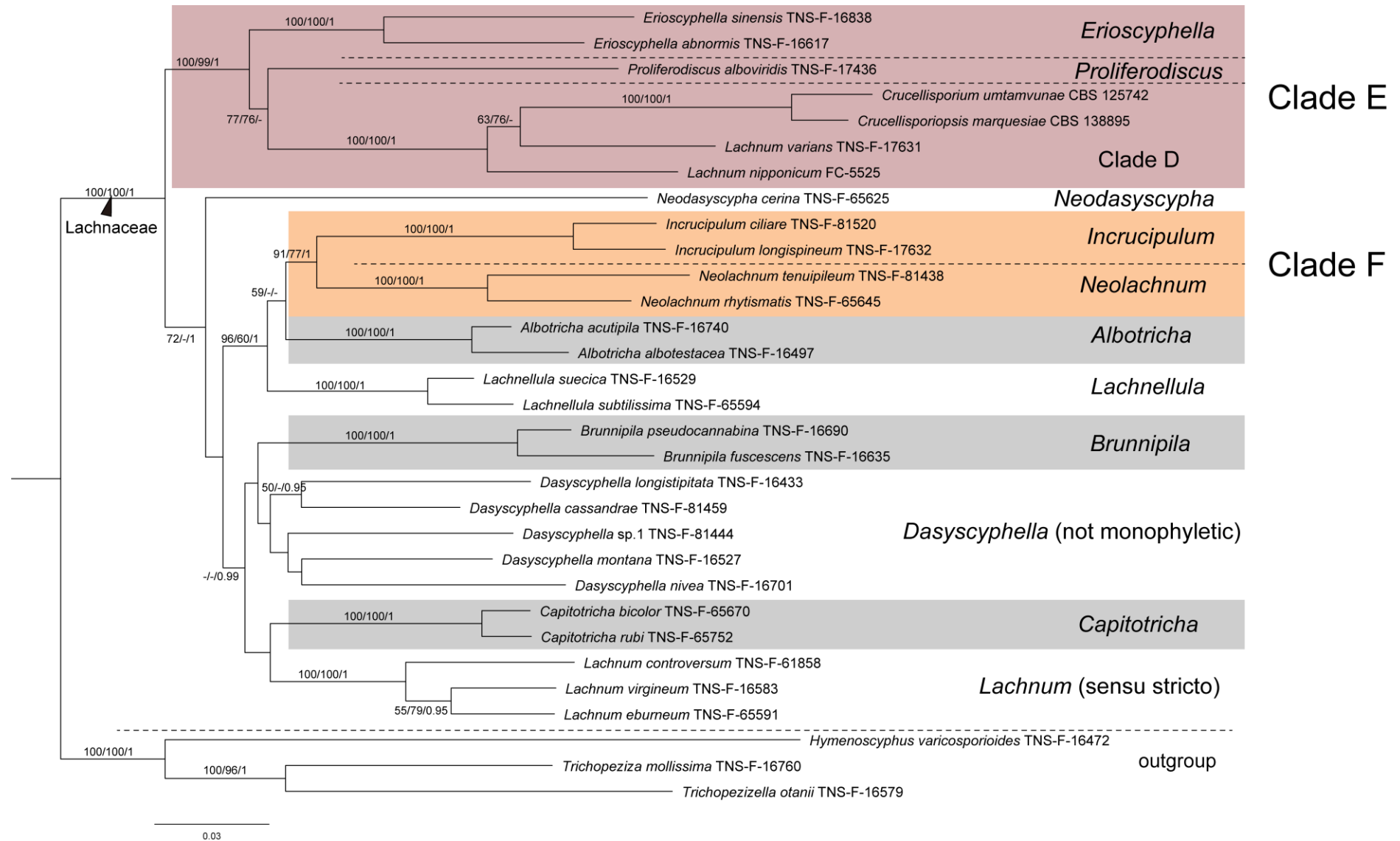


Figure 8. ML best-scored phylogenetic tree based on the concatenated dataset of 7 regions constructed by RAxML-NG. MLBP/MPBP/BPP are represented on branches in this order. Hyphen indicates that MLBP/MPBP < 50% or BPP < 0.95. No evaluation values are shown on branches when MLBP and MPBP < 50% and BPP < 0.95.

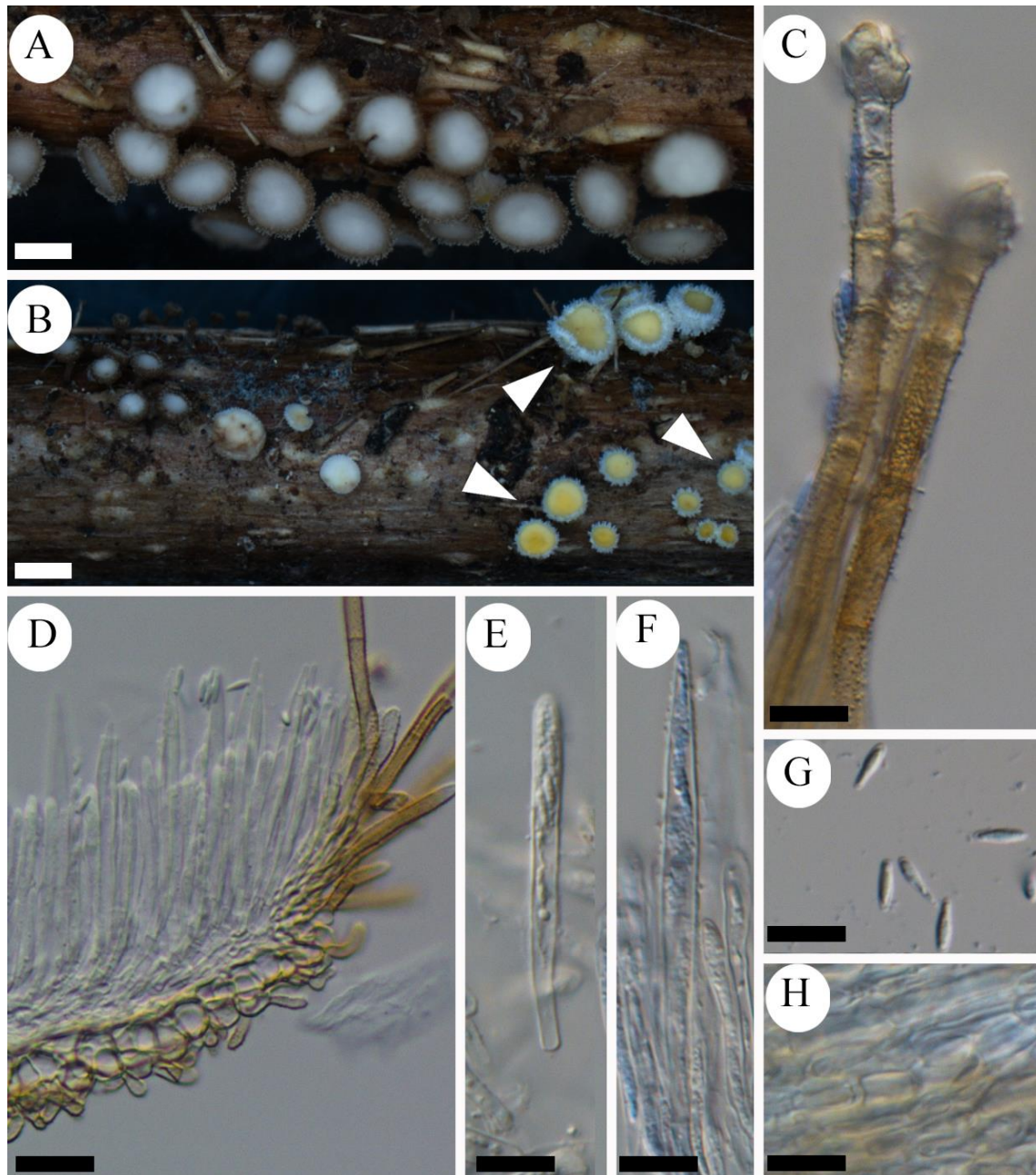


Figure 9. *Brunnipila clandestina* TNS-F-81486. **A** Fresh apothecia **B** *Brunnipila clandestina* with *Capitotricha rubi* (arrowheads) on the same substrate (when fresh) **C** Hairs with crystals **D** Vertical section of an apothecium showing the ectal and medullary excipulum **E** Ascus **F** Paraphysis **G** Ascospores **H** Ectal excipular inner cells. Mounted in CB dissolved with tap water (**D**), CB/LA (**C**, **E**–**H**). Scale bars: 1 mm (**A**, **B**), 10 μ m (**C**, **E**–**H**), 20 μ m (**D**).

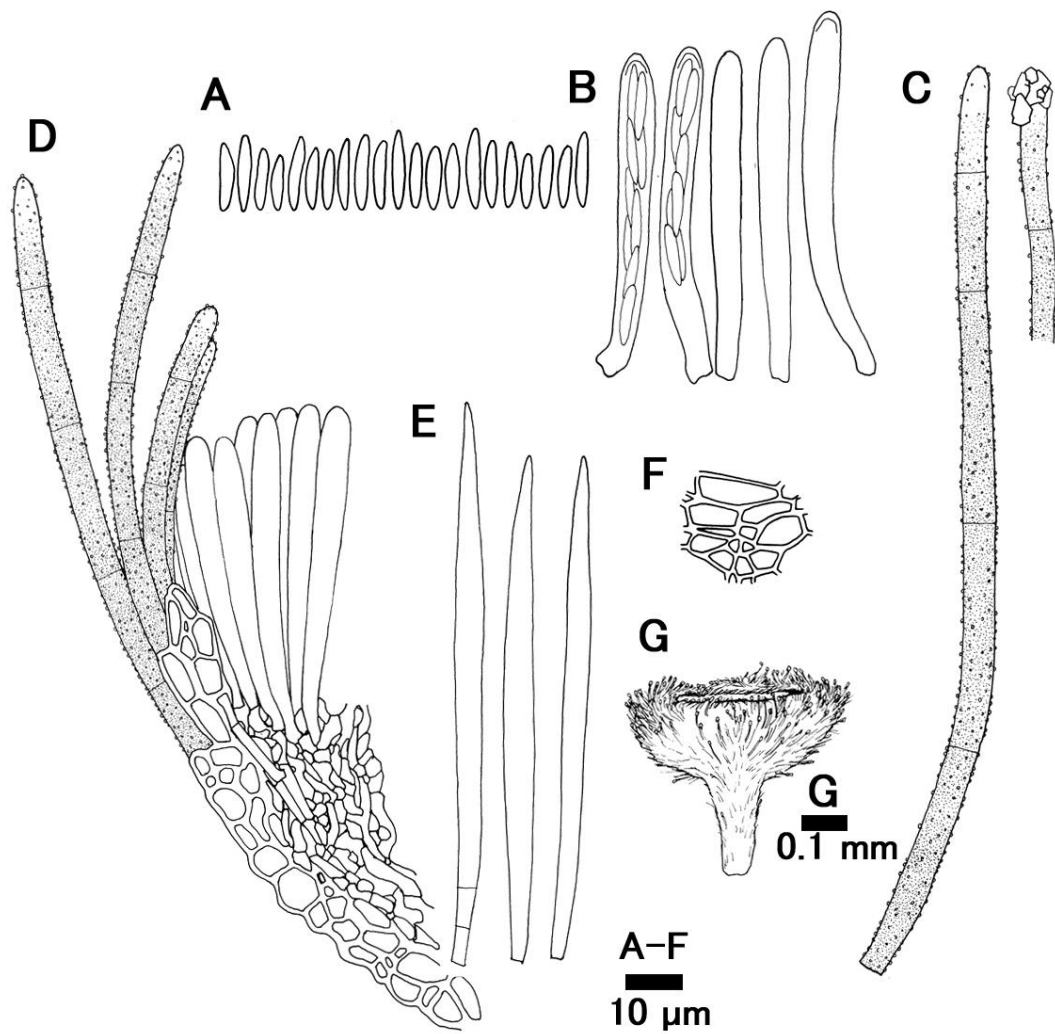


Figure 10. *Brunnipila clandestina* TNS-F-81486. **A** Ascospores **B** Asci **C** Hairs with and detached (left) crystals **D** Vertical section through the margin, showing the hairs and ectal excipulum **E** Paraphyses **F** Ectal excipular cells **G** Apothecium.

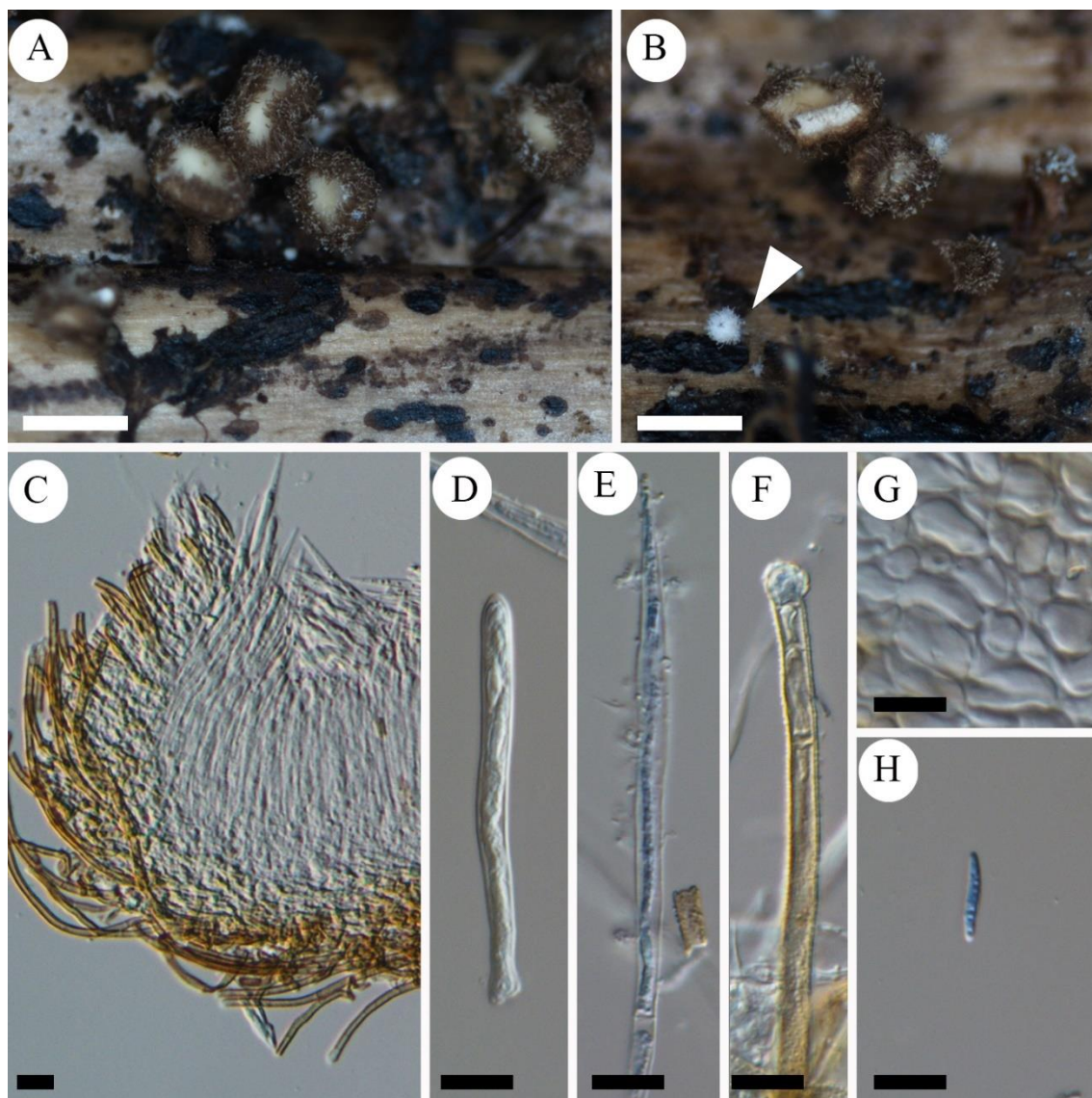


Figure 11. *Brunnipila pseudocannabina* TNS-F-81252. **A** Dried apothecia **B** *Brunnipila pseudocannabina* with *Lachnum nudipes* dried (arrowhead) on the same substrate **C** Vertical section of an apothecium showing the ectal and medullary excipulum **D** Ascus **E** Paraphysis **F** Hair with a crystal cap **G** Ectal excipular cells **H** Ascospore **A–F, H.** TNS-F-81252. **G,** TNS-F-16690. Mounted in CB dissolved with tap water (**C, G**), CB/LA (**D–F, H**). Scale bars: 1 mm (**A, B**), 20 μ m (**C**), 10 μ m (**D–H**).

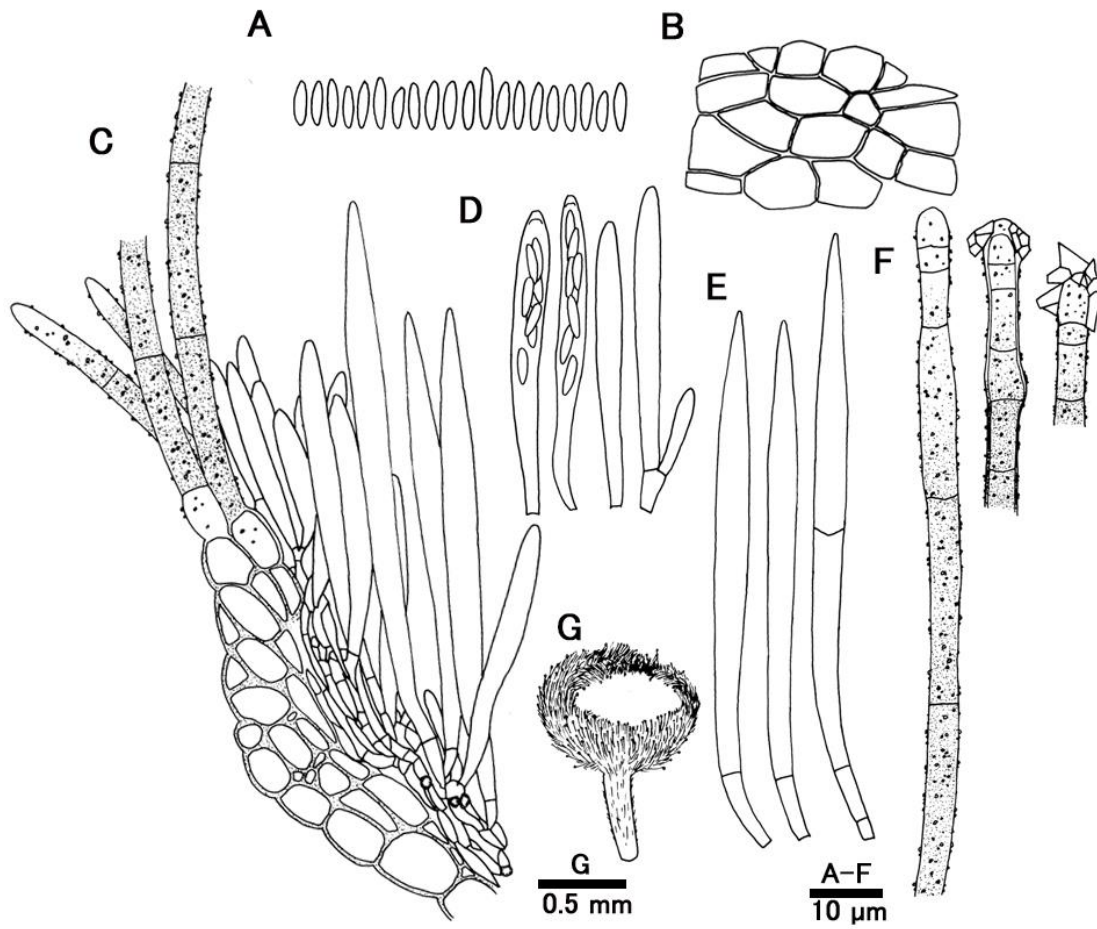


Figure 12. *Brunnipila pseudocannabina* TNS-F-16624, 16771. **A** Ascospores **B** Asci **C** Hairs with and detached (left) crystals **D** Vertical section through the margin, showing the hairs and ectal excipulum **E** Paraphyses **F** Ectal excipular cells **G** Apothecium.

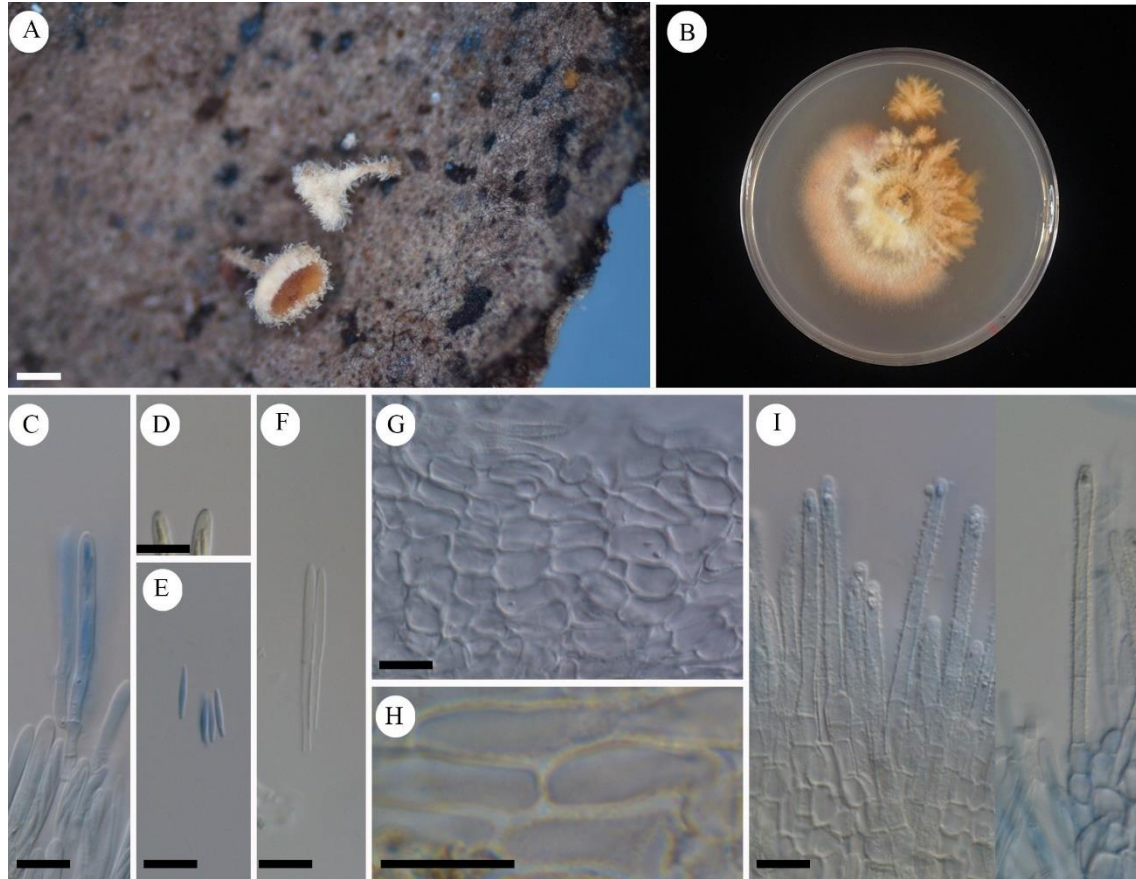


Figure 13. *Erioscyphella boninensis* TNS-F-26520 (Holotype). **A** Dried apothecia **B** Pure culture on PDA (NBRC 114447) **C** Ascus **D** Ascal pore MLZ (+) **E** Ascospores **F** Paraphyses **G** Ectal excipular cells **H** Ectal excipular cells with red granules **I** Hairs with resinous matters arising from ectal excipular cells. Mounted in CB/LA (**C**, **E–I**), MLZ (**D**). Scale bars: 1 mm (**A**), 10 μ m (**C–I**).

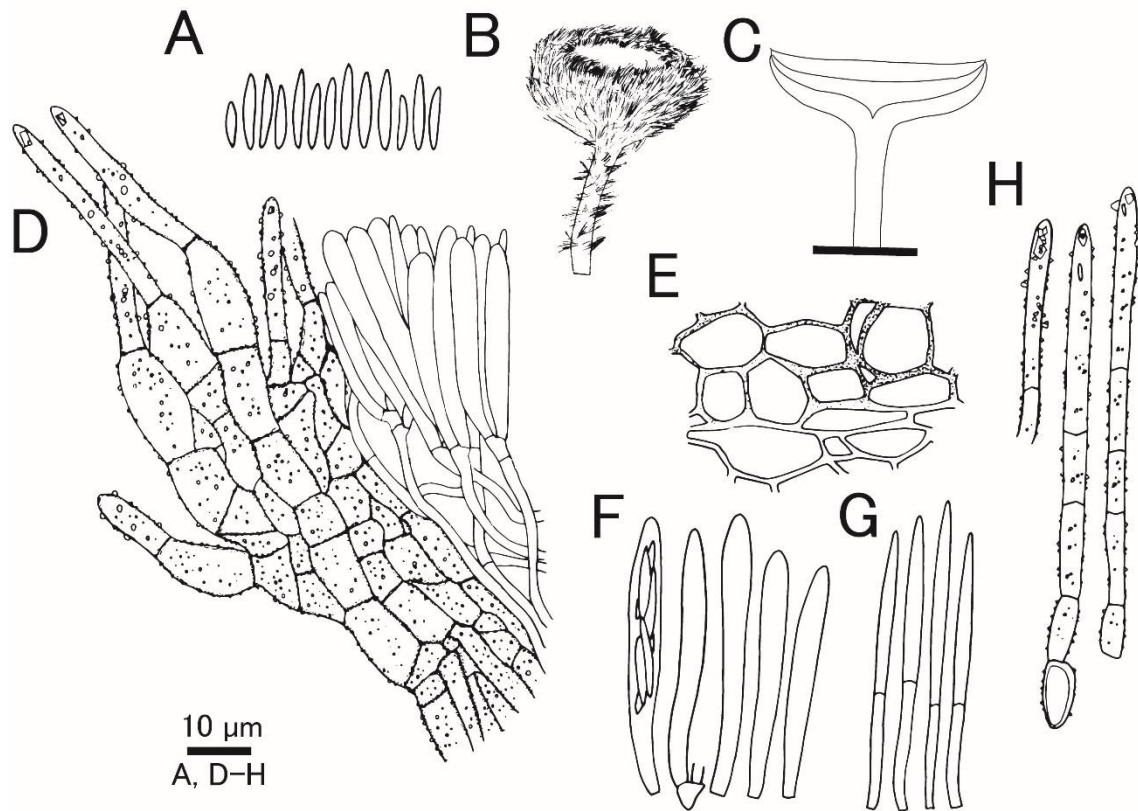


Figure 14. *Erioscyphella boninensis* TNS-F-26520 (Holotype). **A** Ascospores **B** Apothecium **C** Vertical section of an apothecium **D** Expansion of a vertical section of an apothecium **E** Ectal excipular cells **F** Asci **G** Paraphyses **H** Hairs.

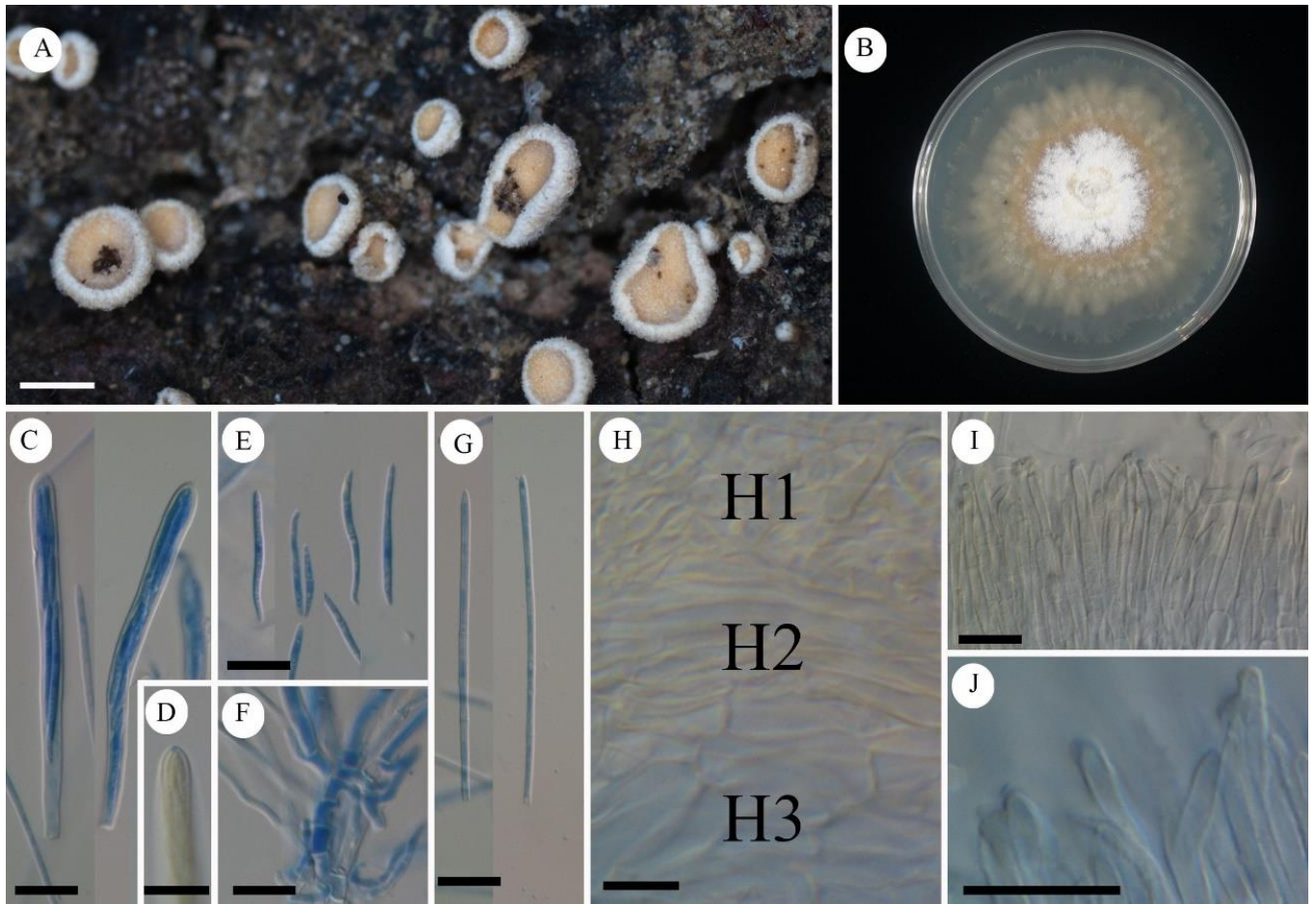


Figure 15. *Erioscyphella insulae* TNS-F-39720 (Holotype). **A** Dried apothecia **B** A pure culture on PDA (NBRC 114459) **C** Asci **D** Ascus pore MLZ (+) **E** Ascospores **F** Ascogenous hyphae **G** Paraphyses **H** Layer structures of excipulum. **H1** Medullary excipulum **H2** Inner layer of ectal excipulum composed of hyphae **H3** Outer layer of ectal excipulum composed of *textura angularis*. **I, J** Hairs with apical amorphous materials. Mounted in CB/LA (**C, E–J**), MLZ (**D**). Scale bars: 1 mm (**A**), 10 μ m (**A–J**).

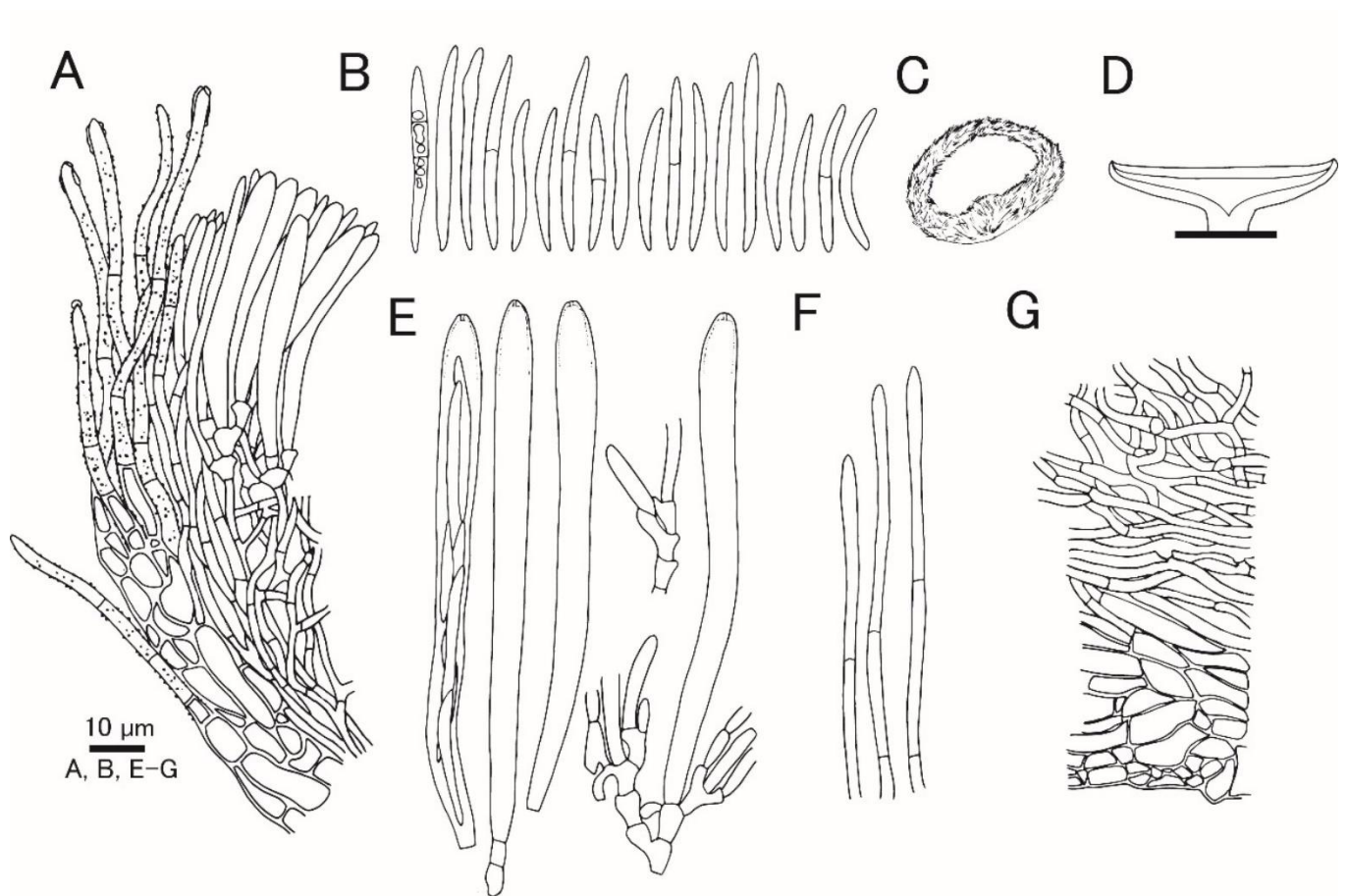


Figure 16. *Erioscyphella insulae* TNS-F-39720 (Holotype). **A** Expansion of a vertical section of an apothecium **B** Ascospores **C** Apothecium **D** Vertical section of an apothecium **E** Asci **F** Paraphyses **G** Layer structures of excipulum.

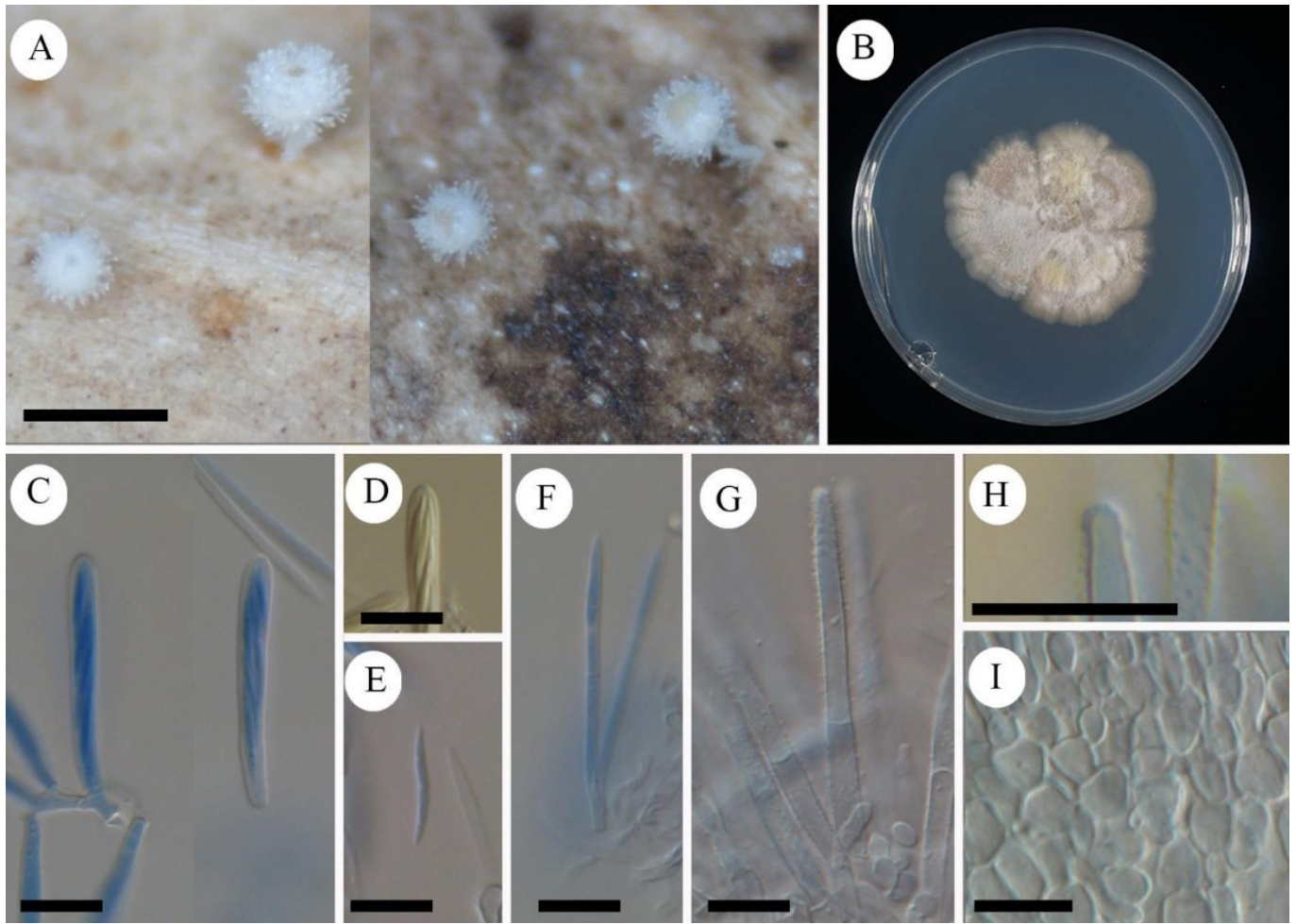


Figure 17. *Erioscyphella otanii* TNS-F-81472 (Holotype). **A** Dried apothecia **B** Pure culture on PDA (NBRC 114476) **C** Asci **D** Ascal pore MLZ (+) **E** Ascospore **F** Paraphyses **G** A hair **H** Hair-apex with an apical amorphous material **I** Ectal excipular cells. Mounted in CB/LA (**C**, **E–I**), MLZ (**D**). Scale bars: 0.5 mm (**A**), 10 μ m (**C–I**).

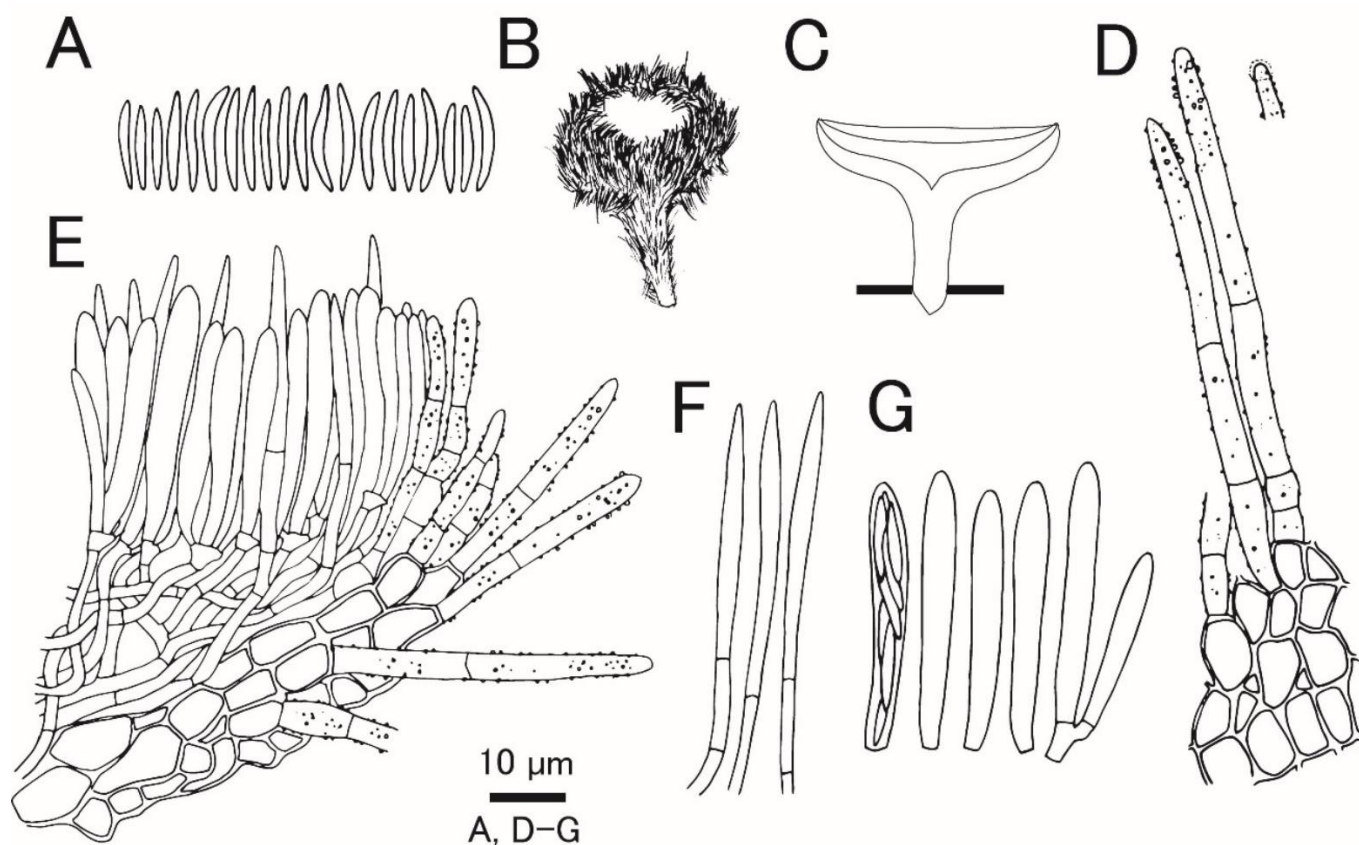


Figure 18. *Erioscyphella otanii* TNS-F-81472 (Holotype). **A** Ascospores **B** Apothecium **C** Vertical section of an apothecium **D** Hairs with cap-like structures arising from ectal excipular cells **E** Expansion of a vertical section of an apothecium **F** Paraphyses **G** Asci.

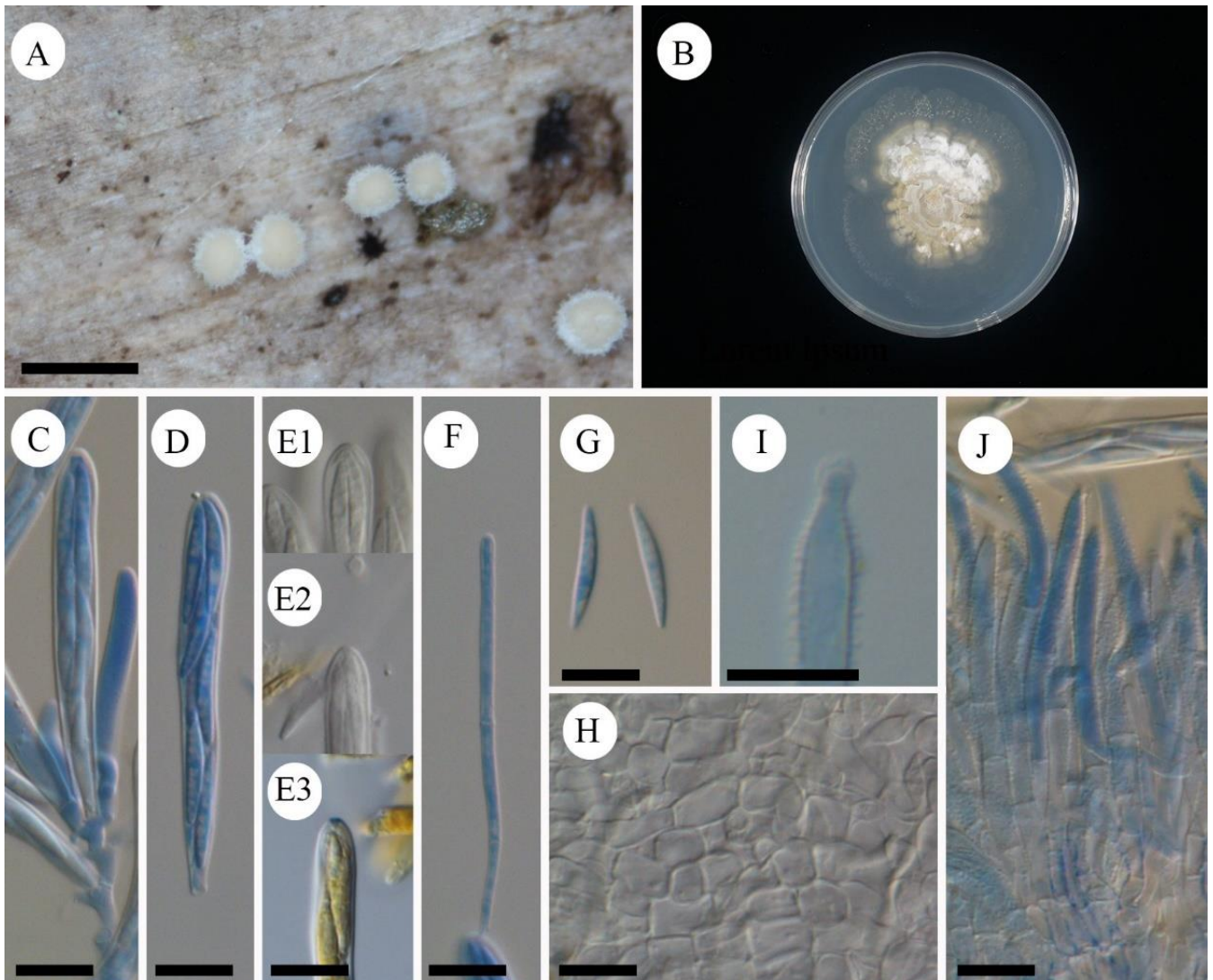


Figure 19. *Erioscyphella papillaris* TNS-F-81272 (Holotype). **A** Dried apothecia **B** Pure culture on PDA (NBRC 113937) **C** Ascus arising from ascogenous hyphae **D** An ascus **E** Ascus pore iodine reactions **E1** MLZ (–) without 3% KOH pretreatment **E2** MLZ (–) with 3% KOH pretreatment **E3** IKI (+) without 3% KOH pretreatment **F** Paraphysis **G** Ascospores with guttules **H** Ectal excipulum **I** Hair-apex with a apical amorphous material **J** Hairs. Mounted in CB/LA (**C**, **D**, **F–J**), MLZ (**E1**, **E2**), IKI (**E3**). Scale bars: 0.5 mm (**A**), 10 μ m (**C–J**).

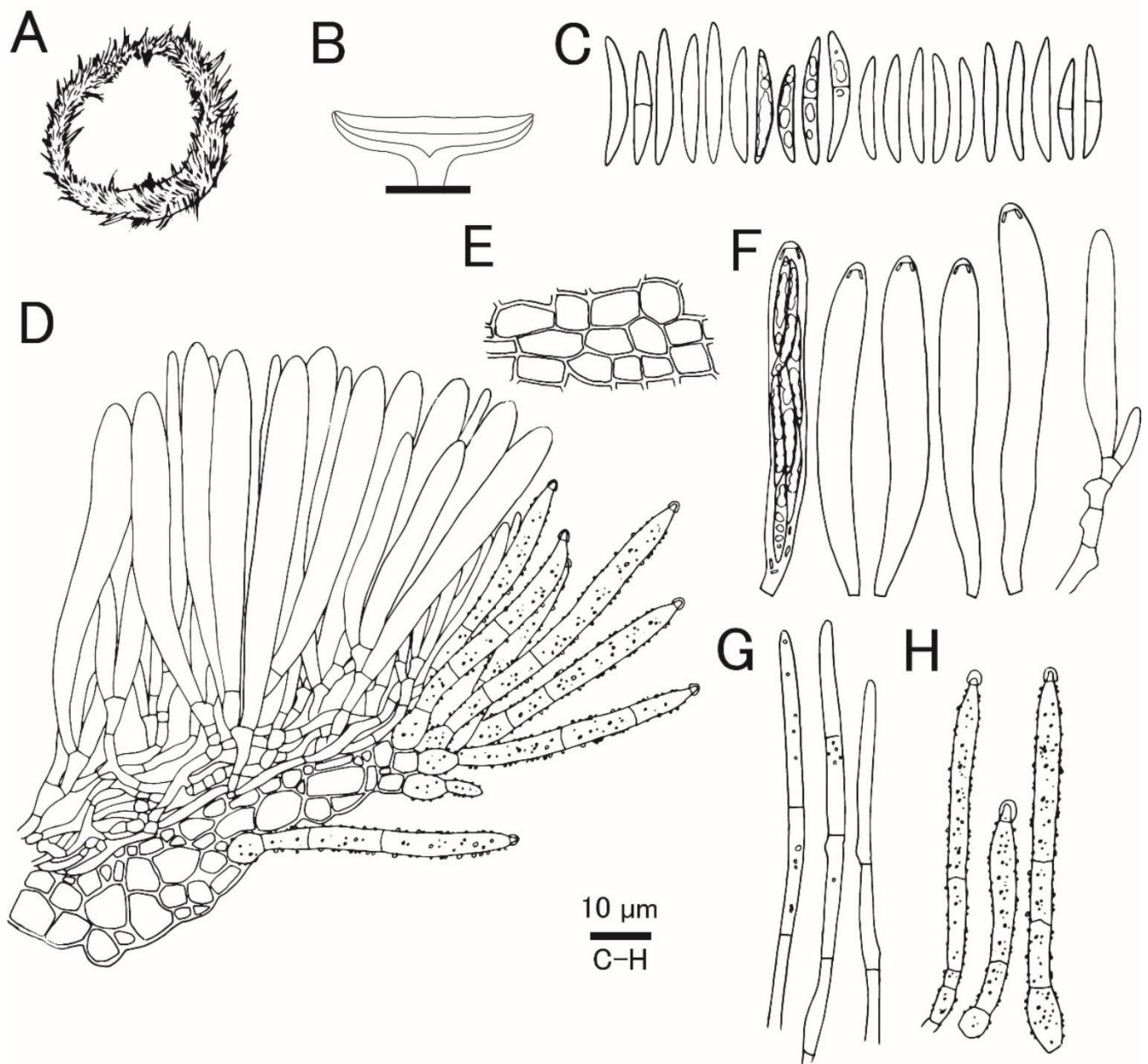


Figure 20. *Erioscyphella papillaris* TNS-F-81272 (Holotype). **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores **D** Expansion of a vertical section of an apothecium **E** Ectal excipular cells **F** Asci **G** Paraphyses **H** Hairs with cap-like structures.

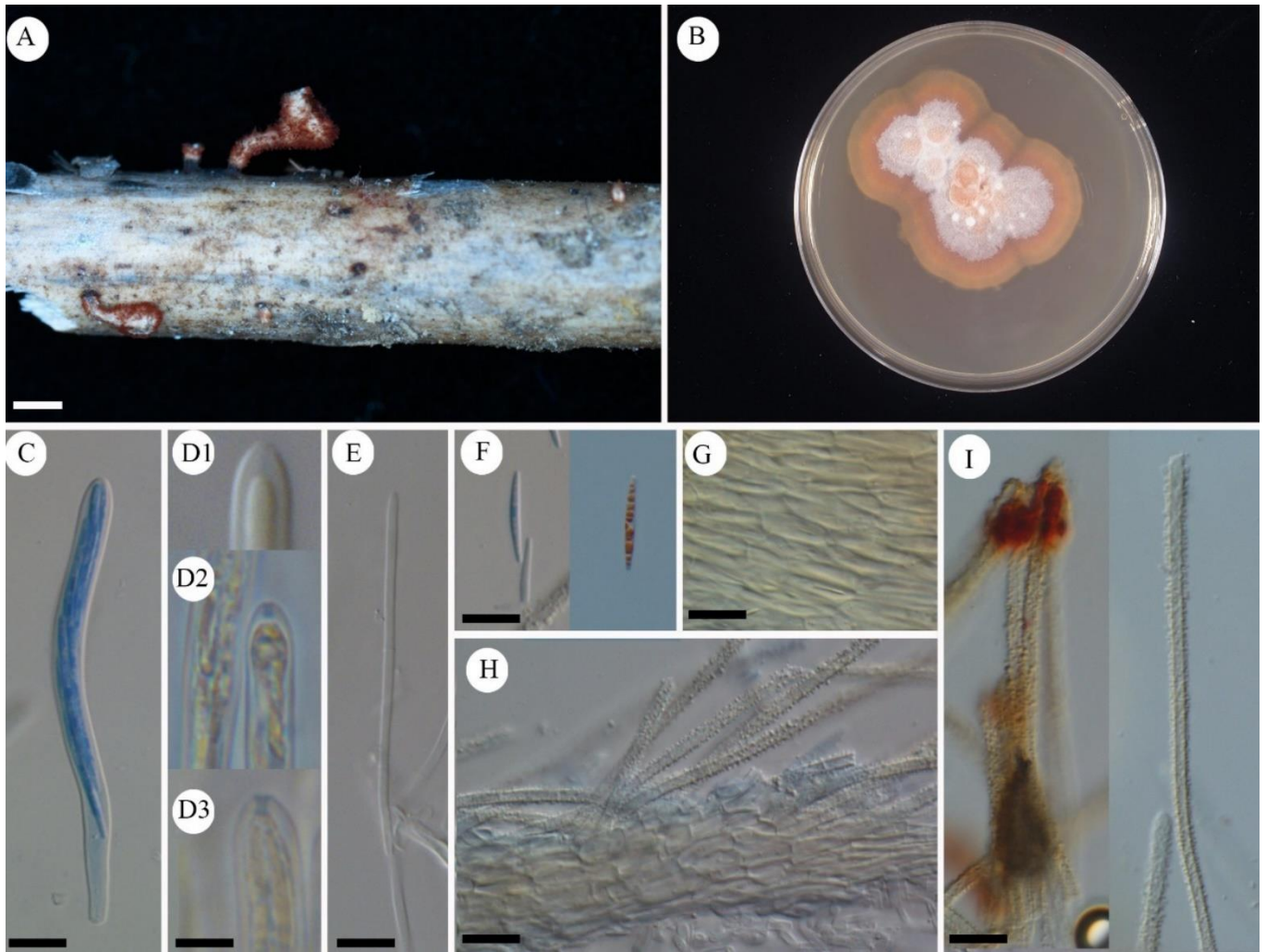


Figure 21. *Erioscyphella paralushanensis* TNS-F-61920 (Holotype). **A** Apothecia **B** Pure culture on PDA (NBRC 114468) **C** Ascus **D** Ascospore iodine reactions **D1** MLZ (faintly +) without 3% KOH pretreatment **D2** MLZ (+) with 3% KOH pretreatment **D3** IKI (+) without 3% KOH pretreatment **E** Paraphysis **F** Ascospores **G** Ectal excipular cells **H** Marginal section of an apothecium generating hairs **I** Hairs with red resinous materials **J** Apical amorphous materials of hairs. Mounted in CB/LA (**C**, **E–J**), MLZ (**D1**, **D2**), IKI (**D3**). Scale bars: 0.5 mm (**A**), 10 μ m (**C–J**).

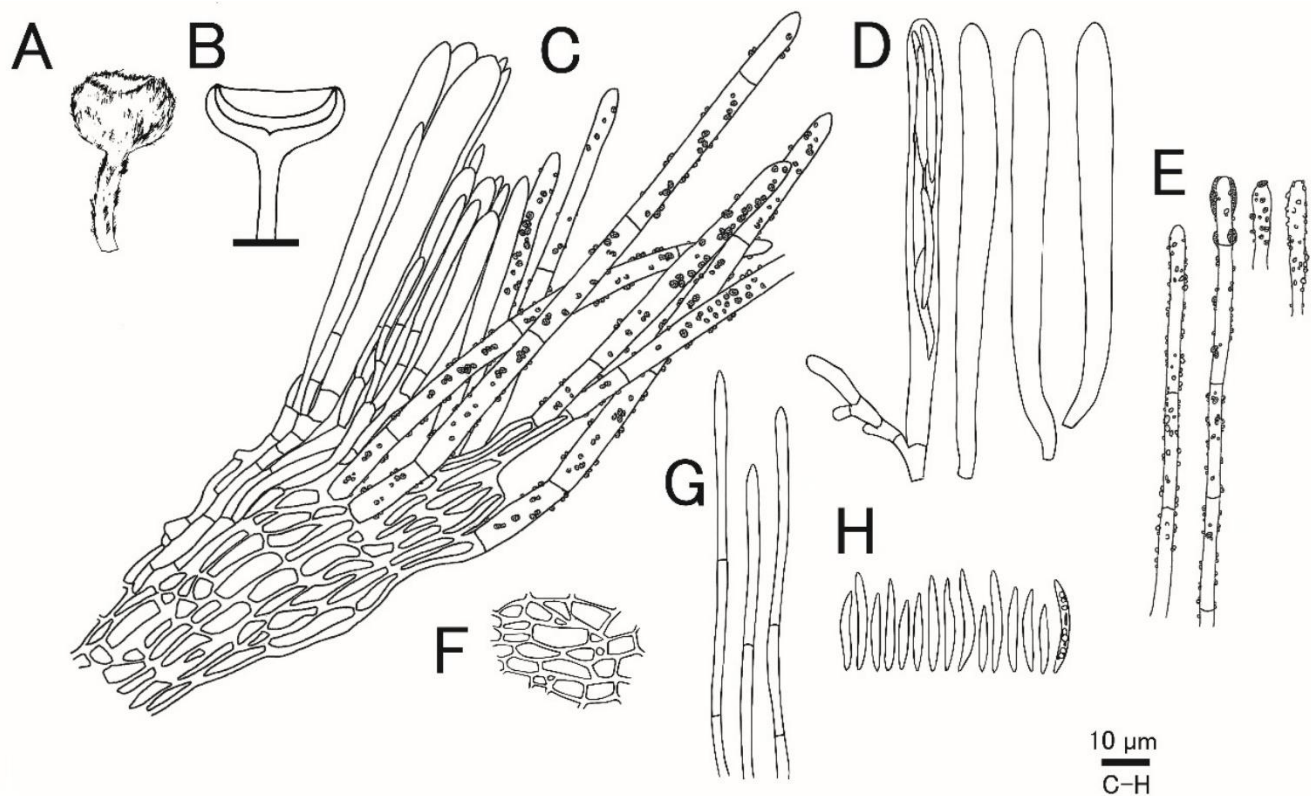


Figure 22. *Erioscyphella paralushanensis* TNS-F-61920 (Holotype). **A** Apothecia **B** Vertical section of an apothecium **C** Expansion of a vertical section of an apothecium **D** Asci **E** Hairs **F** Ectal excipulum **G** Paraphyses **H** Ascospores.

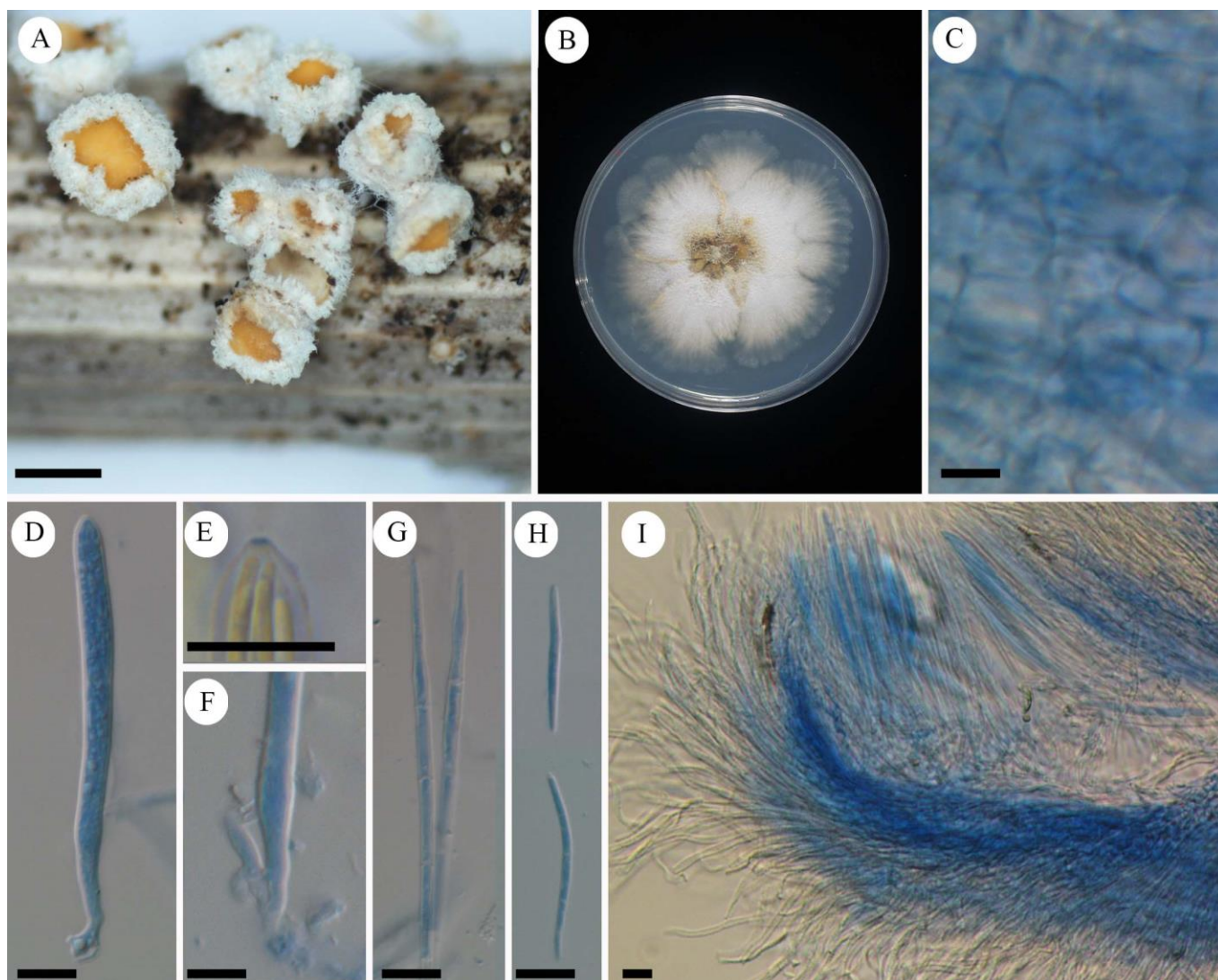


Figure 23. *Erioscyphella sasibrevispora* TNS-F-81401 (Holotype). **A** Dried apothecia **B** A pure culture on PDA (NBRC 114475) **C** Ectal excipular cells **D** Ascus **E** An ascus pore MLZ (+) **F** Ascus base with a perforated crozier **G** Septated paraphyses **H** Ascospores **I** Vertical section through the apothecium. Mounted in CB/LA (**D**, **F–I**), MLZ (**E**). Scale bars: 1 mm (**A**), 10 μ m (**C–I**).

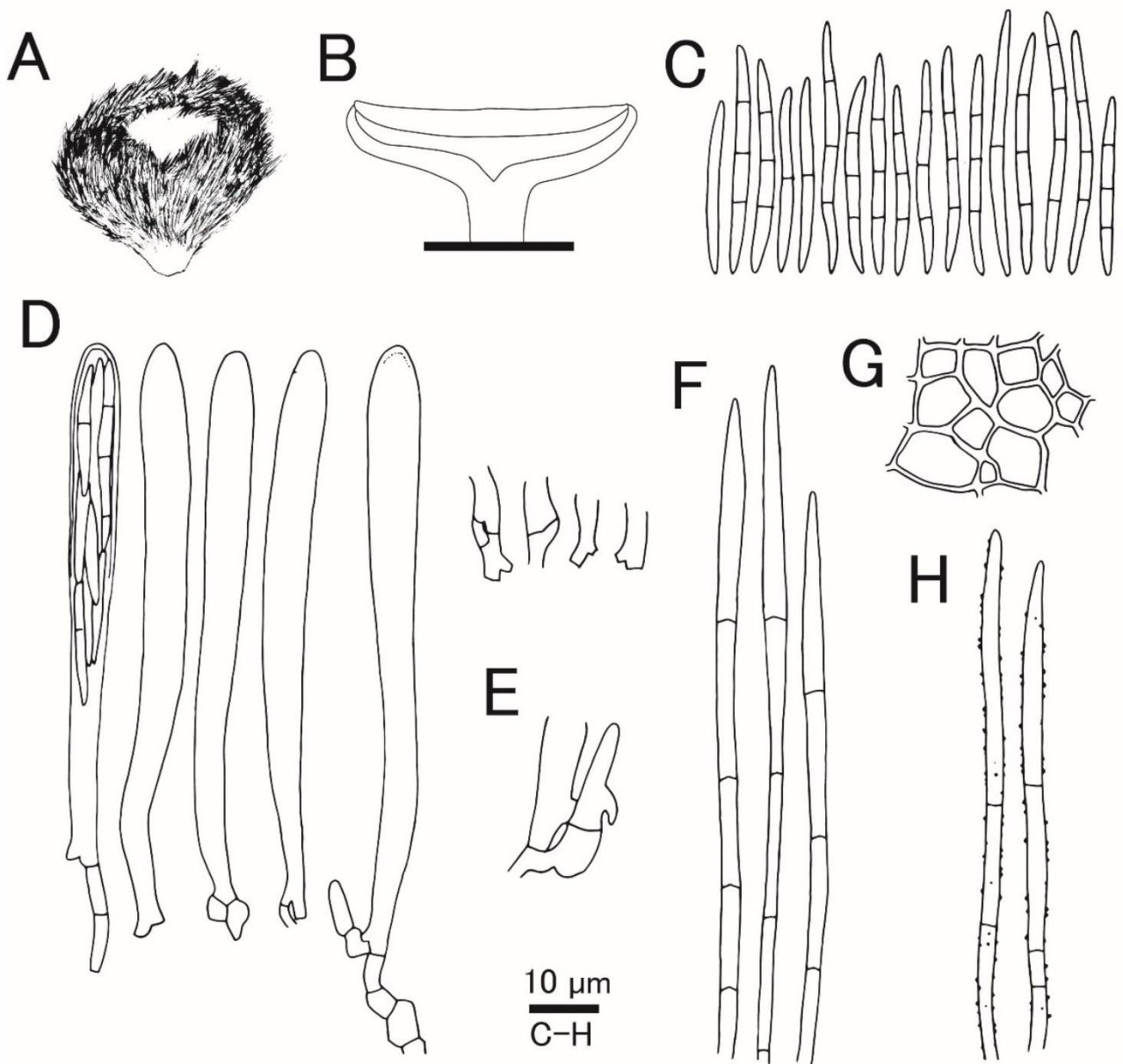


Figure 24. *Erioscyphella sasibrevispora* TNS-F-81401 (Holotype). **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores **D** Asci (with basal structures sometimes with perforation) **E** Ascus base arising from a crozier with perforation **F** Paraphyses **G** Ectal excipular cells **H** Hairs.

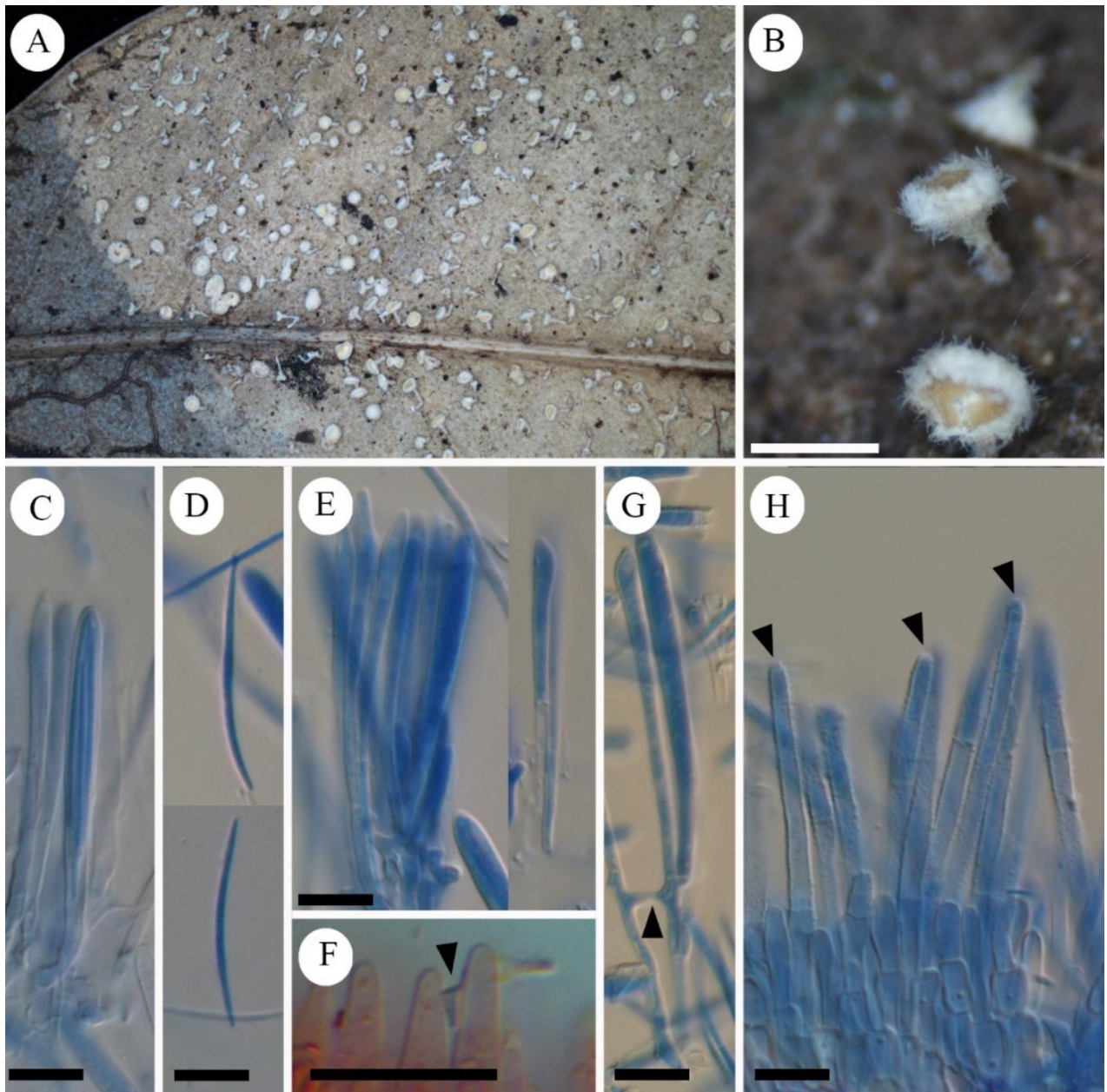


Figure 25. *Erioscyphella sinensis* TNS-F-81383 **A** Apothecia scattered on a substrate **B** Apothecia **C** Asci **D** Ascospores **E** Paraphyses **F** Anastomosed parts near paraphysis apices (arrowhead) **G** Anastomosed parts near basal parts of paraphyses (arrowhead) **H** Ectal excipular cells and producing hairs with apical amorphous materials (arrowheads). Mounted in CB/LA (**C–H**). Scale bars: 1 mm (**B**), 10 µm (**C–H**).

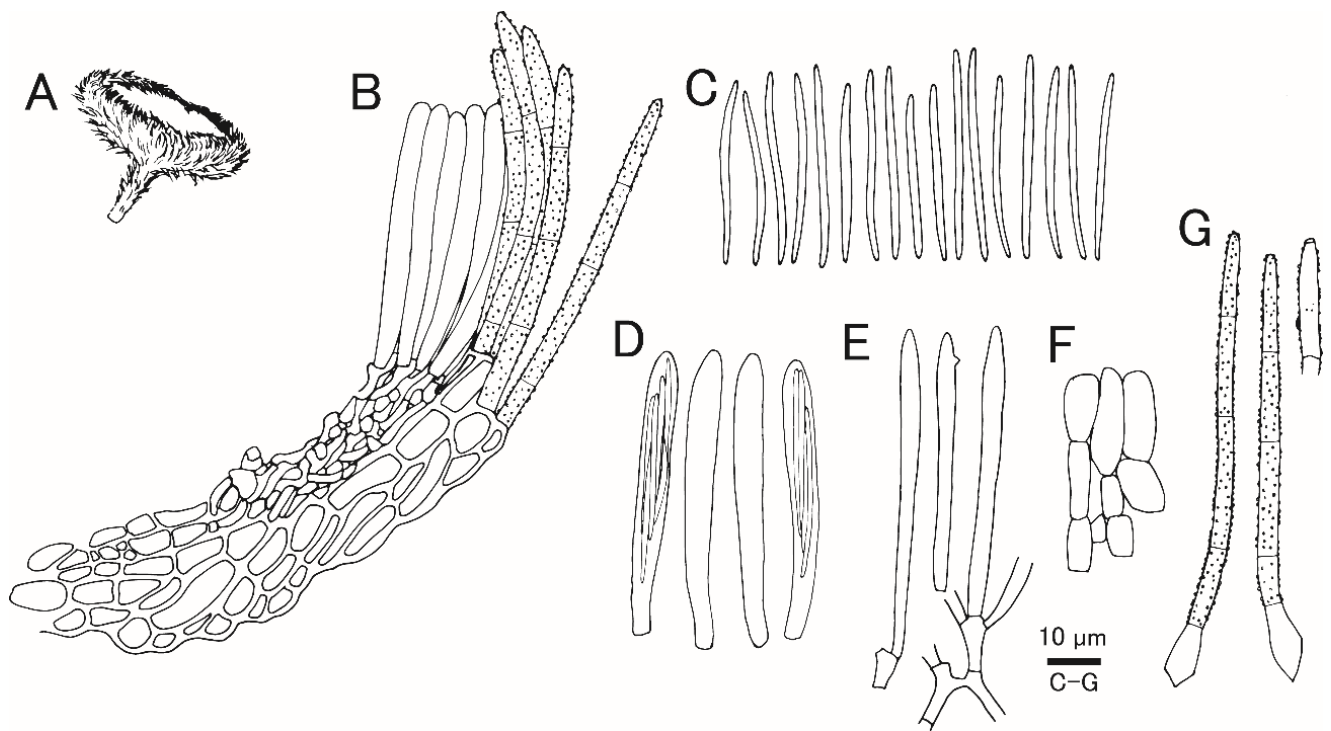


Figure 26. *Erioscyphella sinensis* TNS-F-81383; 16838 **A** Apothecia **B** Vertical section through the apothecium **C** Ascospores **D** Asci **E** Paraphyses **F** Ectal excipular cells **G** Hairs with apical amorphous materials. Illustrated by Rei Sasagawa (**A–D**).

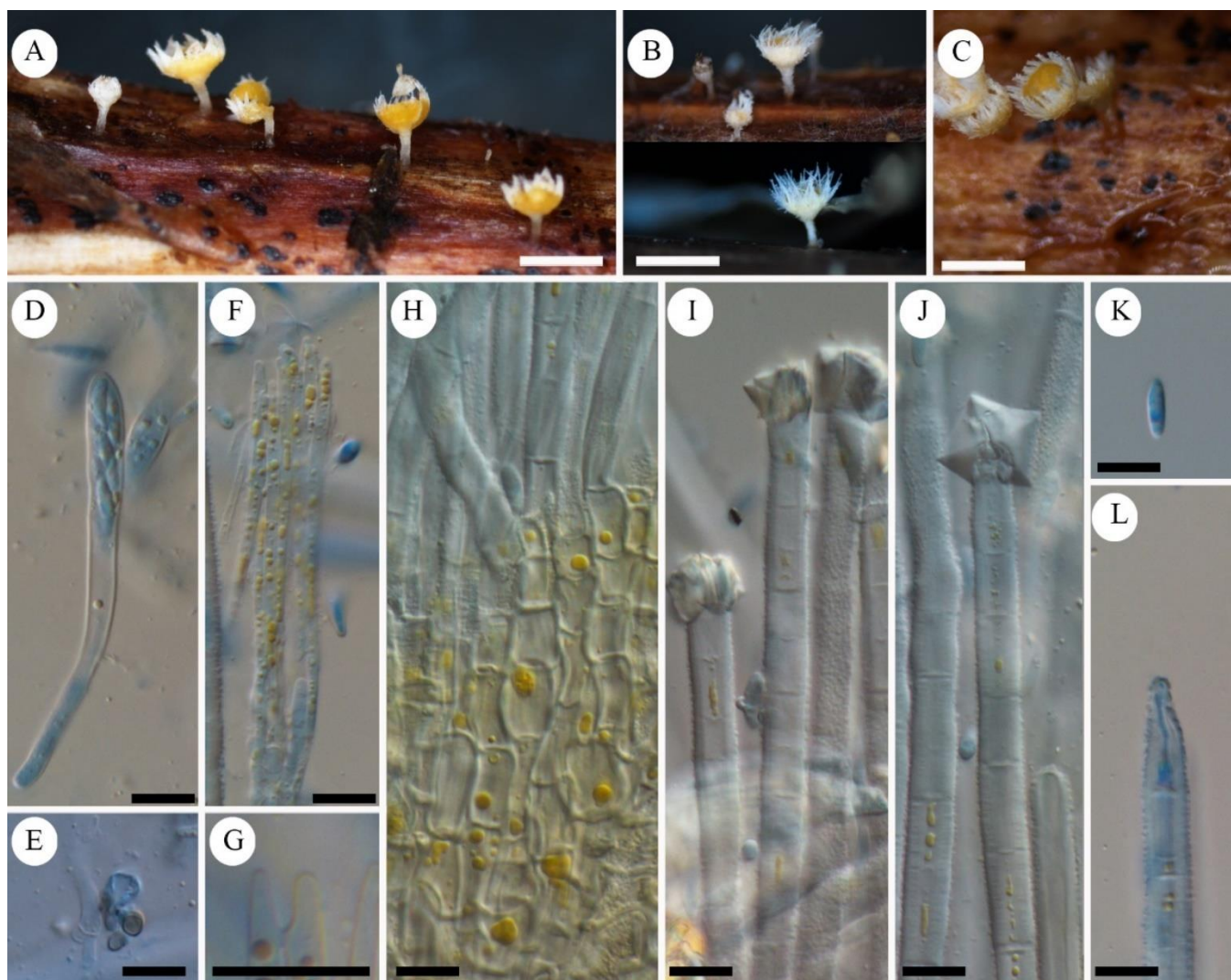


Figure 27. *Incrucipulum aurantiacum* TNS-F-81851 (Holotype). **A–C** Fresh apothecia **D** Ascus **E** Crozier at the base of ascus **F** Paraphyses containing orange oil drops **G** Paraphysis connection **H** Ectal excipular cells containing orange oil drops **I, J** Hairs with crystals **K** Ascospore **K** Hair apex with acute apex. Mounted in CB/LA (**D–L**). Scale bars: 1 mm (**A–C**), 10 μm (**D–L**).

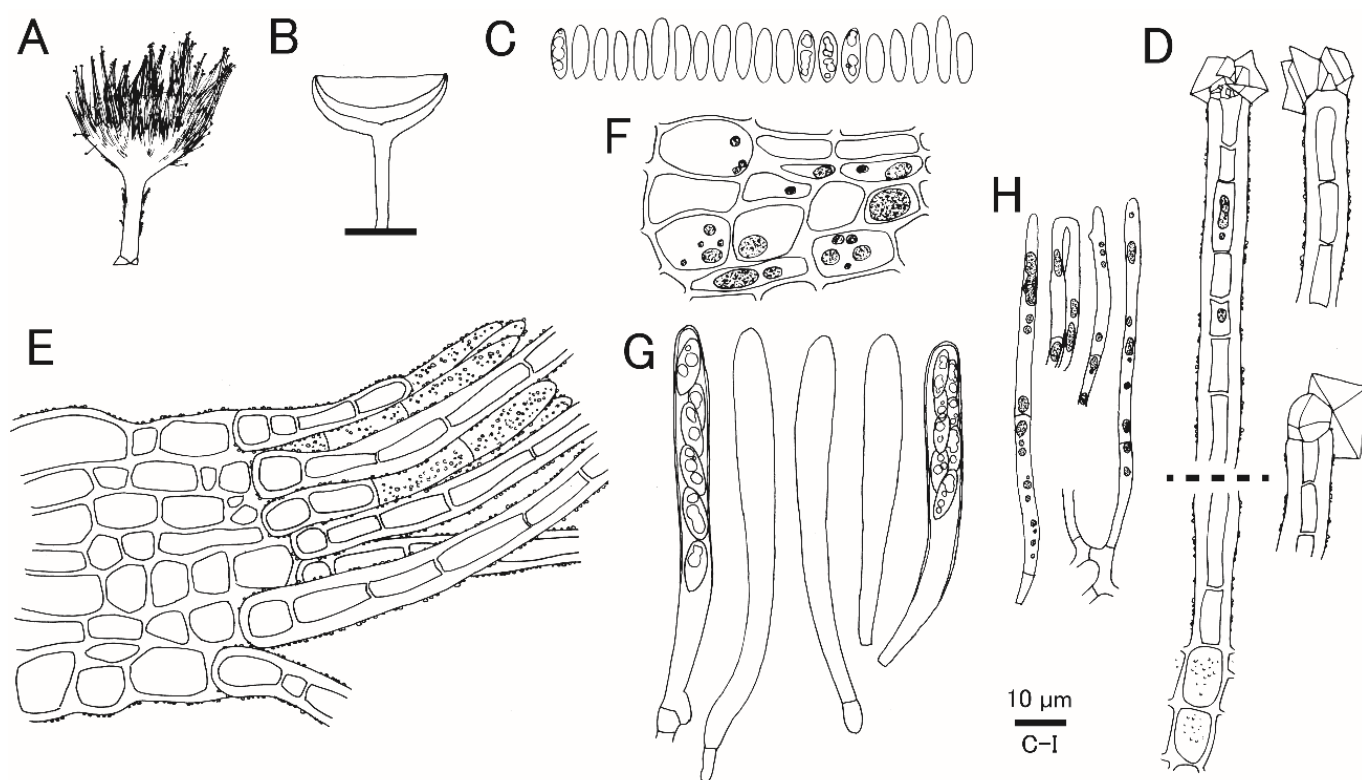


Figure 28. *Incrucipulum aurantiacum* TNS-F-81851 (Holotype). **A** Apothecium **B** Marginal section of an apothecium **C** Ascospores **D** Hairs with crystals **E** Vertical section through the apothecium producing thick-walled hairs **F** Ectal excipular cells containing orange oil drops **G** Asci **H** Anastomosed paraphyses.

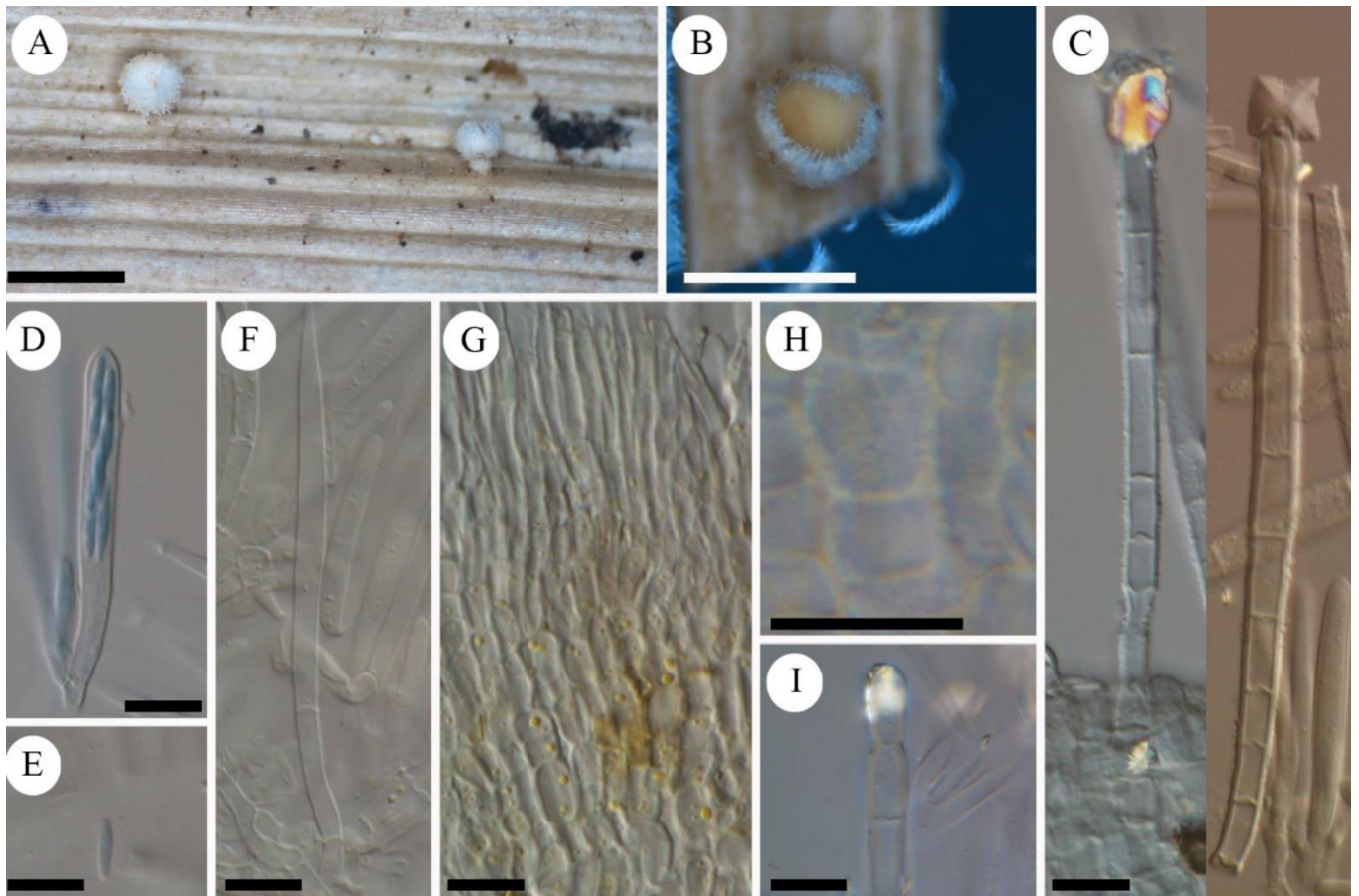


Figure 29. *Incrucipulum brevistipitatum* TNS-F-81832 (Holotype). **A** Fresh apothecia on substrates **B** Apothecium **C** Hairs with crystals **D** Ascus **E** Ascospore **F** Paraphysis **G** Ectal excipular cells containing orange oil drops **H** Granulated ectal excipular cells **I** Hair apex. Mounted in CB/LA (**D–I**). Scale bars: 1 mm (**A, B**), 10 μ m (**D–I**).

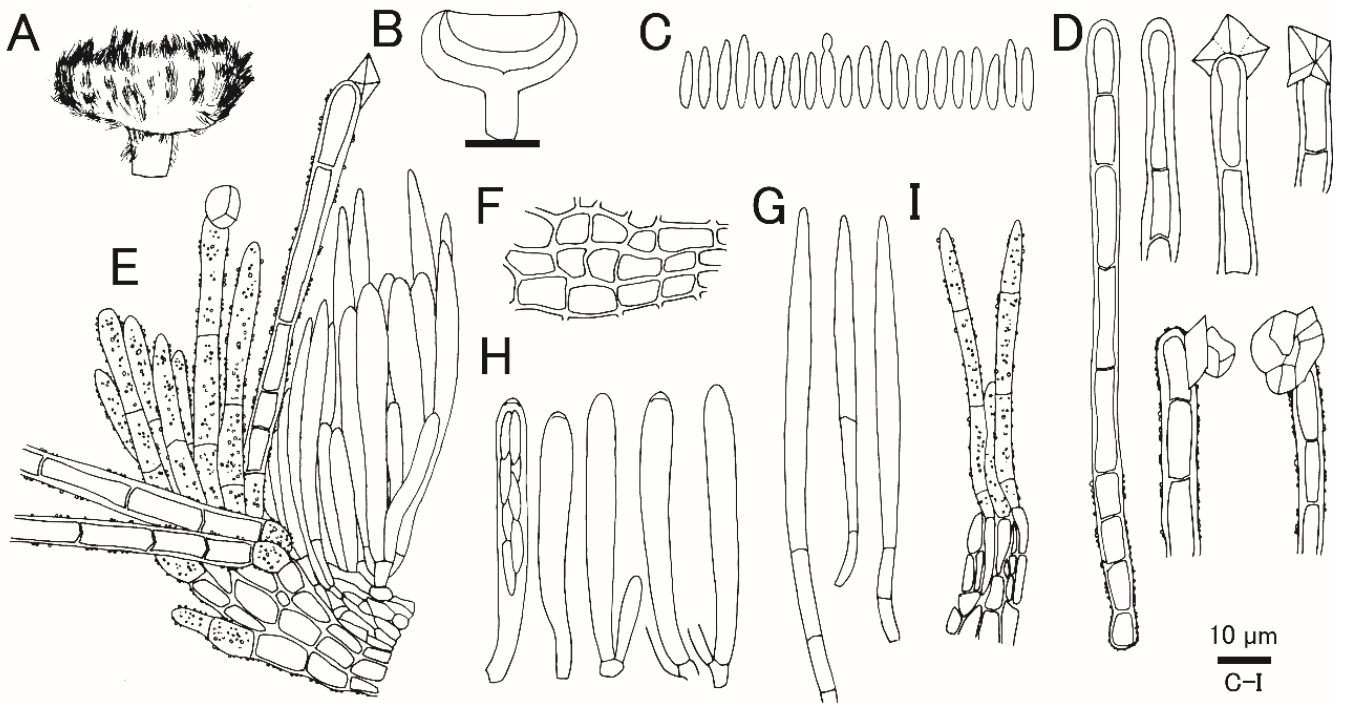


Figure 30. *Incrucipulum brevistipitatum* TNS-F-81832 (Holotype). **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores (with germination) **D** Thick-walled hairs with crystals **E** Vertical section through the apothecium producing thick-walled hairs **F** Ectal excipular cells **G** Paraphyses **H** Asci **I** Young hairs lacking crystals.

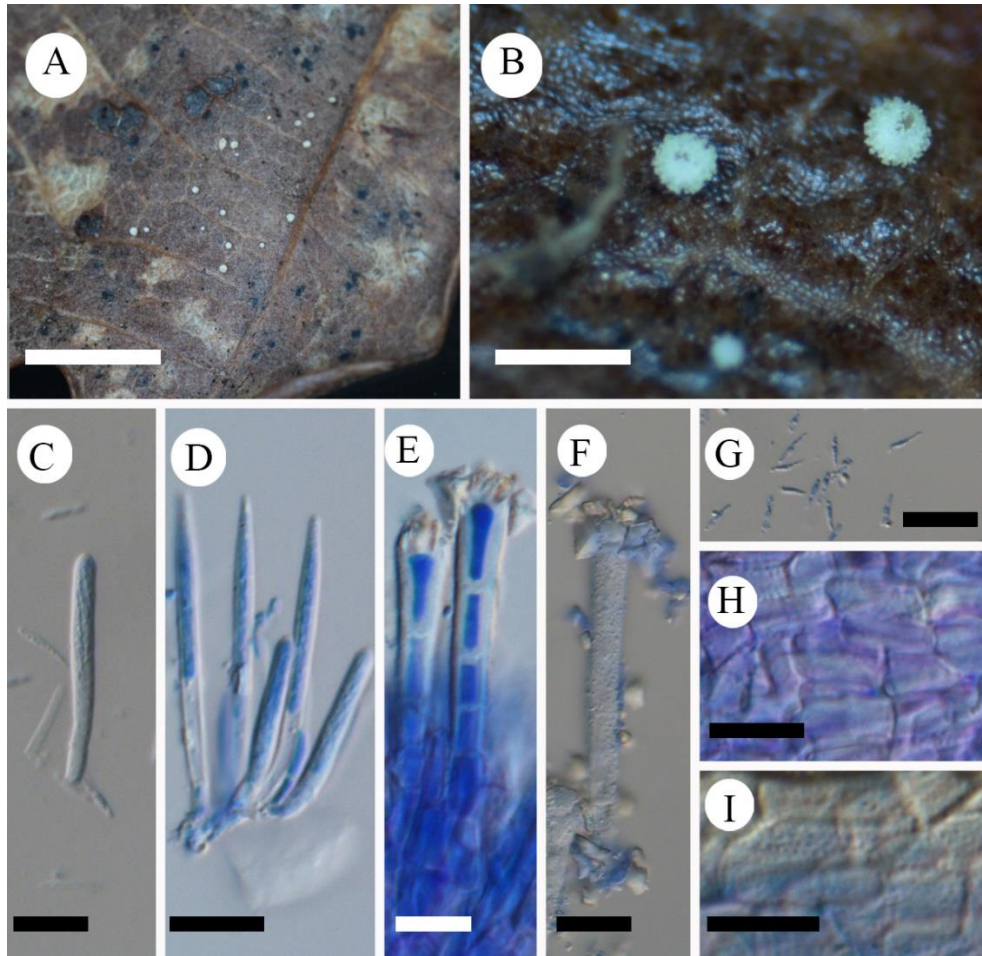


Figure 31. *Incrucipulum capitatum* TNS-F-81420. **A** Apothecia scattered on the substrates **B** Dried apothecia **C** Ascus **D** Paraphyses **E**, **F** Hairs with crystals **G** Ascospores **H** Ectal excipular cells **I** Surface of ectal excipular cells with granules. Mounted in CB/LA (C–I). Scale bars: 1 cm (A), 1 mm (B), 10 μ m (C–I).

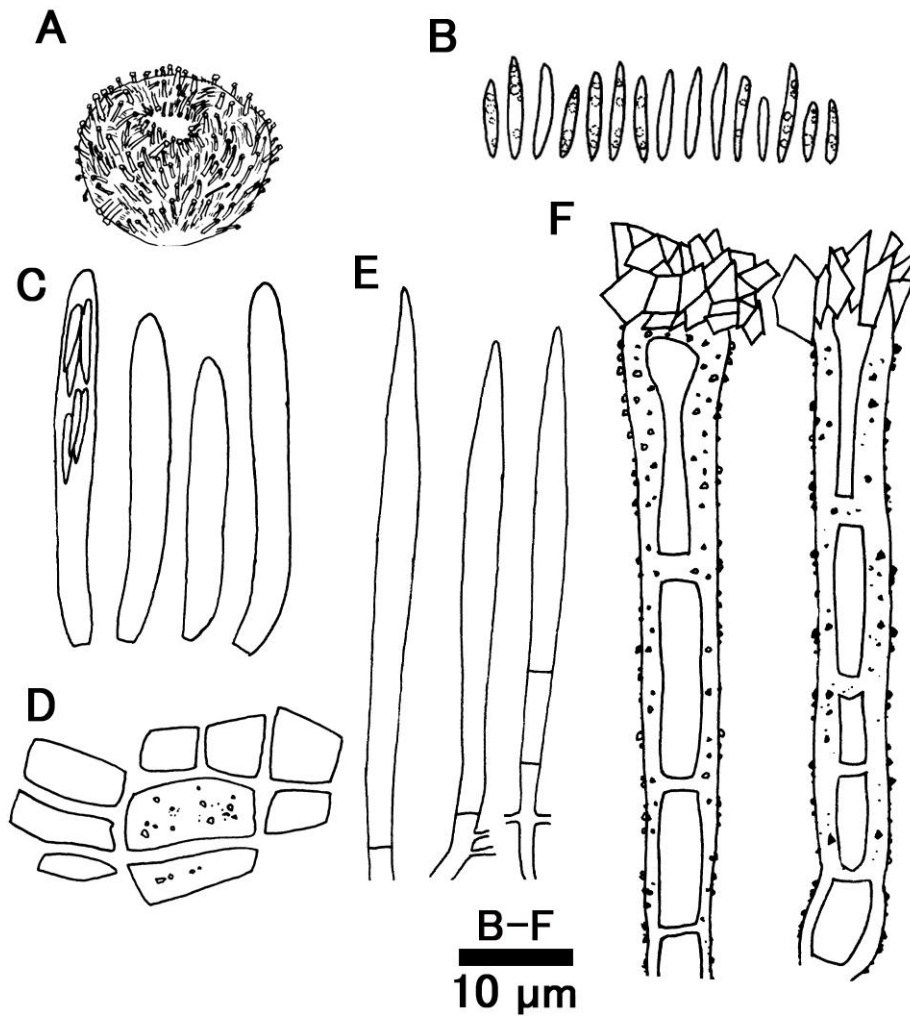


Figure 32. *Incrucipulum capitatum* TNS-F-81420. **A** Apothecium **B** Ascospores **C** Asci **D** Paraphyses **E** Thick-walled hairs with crystals **F** Ectal excipular cells.

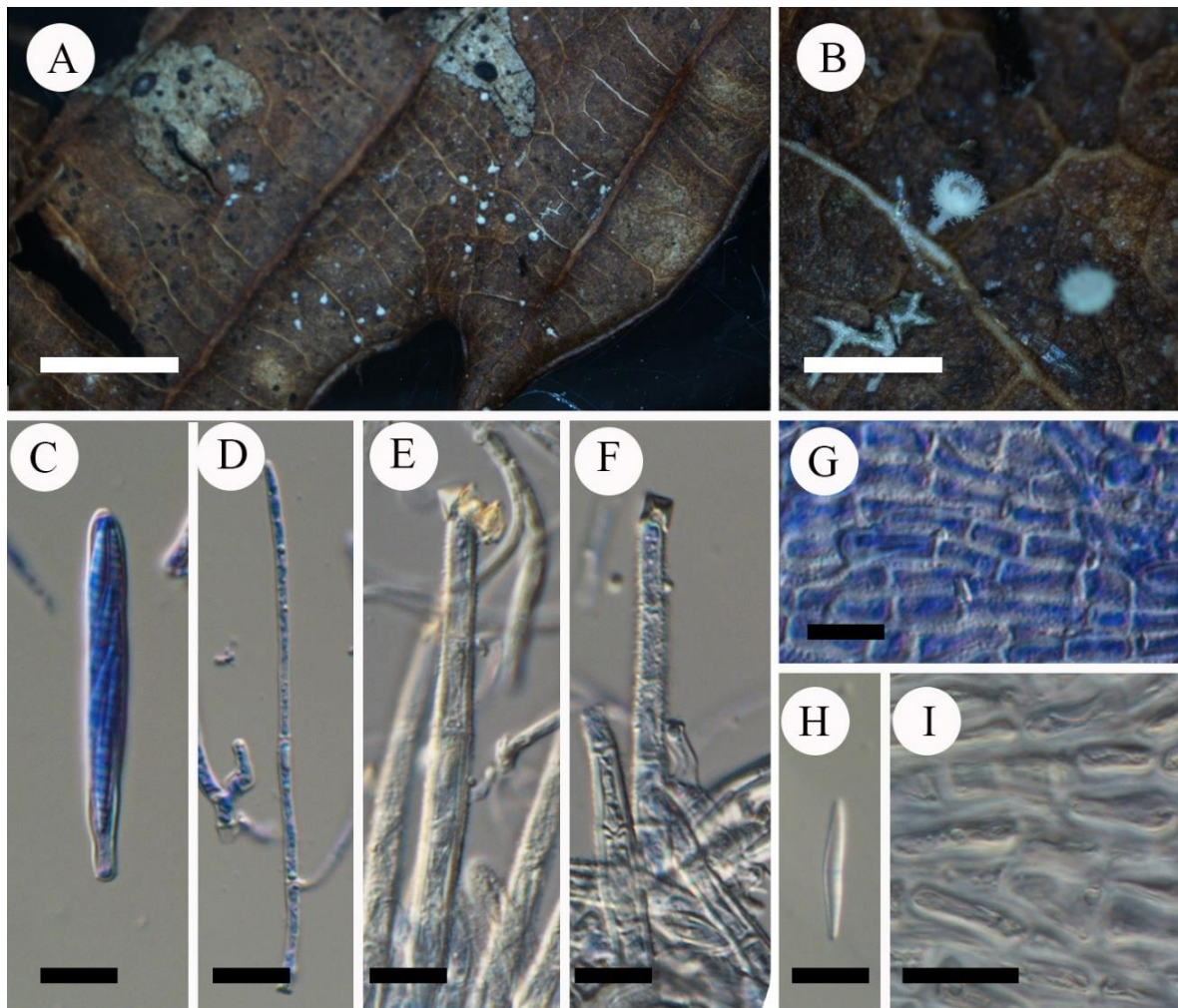


Figure 33. *Incrucipulum ciliare* TNS-F-81520. **A** Apothecia scattered on the substrate **B** Dried apothecia **C** Ascus **D** Paraphysis **E**, **F** Hairs with crystals **G** Ectal excipular cells with granulated surfaces **H** Ascospore **I** Thick-walled ectal excipular cells. Mounted in CB/LA (**C–I**). Scale bars: 1 cm (**A**), 1 mm (**B**), 10 μ m (**C–I**).

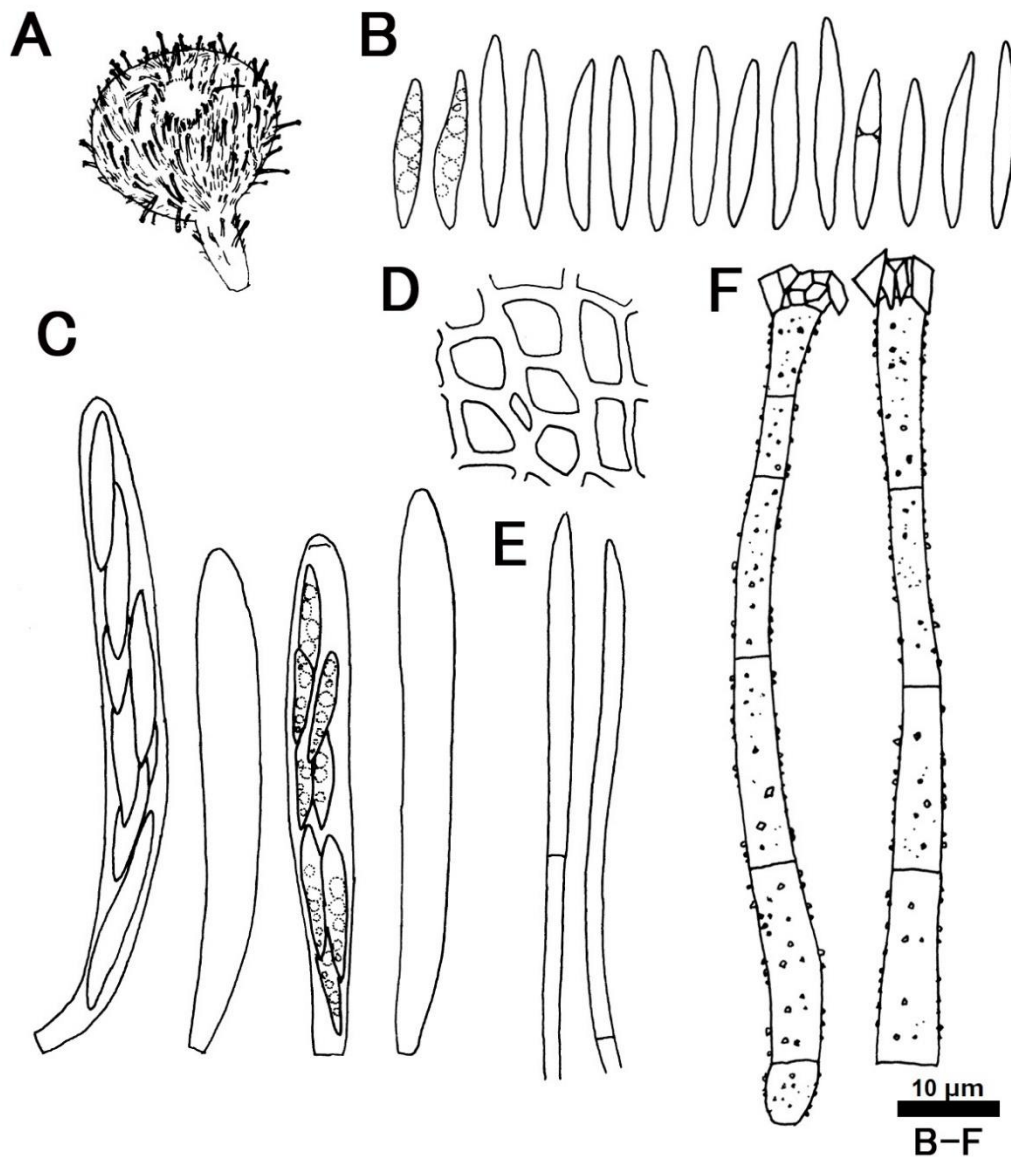


Figure 34. *Incrucipulum ciliare* TNS-F-81520. **A** Apothecium **B** Ascospores **C** Asci **D** Ectal excipular cells **E** Paraphyses **F** Hairs with crystals.

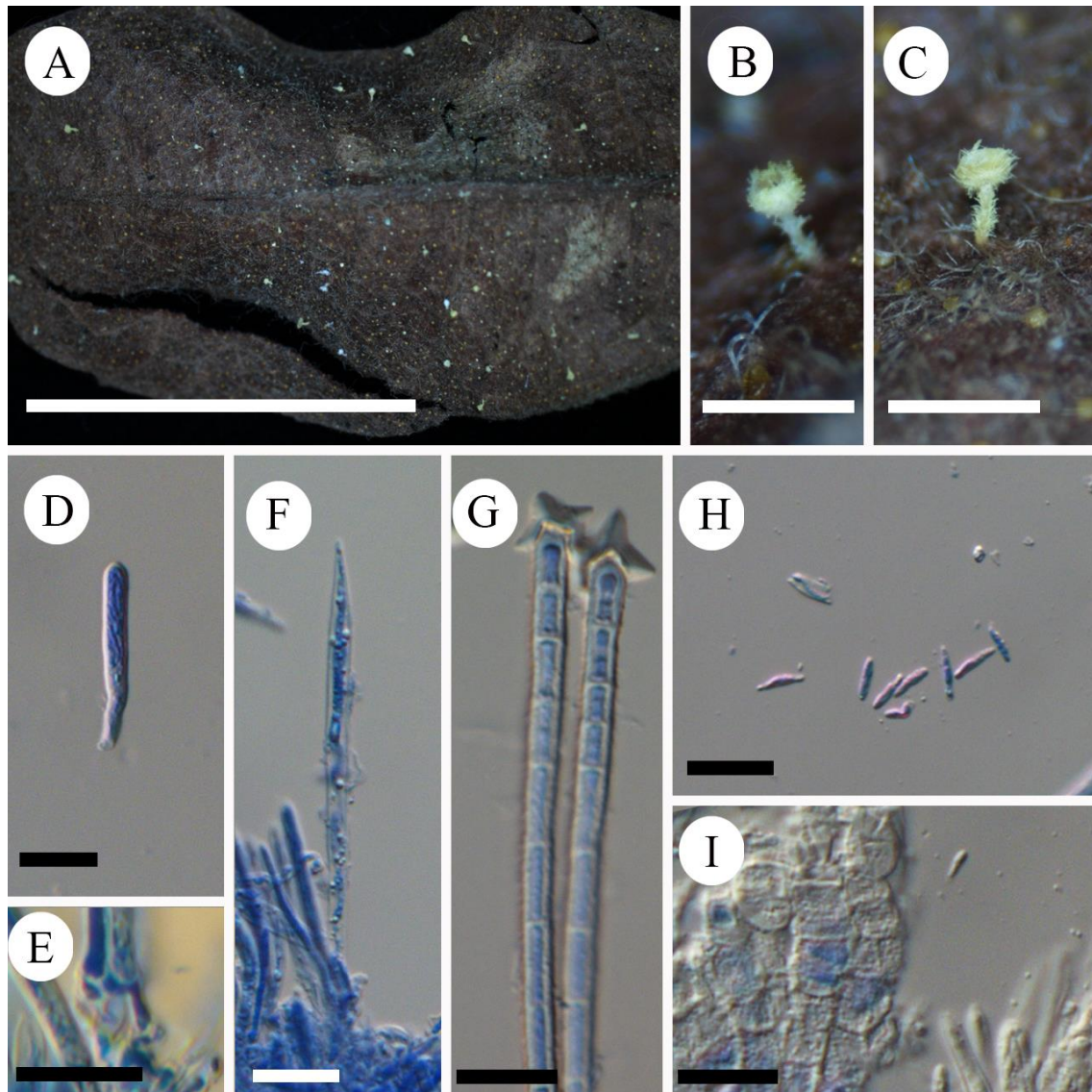


Figure 35. *Incrucipulum foliicola* TNS-F-81508 (A, C–I), TNS-F-81526 (Holotype; B). **A** Apothecia scattered on the substrate. **B** Fresh apothecium **C** Dried apothecium **D** Ascus **E** Ascus bases with croziers **F** Paraphysis **G** Hairs with cruciate crystals like ‘cross shuriken’ **H** Ascospores **I** Ectal excipular cells with granulate surface. Mounted in CB/LA (D–I). Scale bars: 1 cm (A), 0.5 mm (B, C), 10 μ m (D–I).

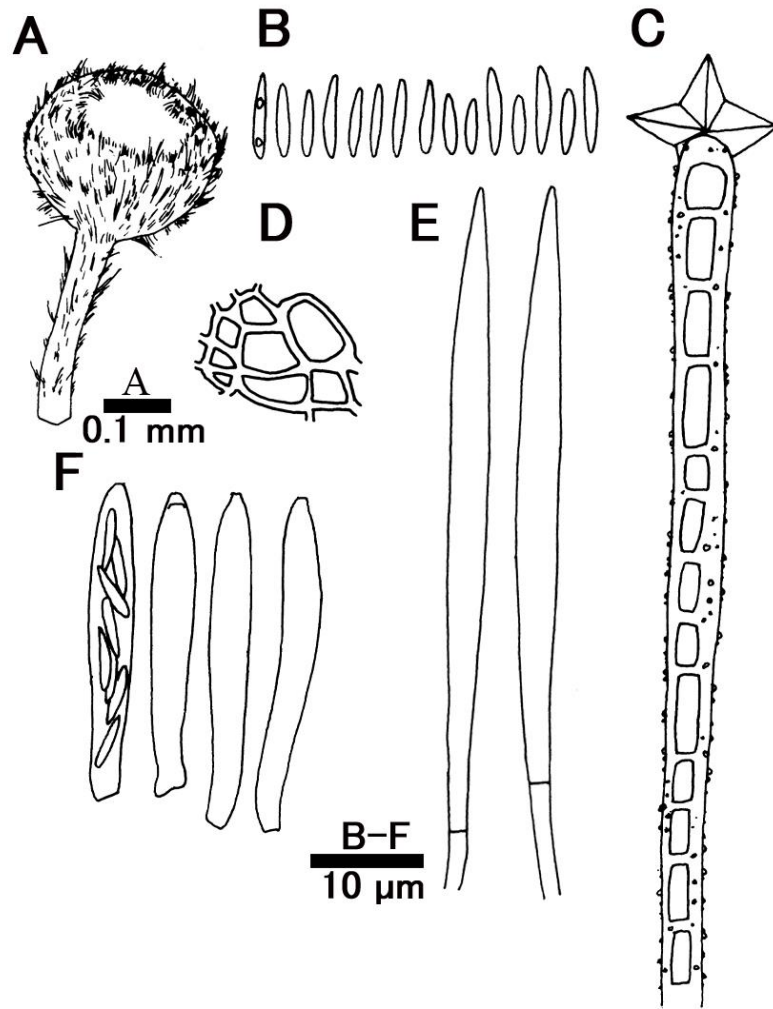


Figure 36. *Incrucipulum foliicola* TNS-F-81508. **A** Apothecium **B** Ascospores **C** Hair with a crystal **D** Ectal excipular cells **E** Paraphyses **F** Asci.

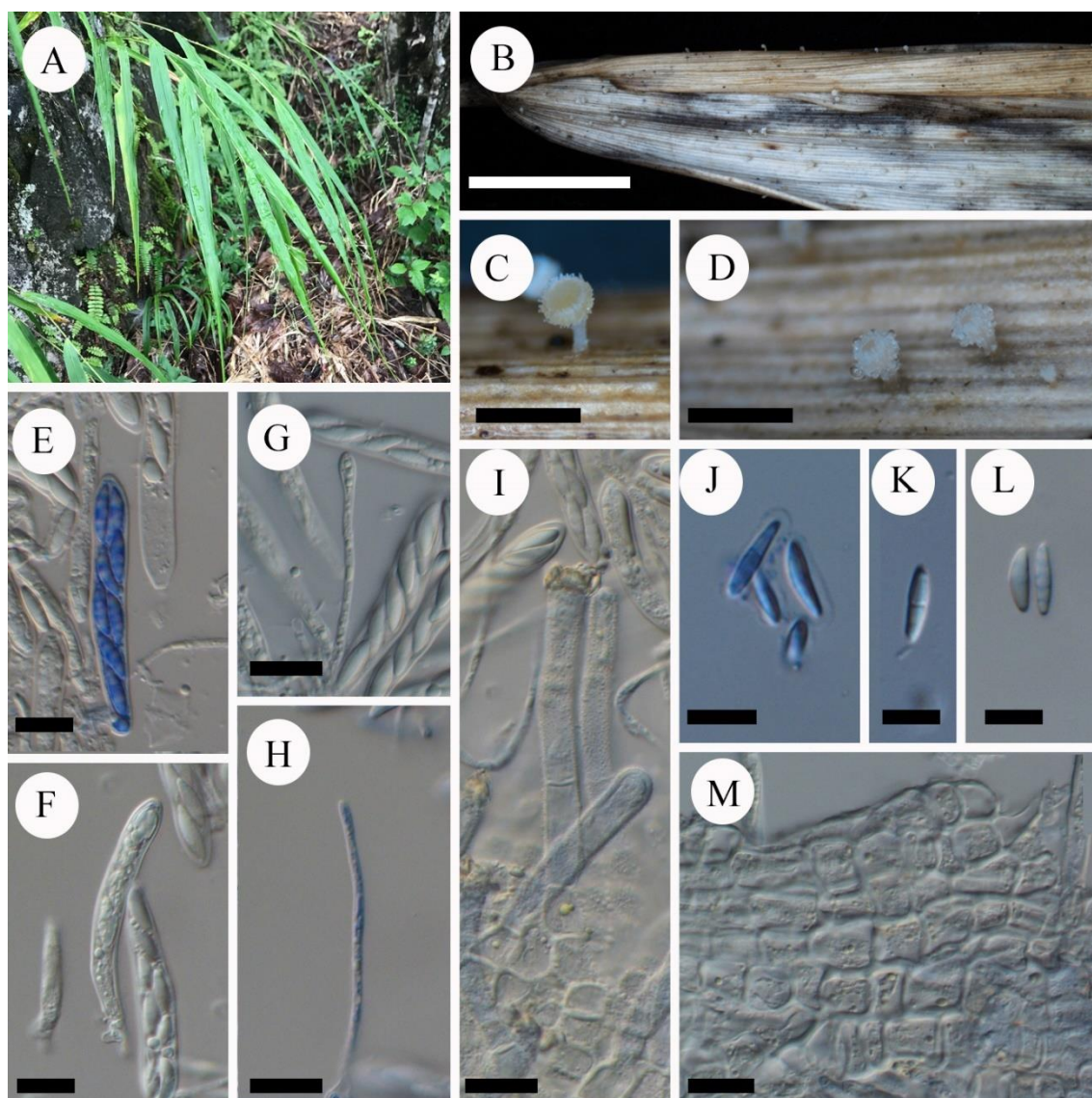


Figure 37. *Incrucipulum hakonechloae-macrae* TNS-F-81512 (Holotype). **A** The host *Hakonechloa macra* and its litter in the collection site of *I. hakonechloae-macrae* **B** Scattered apothecia of *I. hakonechloae-macrae* occurring on the host leaf **C**, **D** Fresh apothecia **E** Ascus **F** Ascus arising from a crozier **G**, **H** Paraphysis **I** Hair arising from ectal excipular cells with granulate surface **J** Ascospores packed by gelatinous sheath **K** Ascospore with a septum **L** Ascospores containing large lipid bodies **M** Thick-walled ectal excipular cells containing orange oil drops. Mounted in CB/LA (**E–M**). Scale bars: 1 cm (**B**), 0.5 mm (**C**, **D**), 10 μ m (**E–M**).

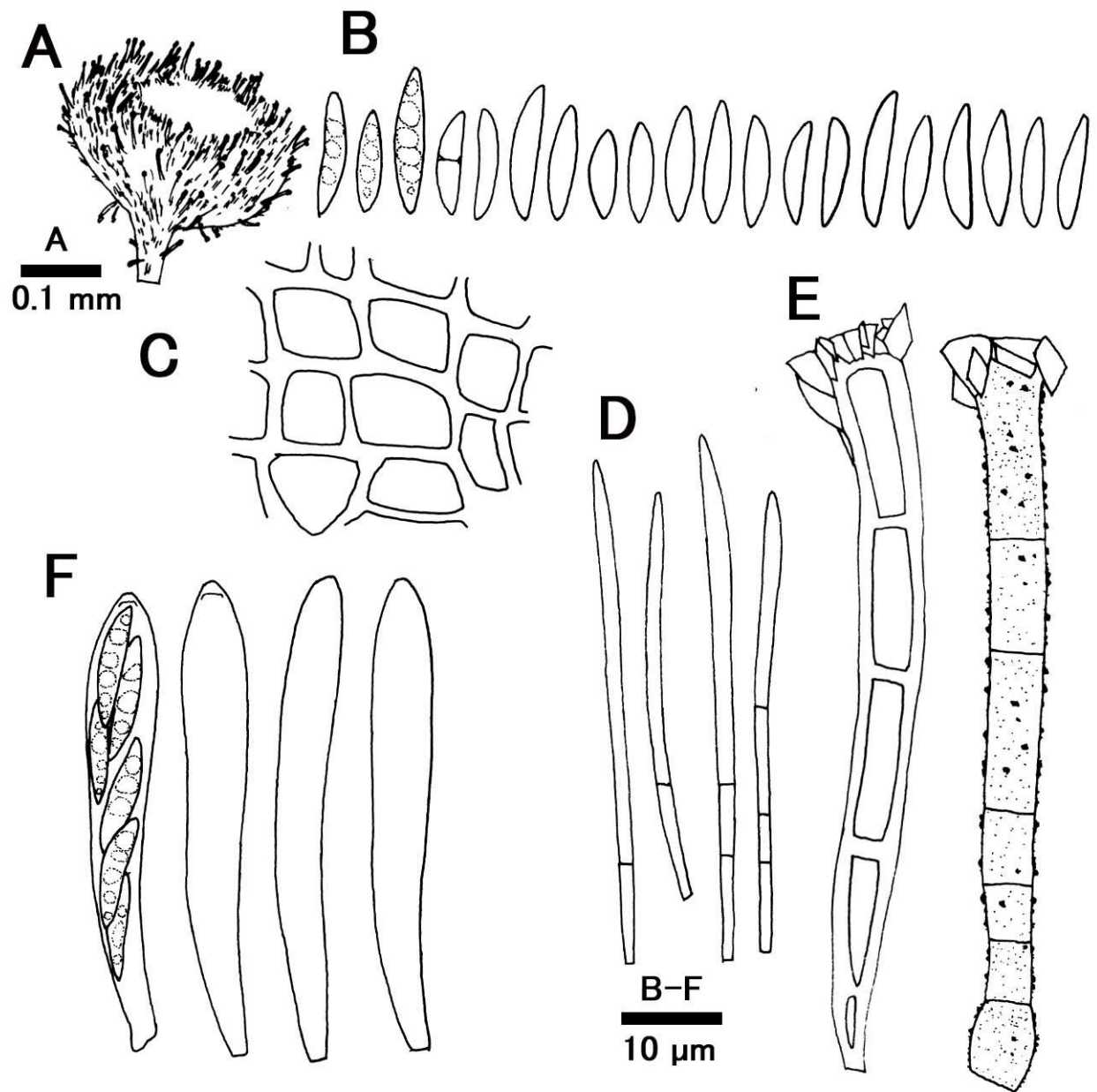


Figure 38. *Incrucipulum hakonechloae-macrae* TNS-F-81512 (Holotype). **A** Apothecium **B** Ascospores **C** Ectal excipular cells **D** Paraphyses **E** Hairs with crystals **F** Asci.

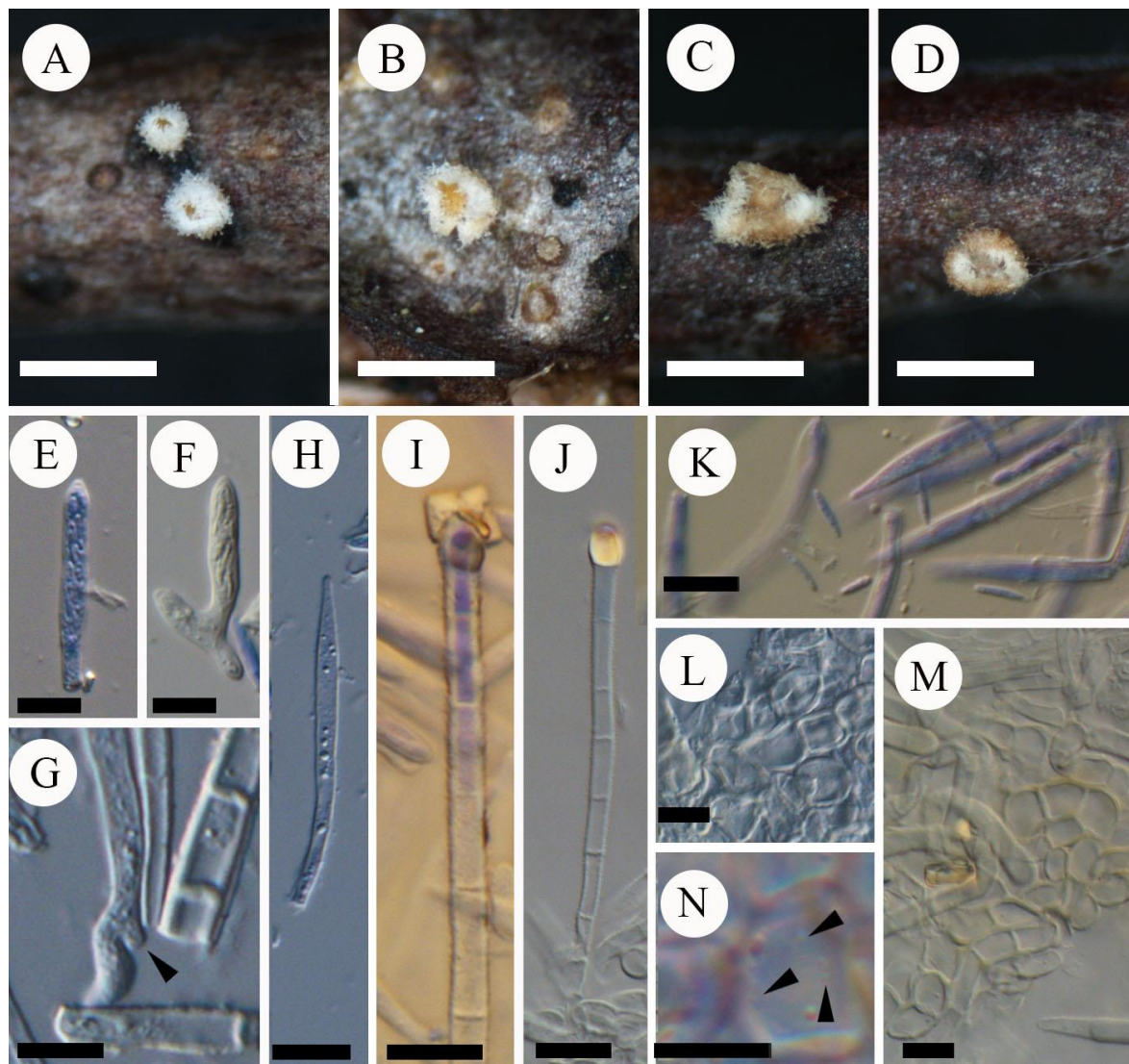


Figure 39. *Incrucipulum pseudosulphurellum* TNS-F-81441 (Holotype). **A, B** Fresh apothecia **C, D** Dried apothecium **E** Ascus **F** Ascus with an irregularly branched base **G** Immature ascus with a crozier-like structure (arrowhead) **H** Paraphysis **I** Hair with a tetrahedral crystal **J** Hair with an irregular-shaped crystal **K** Ascospores **L** Hyaline ectal excipular cells **M** Subhyaline ectal excipular cells **N** Surface of ectal excipular cells with granules (arrowheads). Mounted in CB/LA (**E–M**). Scale bars: 0.5 cm (**A–D**), 10 µm (**E–N**).

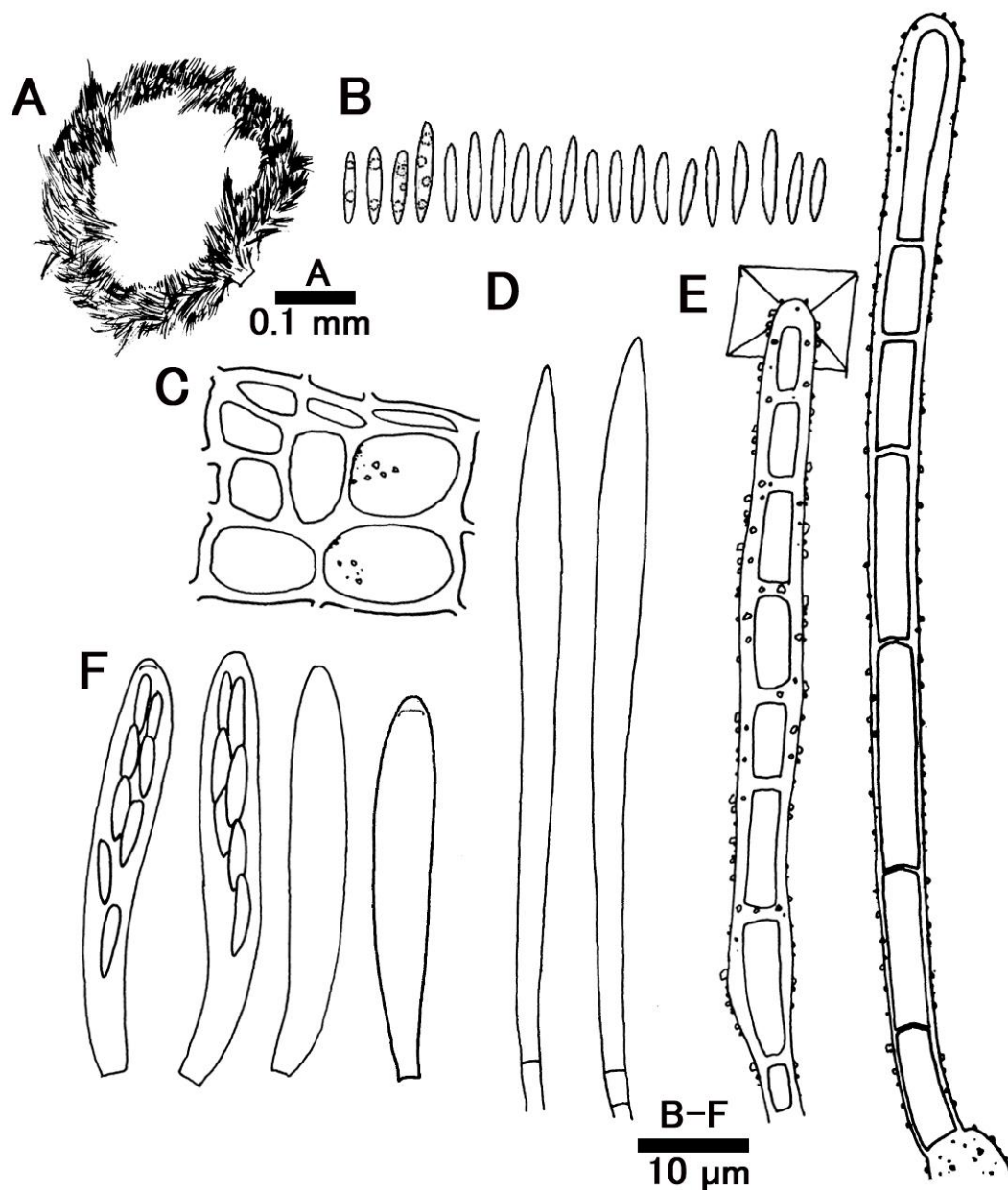


Figure 40. *Incrucipulum pseudosulphurellum* TNS-F-81441 (Holotype). **A** Apothecium **B** Ascospores **C** Ectal excipular cells **D** Paraphyses **E** Hairs with and without crystals **F** Asci.

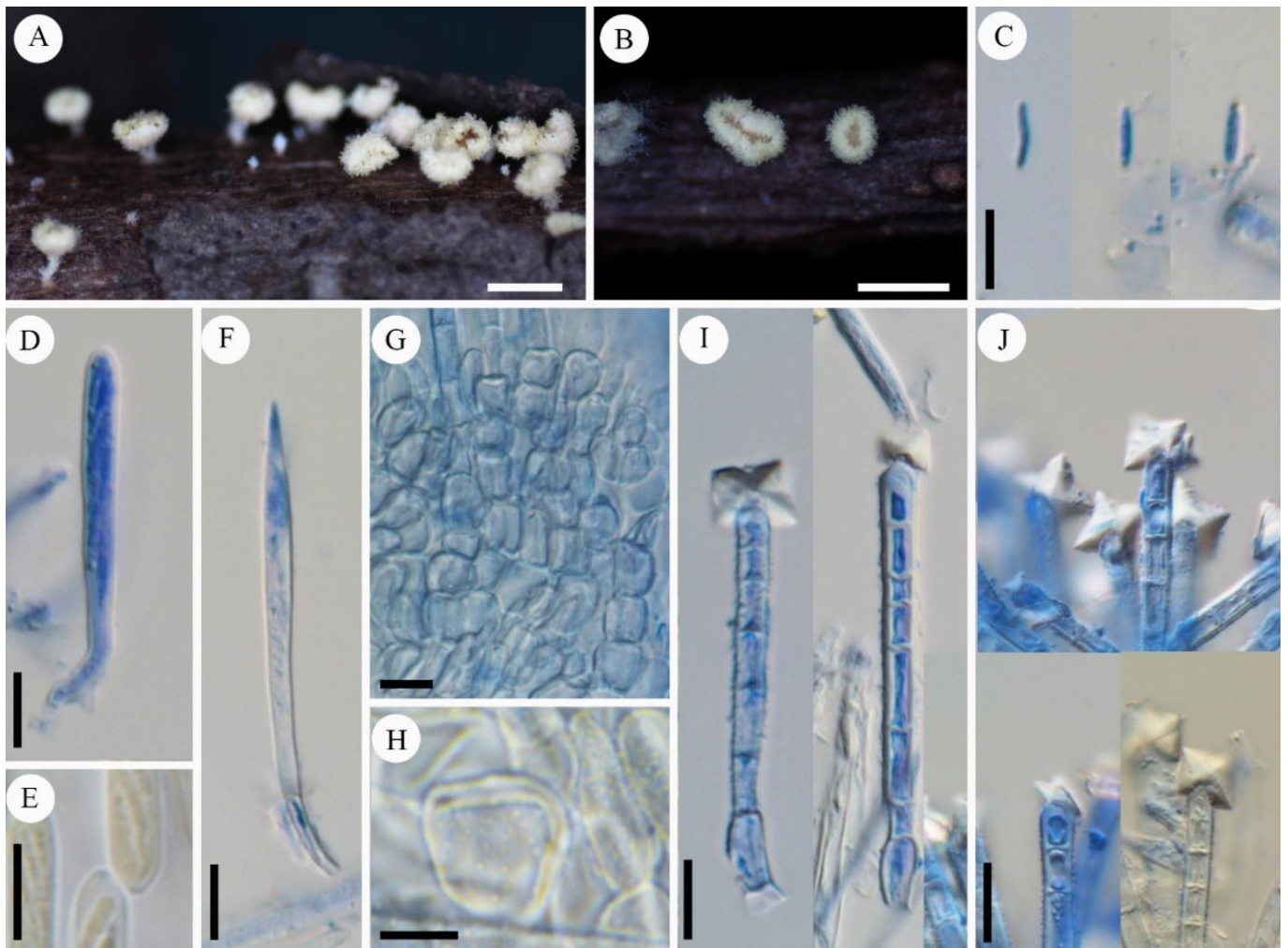


Figure 41. *Incrucipulum sulphurellum* TNS-F-81698 (A, C–J); TNS-F-81695 (B). A, B Apothecia C Ascospores D Ascus E Ascal pore stained blue by MLZ F Paraphysis G Ectal excipular cells H Ectal excipular cells with granulate surfaces I Hairs J Morphological variation of hair crystals. Mounted in CBW (C, D, F–J); MLZ (E). Scale bars: A, B 0.5 mm, C–J 10 μ m.

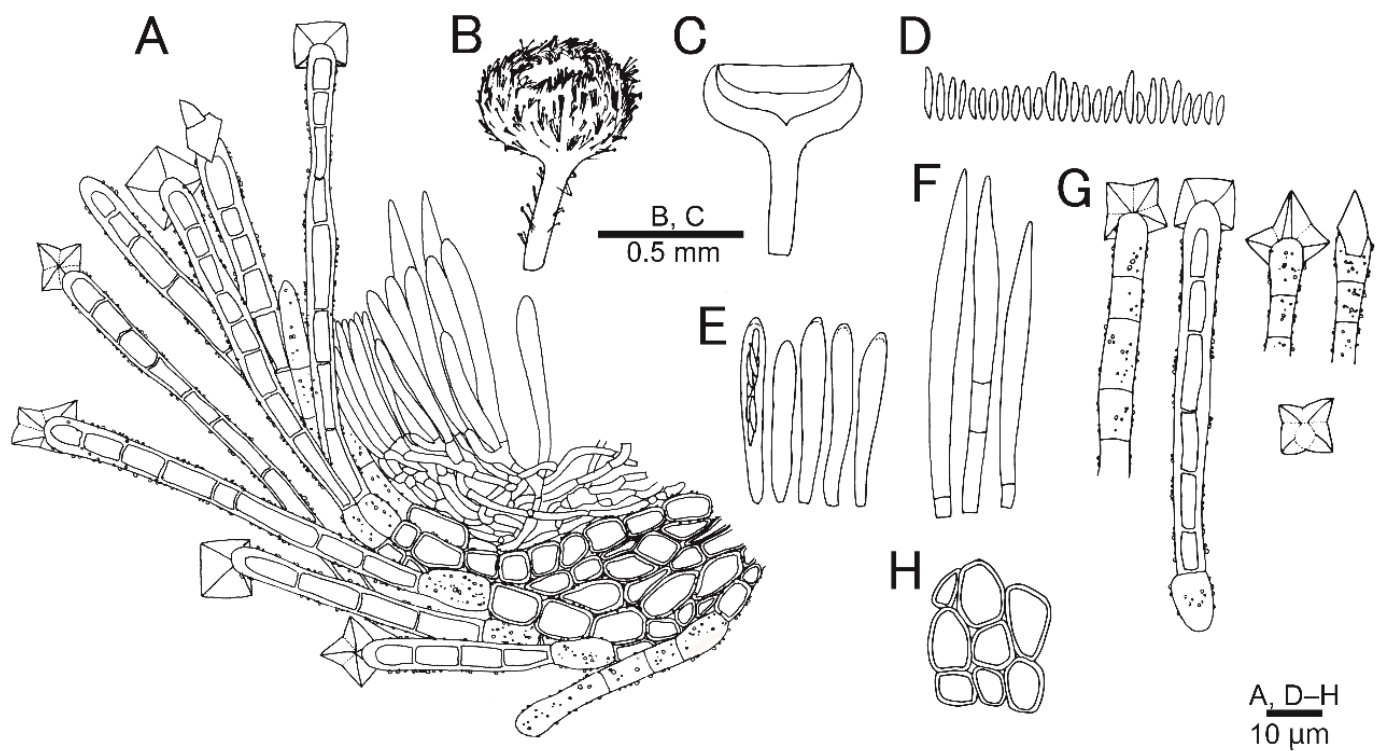


Figure 42. *Incrucipulum sulphurellum* TNS-F-81698. **A** Marginal part of the vertical section of the apothecium **B** Apothecium **C** Vertical section of apothecium **D** Ascospores **E** Asci **F** Paraphyses **G** Hairs with crystals **H** Ectal excipular cells.

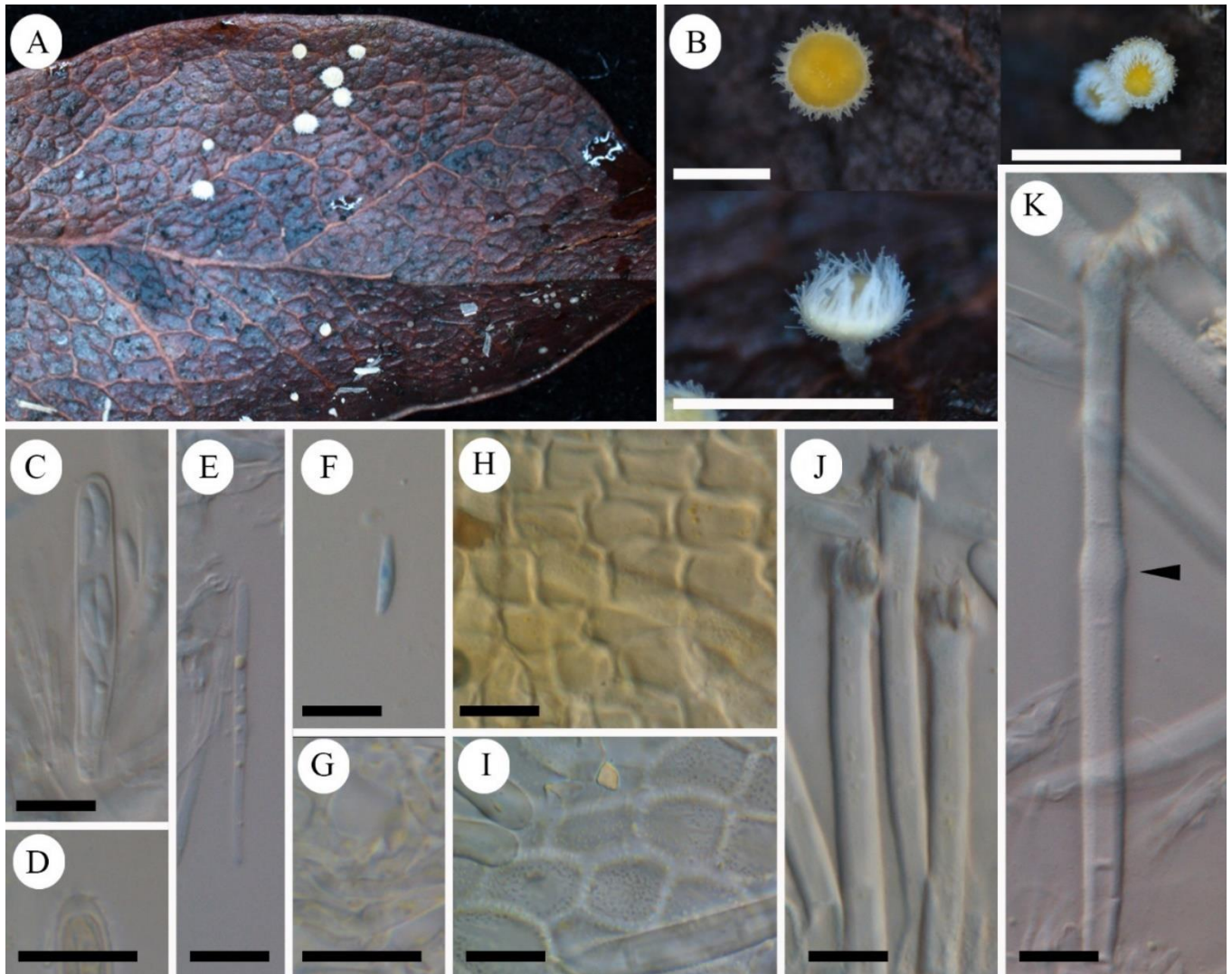


Figure 43. *Incrucipulum virtembergense* TNS-F-80905. **A** Apothecia scattered on the substrate. **B** Apothecia (when fresh) **C** Ascus **D** Ascus pore which turned blue by MLZ after KOH pretreatment **E** Paraphysis containing yellow oil drops **F** Ascospore **G** Hyphae of the medullary excipulum containing yellow oil drops **H** Ectal excipular cells like stone pavings containing yellow oil drops **I** Ectal excipular cells with granulate surfaces. **J** Crystallized hairs containing yellow oil drops **K** Hair with partial inflation (arrowhead). Mounted in CB/LA (**C–K**). Scale bars: 1 mm (**B**), 10 μ m (**C–K**).

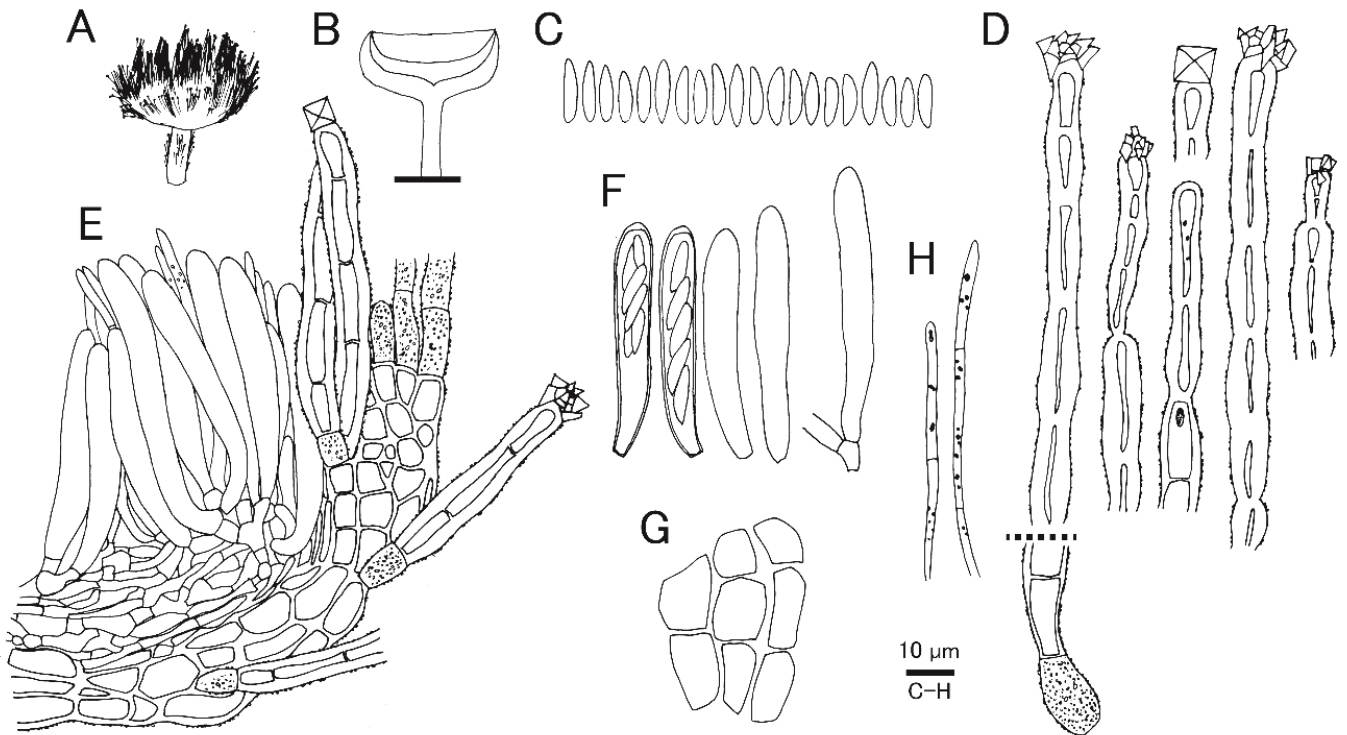


Figure 44. *Incrucipulum virtembergense* TNS-F-80905. **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores **D** Hairs with crystals **E** Expansion of a vertical section of an apothecium **F** Thick-walled asci **G** Ectal excipular cells **H** Paraphyses.

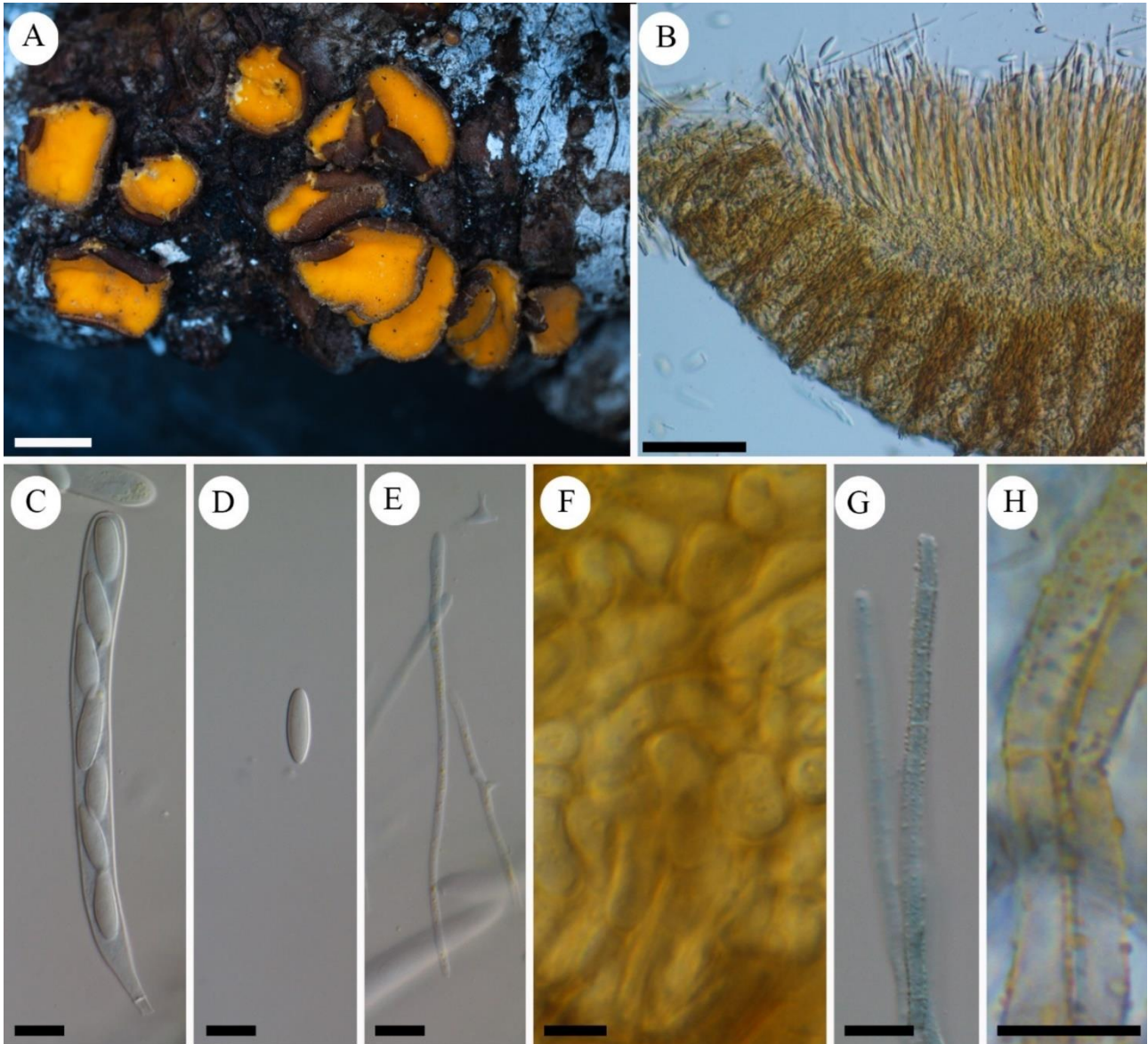


Figure 45. *Lachnellula pini* TNS-F-81833. **A** Dried apothecia **B** Vertical section of an apothecium **C** Ascus **D** Ascospore **E** Paraphyses containing orange oil drops **F** Ectal excipular cells **G** Hairs **H** Brown granules on hair surfaces. Mounted in CB/LA (**C–H**). Scale bars: 1 mm (**B**), 100 μ m (**B**), 10 μ m (**C–H**).

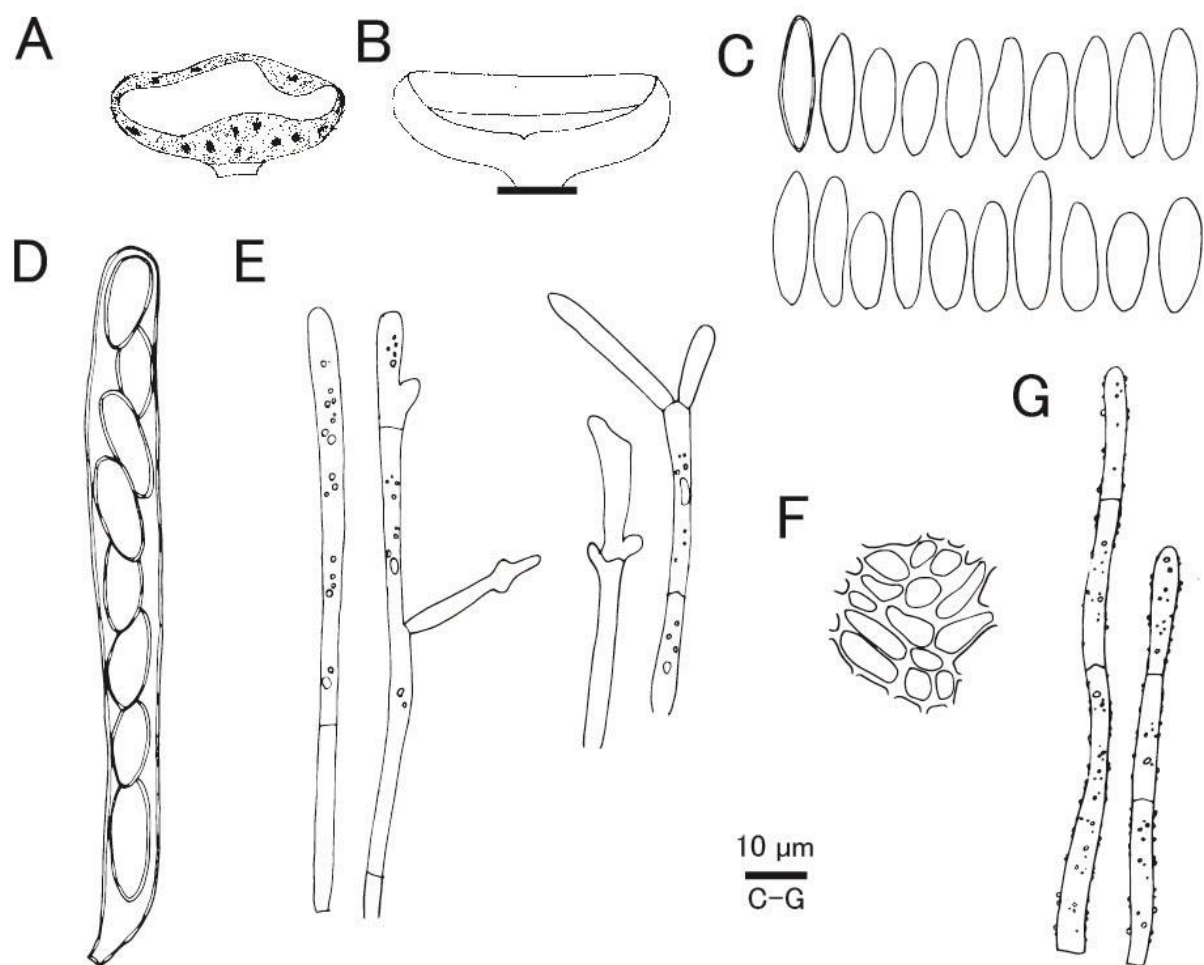


Figure 46. *Lachnellula pini* TNS-F-81833. **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores **D** Ascus **E** Paraphyses branching like fingers **F** Ectal excipular cells **G** Hairs.

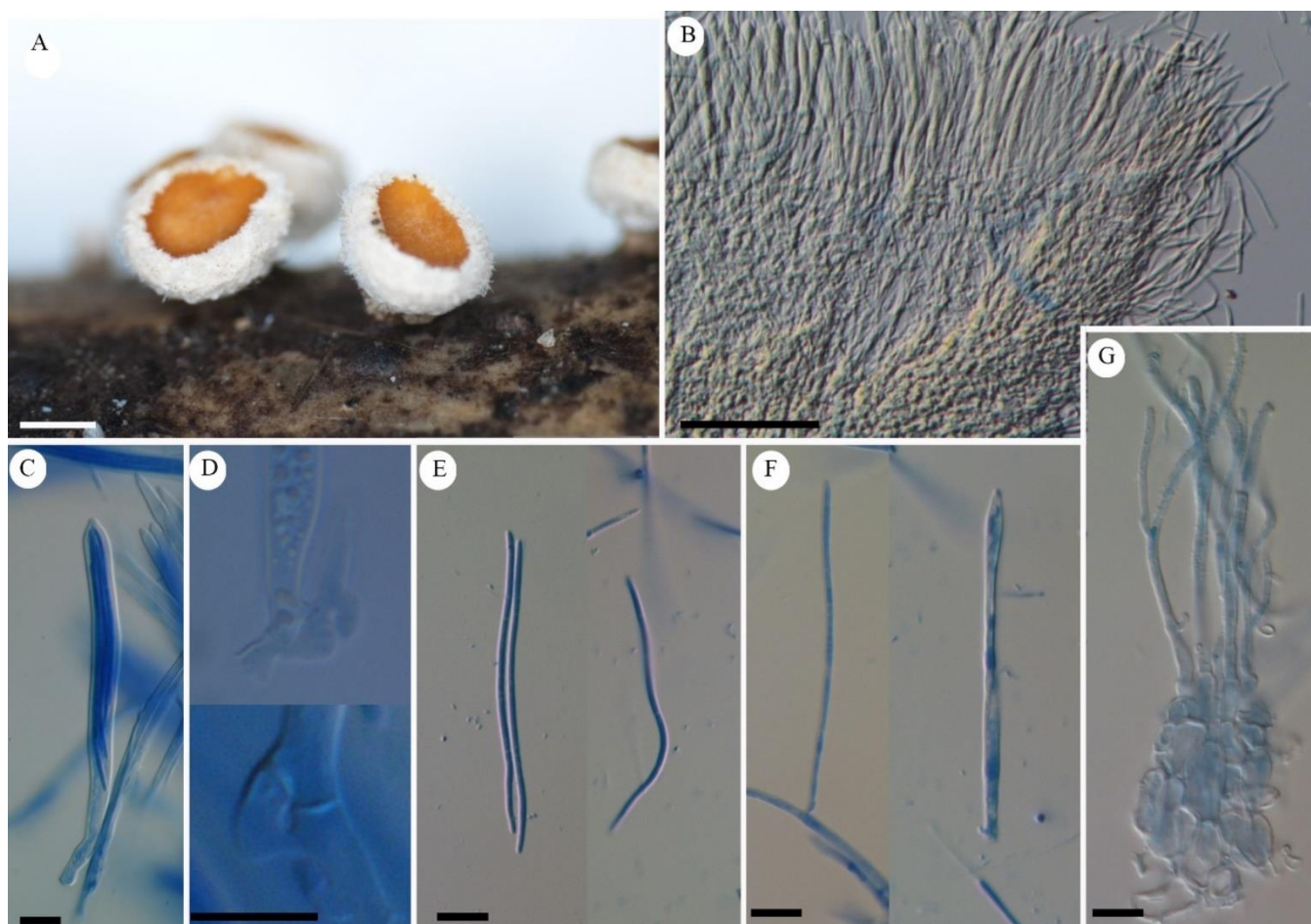


Figure 47. *Lachnum* *novoguineense* var. *yunnanicum* TNS-F-16442. **A** Dried apothecia **B** Expansion of a vertical section of an apothecium **C** Ascus **D** Ascus bases with croziers with perforation **E** Ascospores **F** Paraphyses **G** Hairs arising from ectal excipular cells. Mounted in CB/LA (C–G). Scale bars: 50 μm (B), 10 μm (C–G).

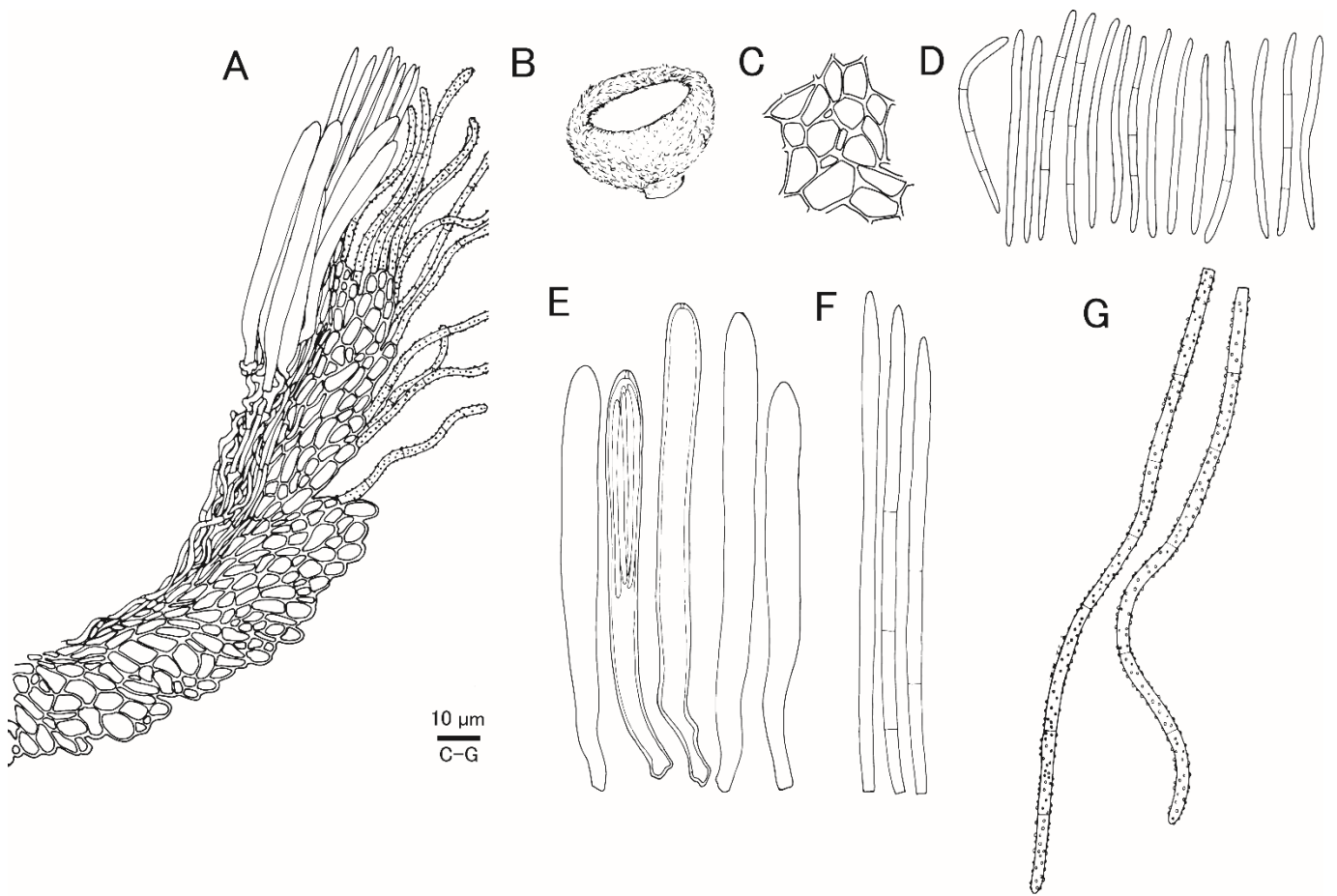


Figure 48. *‘Lachnum’ novoguineense* var. *yunnanicum* TNS-F-16442, 16642. **A** Expansion of a vertical section of an apothecium **B** Apothecium **C** Ectal excipular cells **D** Ascospores **E** Asci **F** Paraphyses **G** Hairs. Illustrated by Rei Sasagawa (**A**, **B**, **D–G**)

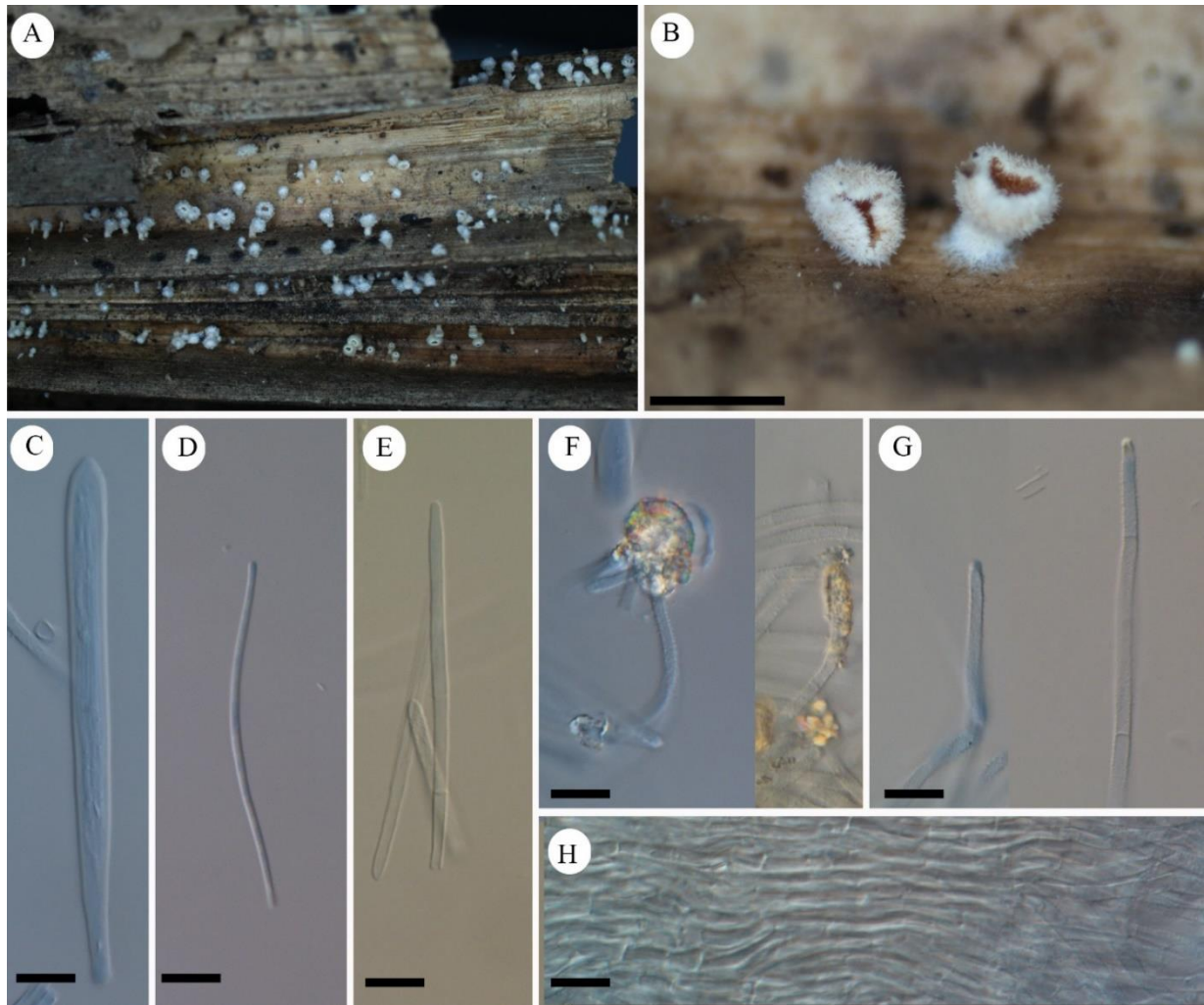


Figure 49. *Lachnum palmae* TNS-F-24600. **A** Dried apothecia scattered on a substrate **B** Dried apothecia **C** Thick-walled ascus **D** Ascospore **E** Paraphyses **F** Hairs with resinous materials **G** Hair with apical amorphous materials **H** Ectal excipular cells. Mounted in CB/LA (**C–H**). Scale bars: 1 mm (**B**), 10 µm (**C–H**).

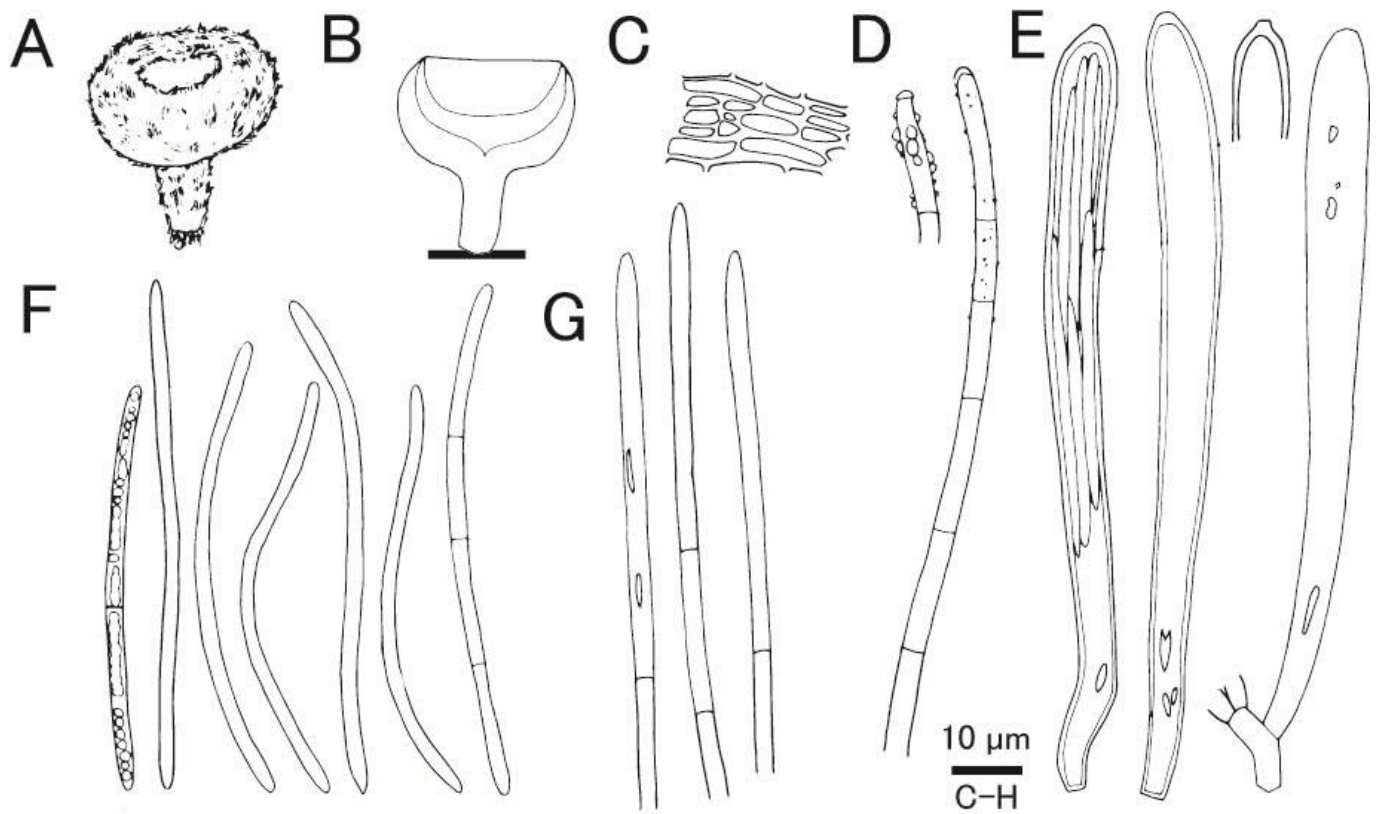


Figure 50. '*Lachnum*' *palmae* TNS-F-24600. **A** Apothecium **B** Vertical section of an apothecium **C** Ectal excipular cells **D** Hairs with apical amorphous materials **E** Thick-walled asci **F** Ascospores **G** Paraphyses.

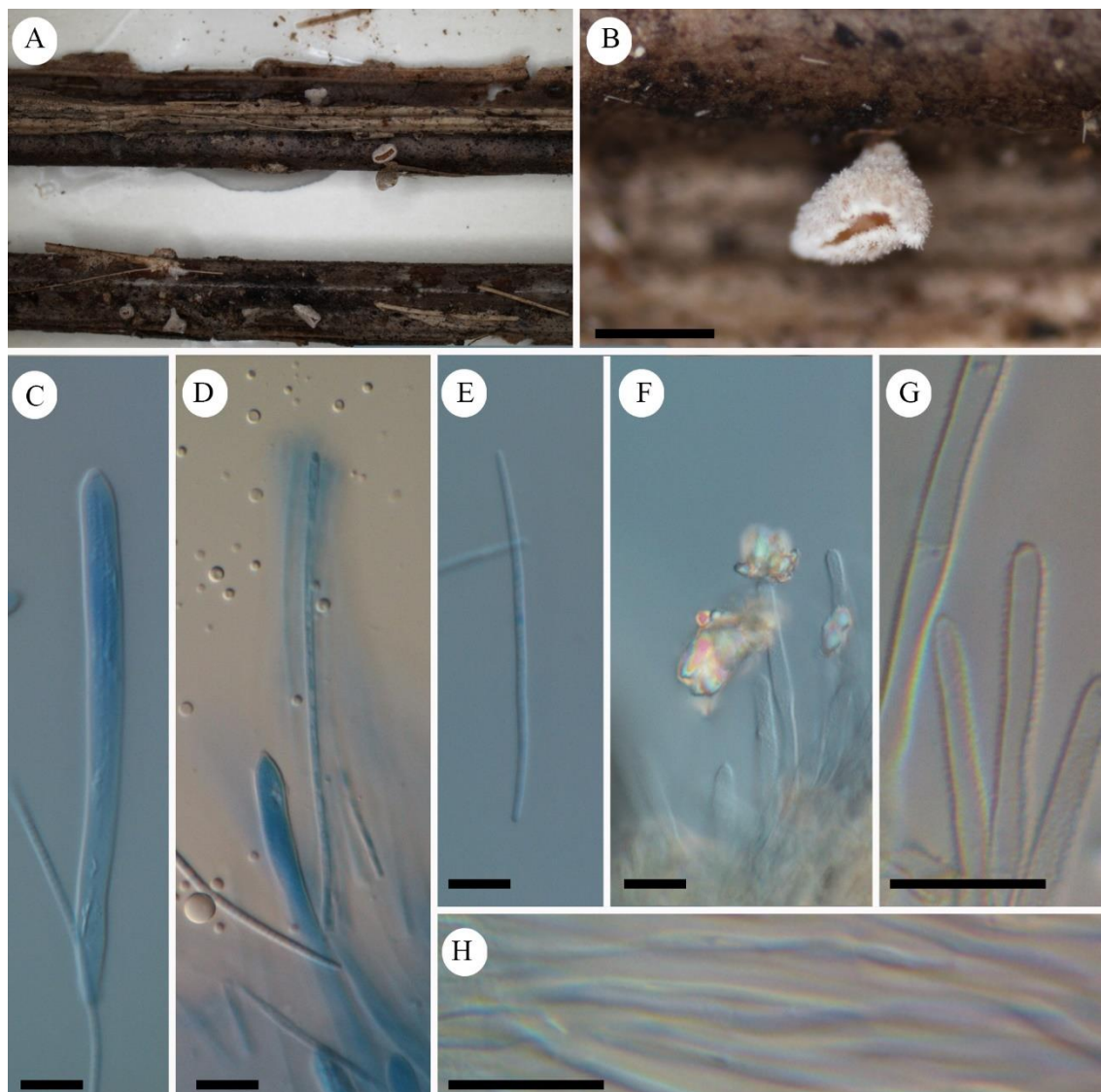


Figure 51. *'Lachnum' palmae* TNS-F-17567. **A** Dried apothecia scattered on substrates **B** Dried apothecium **C** Thick-walled ascus **D** Paraphysis **E** Ascospore **F** Hairs with resinous materials **G** Hairs with apical amorphous materials **H** Ectal excipular cells. Mounted in CB/LA (**C–H**). Scale bars: 1 mm (**B**), 10 μ m (**C–H**).

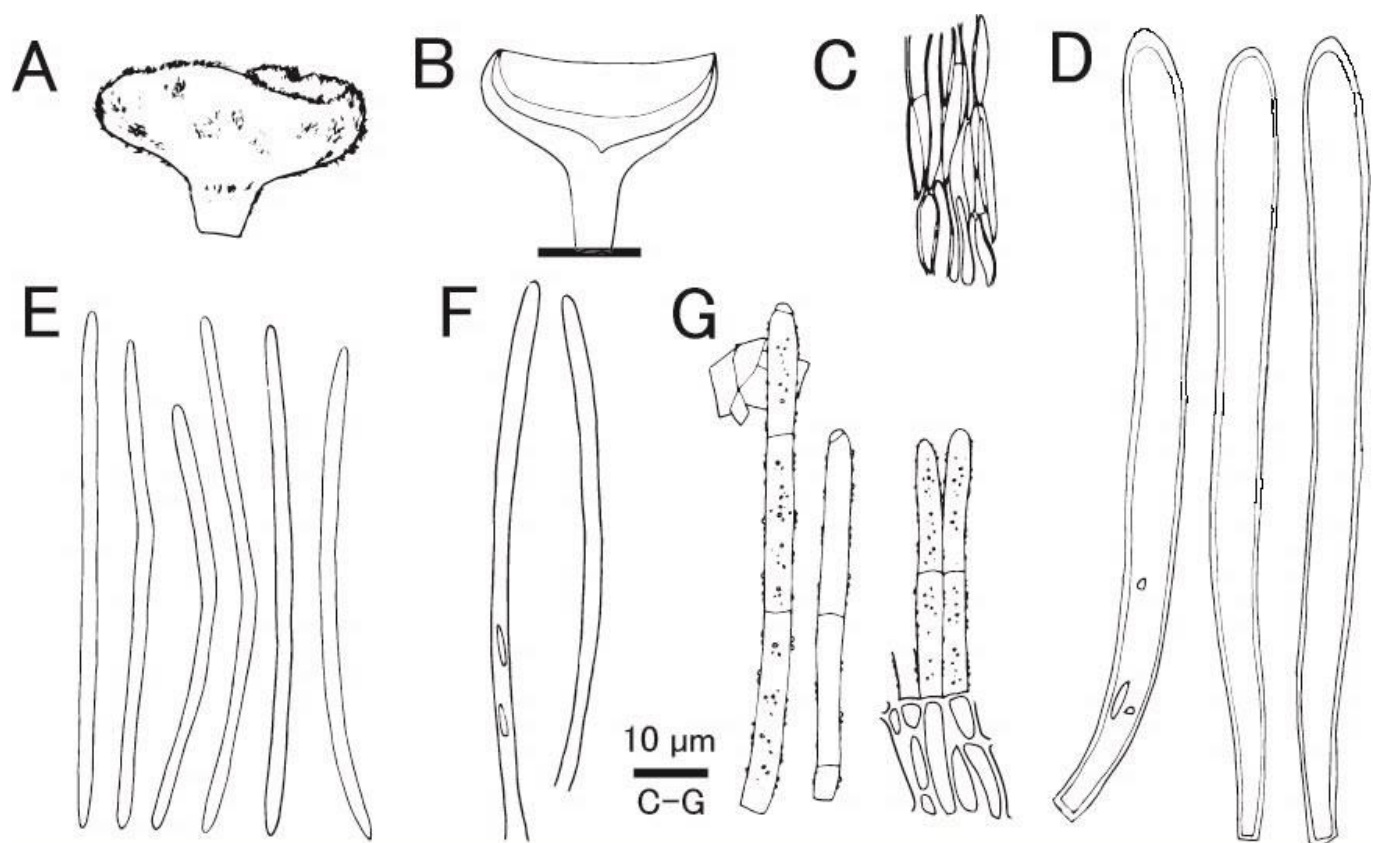


Figure 52. '*Lachnum*' *palmae* TNS-F-17567. **A** Apothecium **B** Vertical section of an apothecium **C** Ectal excipular cells **D** Thick-walled asci **E** Ascospores **F** Paraphyses **G** Hairs with apical amorphous materials and resinous materials.

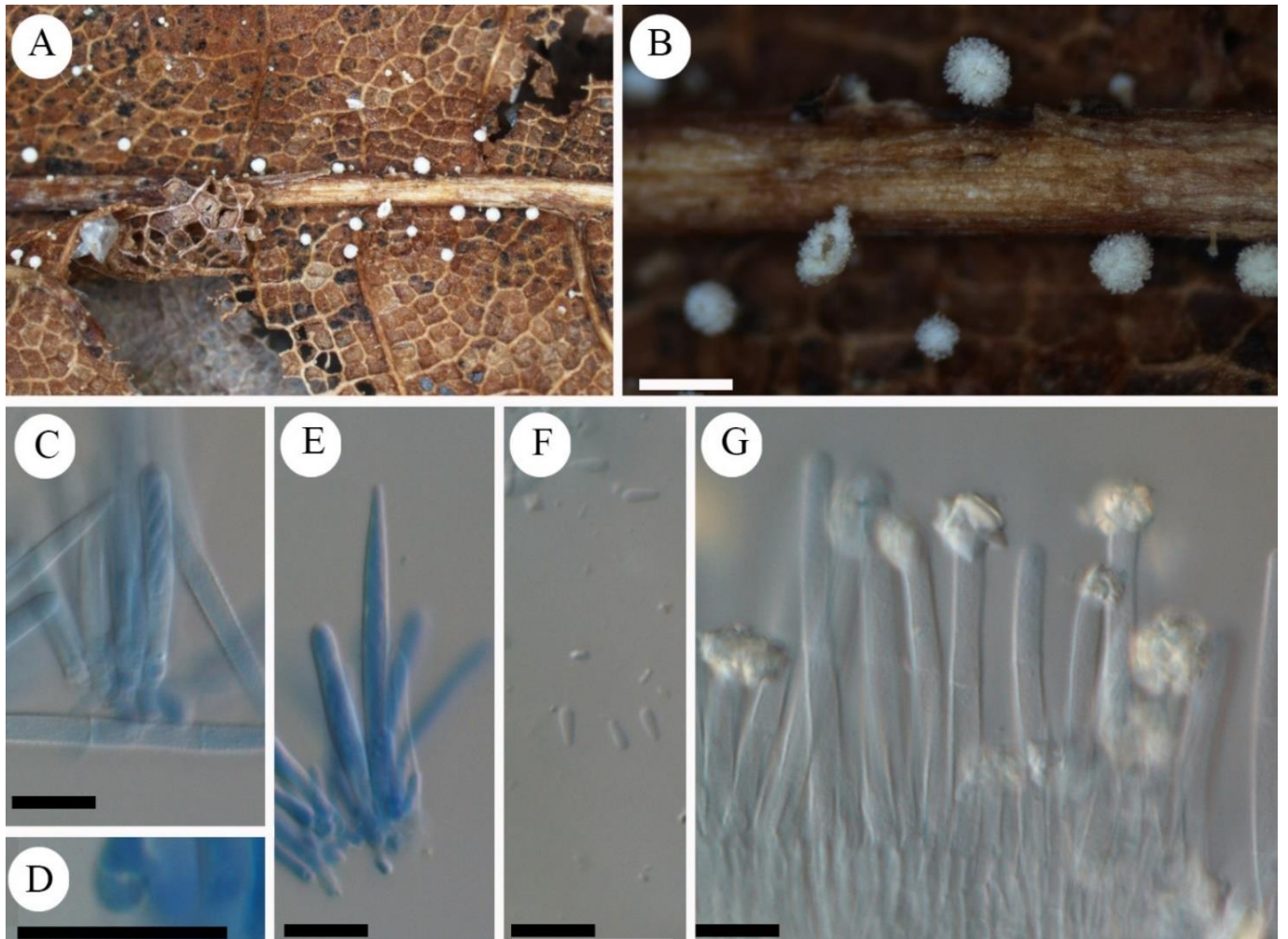


Figure 53. *Neolachnum rhytismatis* TNS-F-65645. **A** Dried apothecia scattered on substrates **B** Dried apothecia **C** Asci **D** Crozier with perforation **E** Paraphysis **F** Ascospores **G** Hairs with crystals. Mounted in CB/LA (C–G). Scale bars: 0.5 mm (B), 10 μ m (C–G).

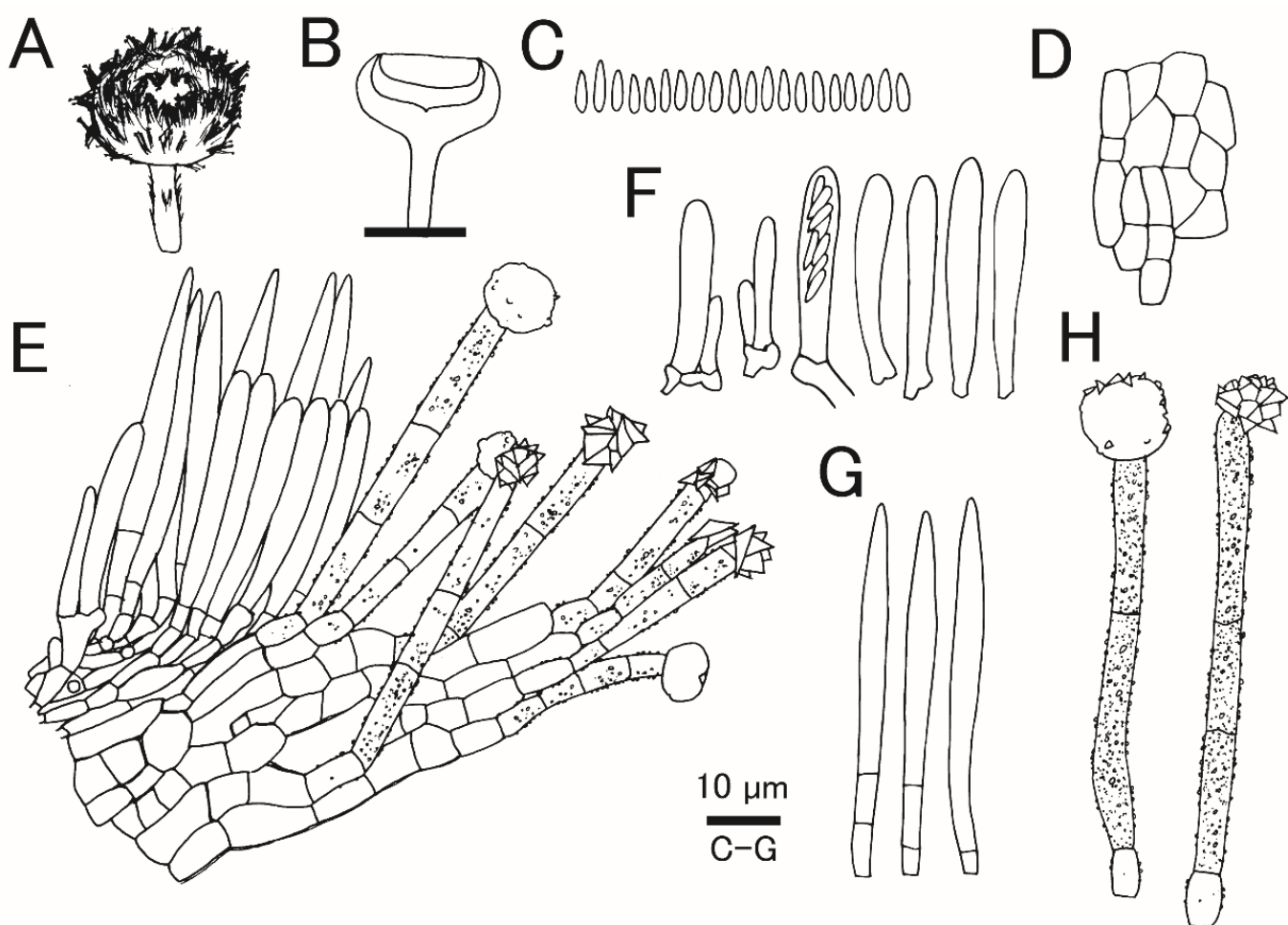


Figure 54. *Neolachnum rhytismatis* TNS-F-65645. **A** Apothecium **B** Vertical section of an apothecium **C** Ascospores **D** Ectal excipular cells **E** Expansion of a vertical section of an apothecium **F** Asci arising from croziers **G** Paraphyses **H** Hairs with crystal masses.

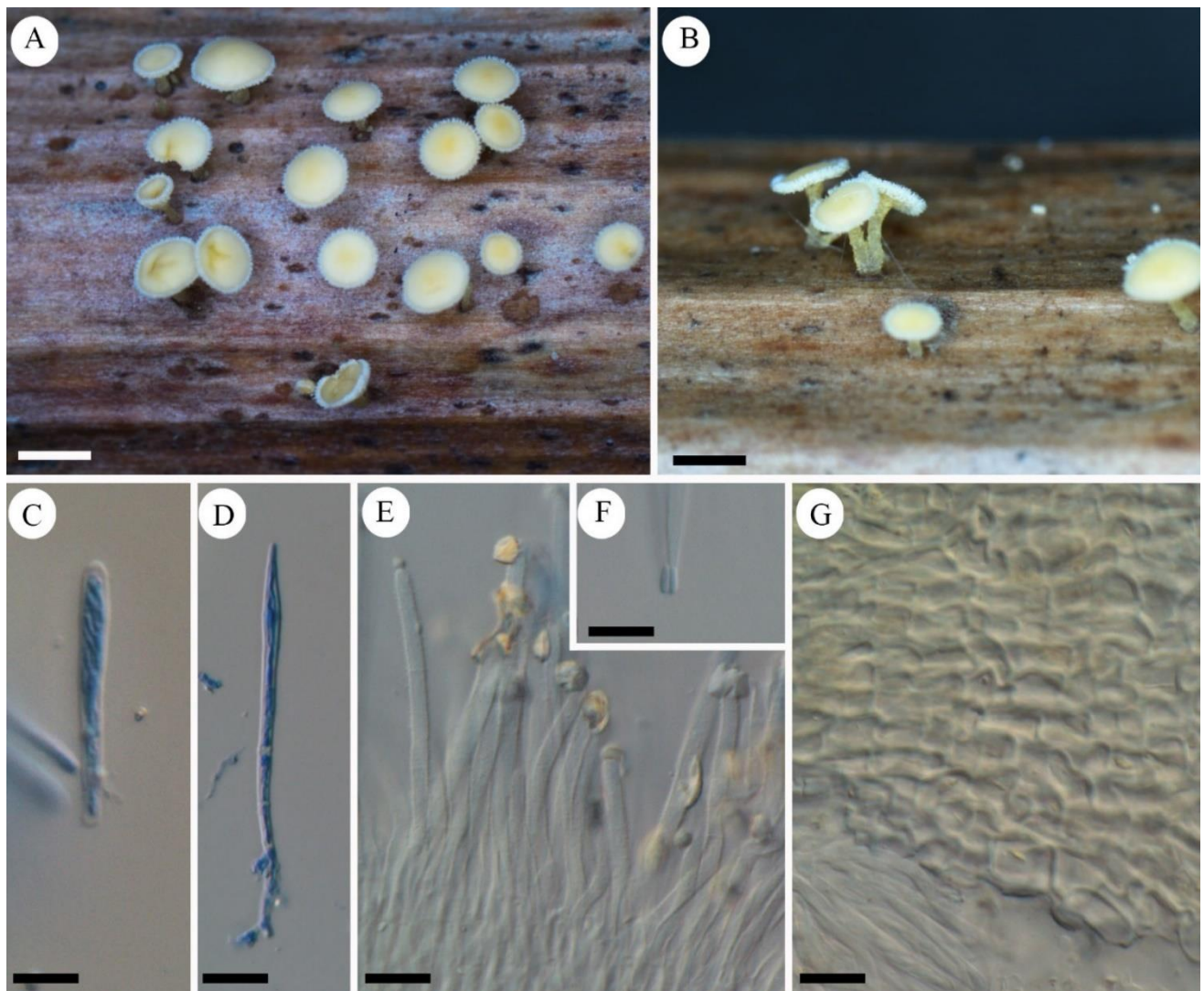


Figure 55. *Neolachnum tenuipileum* TNS-F-81438. **A, B** Fresh apothecia **C** Ascus **D** Paraphysis **E** Hairs with crystals **F** Ascospores **G** Thick-walled ectal excipular cells. Mounted in CB/LA (C–G). Scale bars: 1 mm (A, B), 10 µm (C–G).

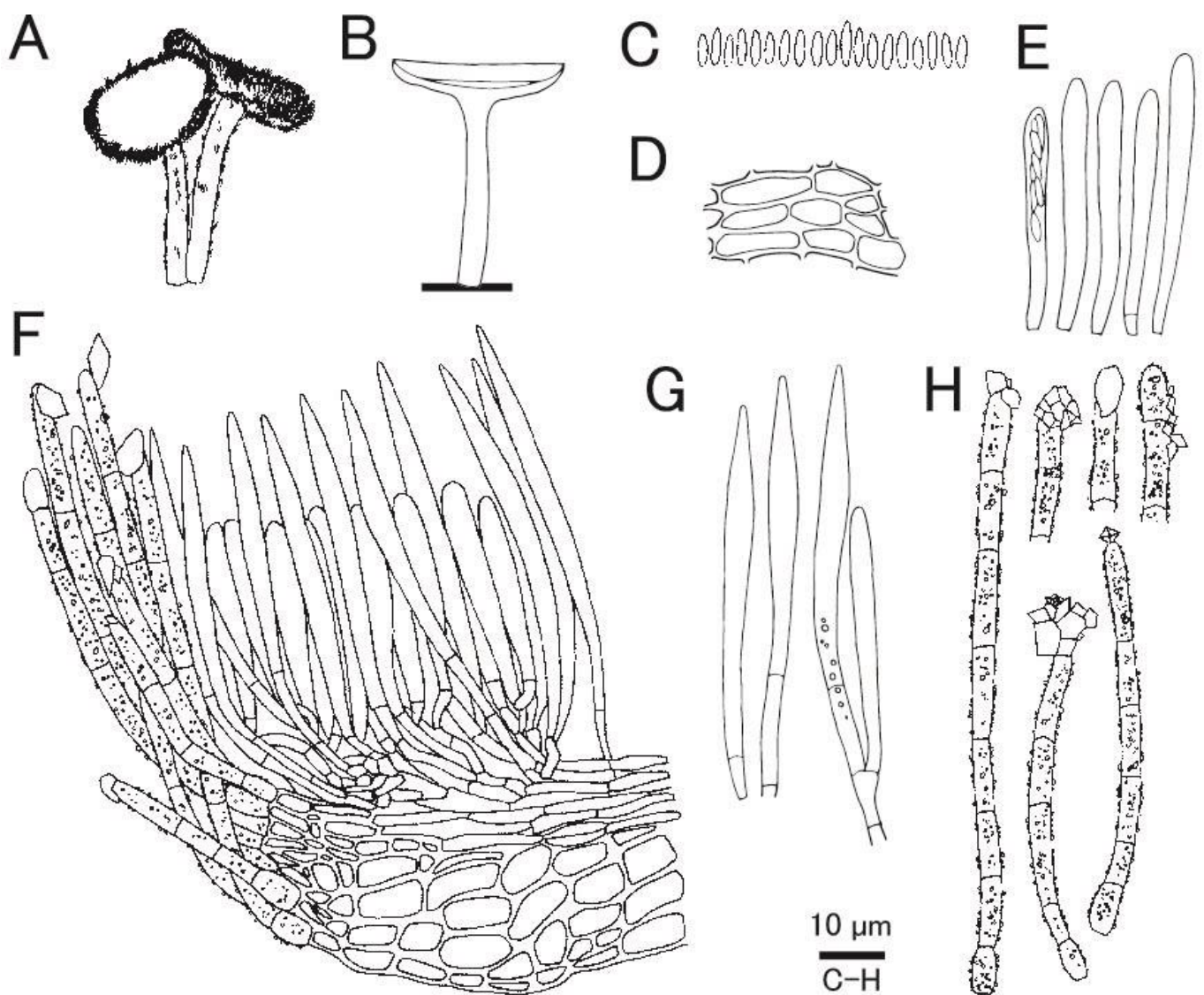


Figure 56. *Neolachnum tenuipileum* TNS-F-81438. **A** Apothecia **B** Vertical section of an apothecium **C** Ascospores **D** Ectal exipular cells **E** Asci **F** Expansion of a vertical section of an apothecium **G** Paraphyses **H** Hairs with crystals.

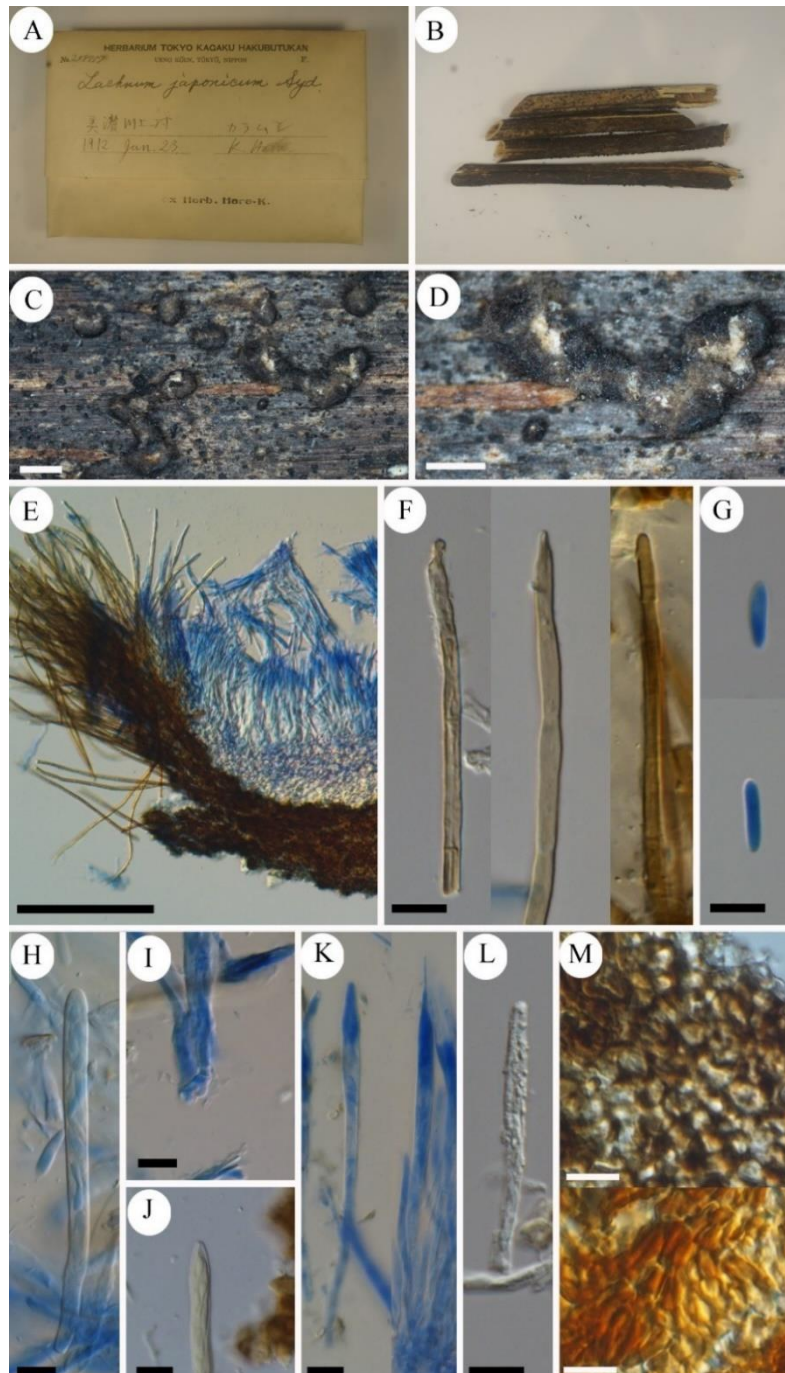


Figure 57. *Trichopezizella japonica* TNS-F-209717. **A** Outer appearance of the specimen **B** Substrates with numerous apothecia **C, D** Apothecia **E** Vertical section through the apothecia, showing hairs, ectal excipulum, medulla and hymenium **F** Hairs **G** Ascospores. **H** Ascus **I** Ascus base with a crozier **J** Ascus pore MLZ (–) without 3% KOH pretreatment **K** Paraphyses from which amorphous matters were detached by squash mounts **L** Paraphysis with amorphous matters **M** Ectal excipulums. Mounted in CB/LA (**E–I, K–M**), MLZ (**J**). Scale bars: 1 mm (**C, D**), 10 μ m (**E–M**).

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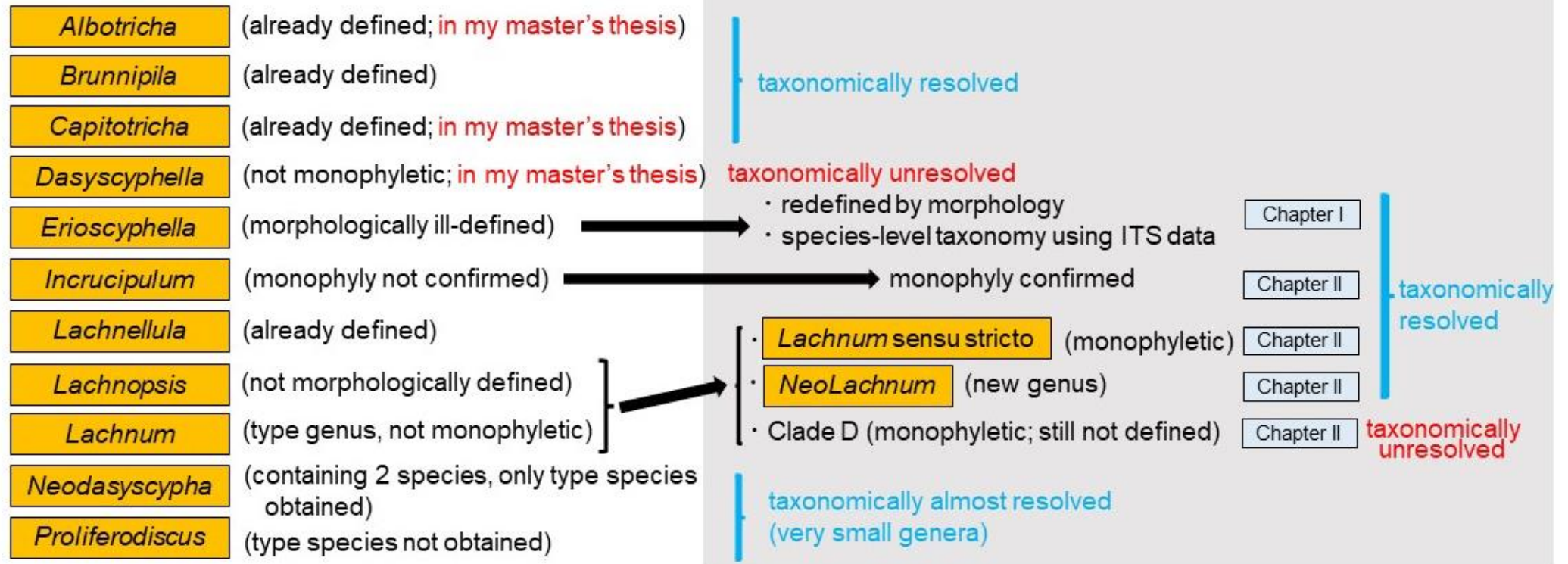
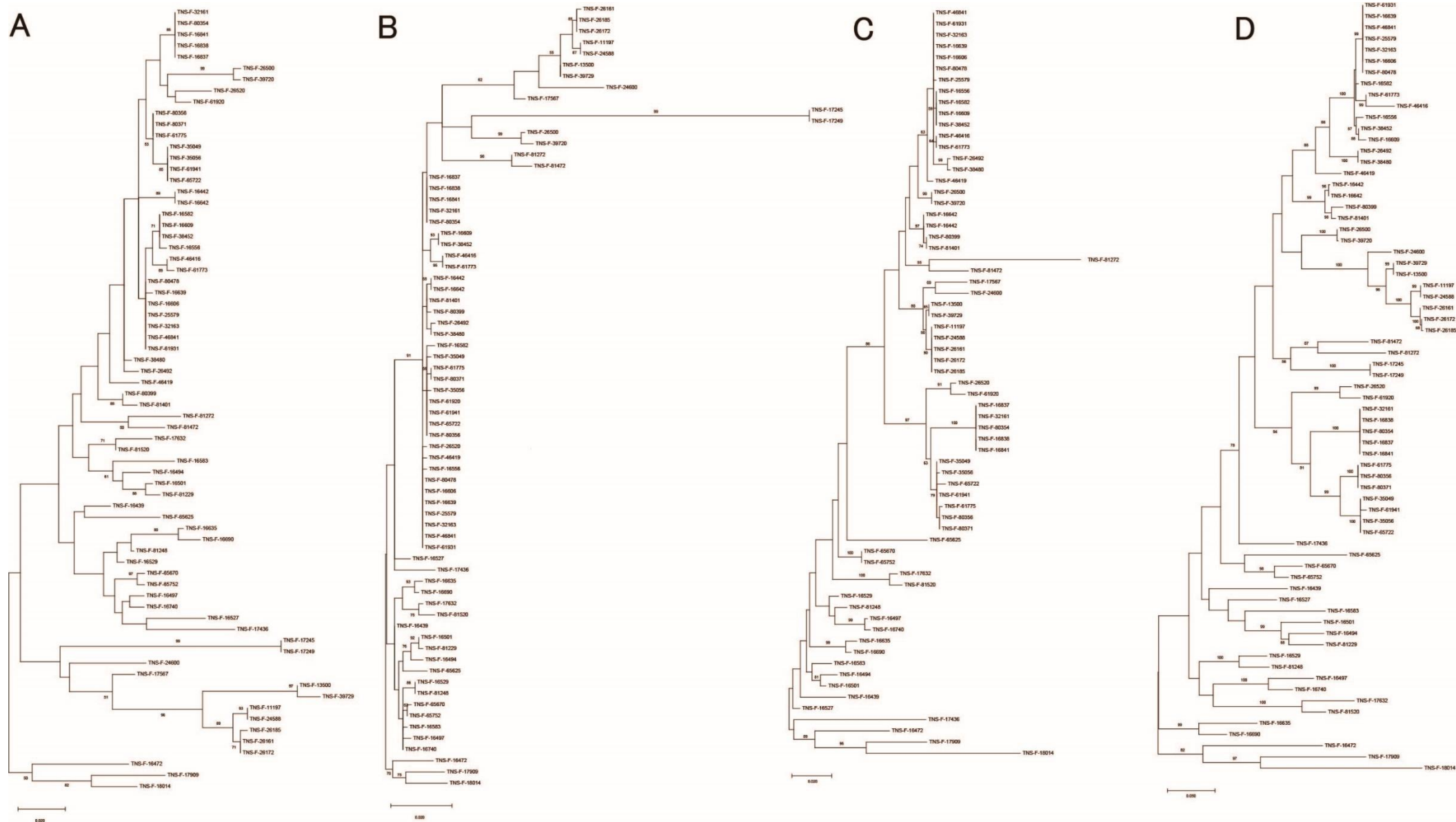
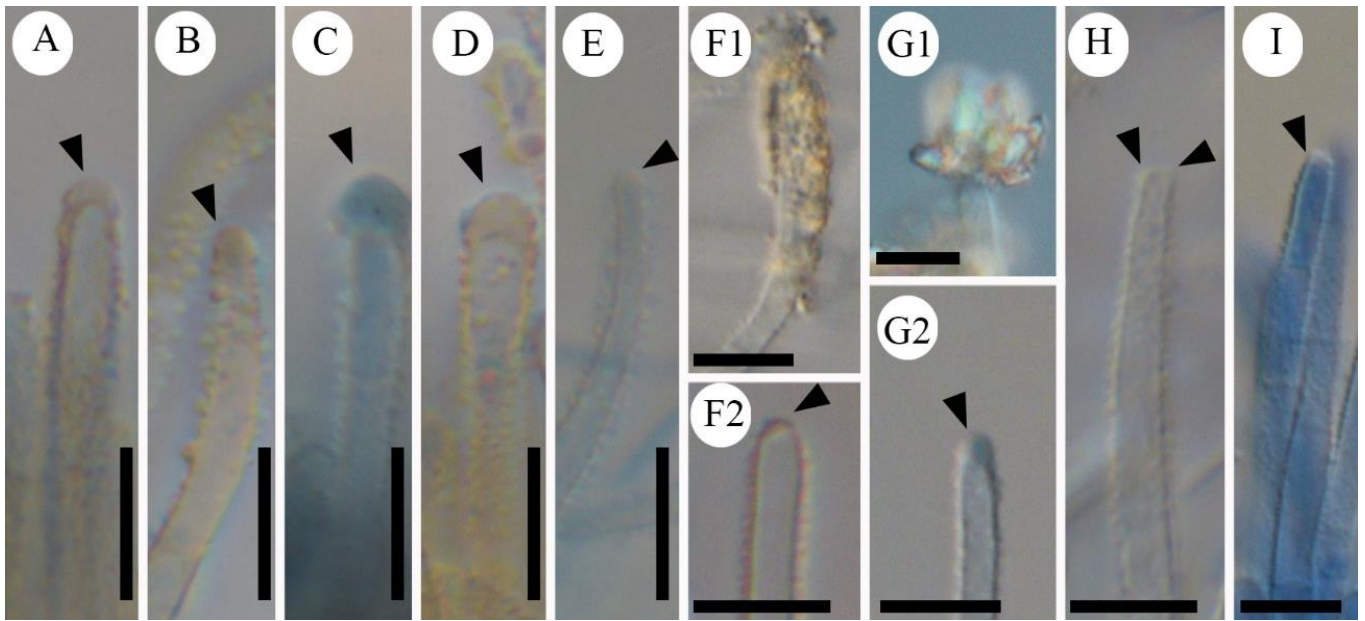


Figure 58. Taxonomic treatment within Lachnaceae carried out in this study.

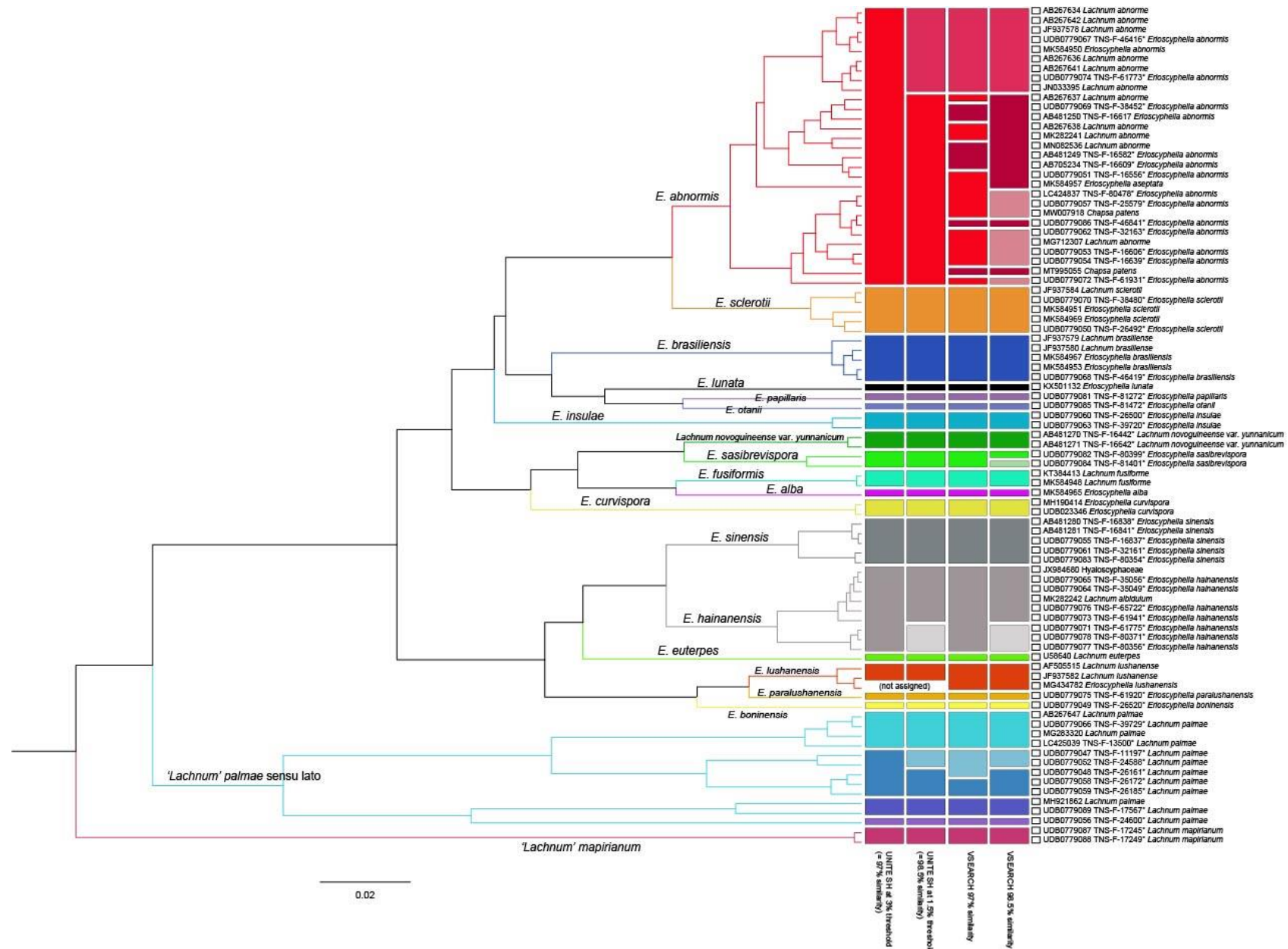
Appendix



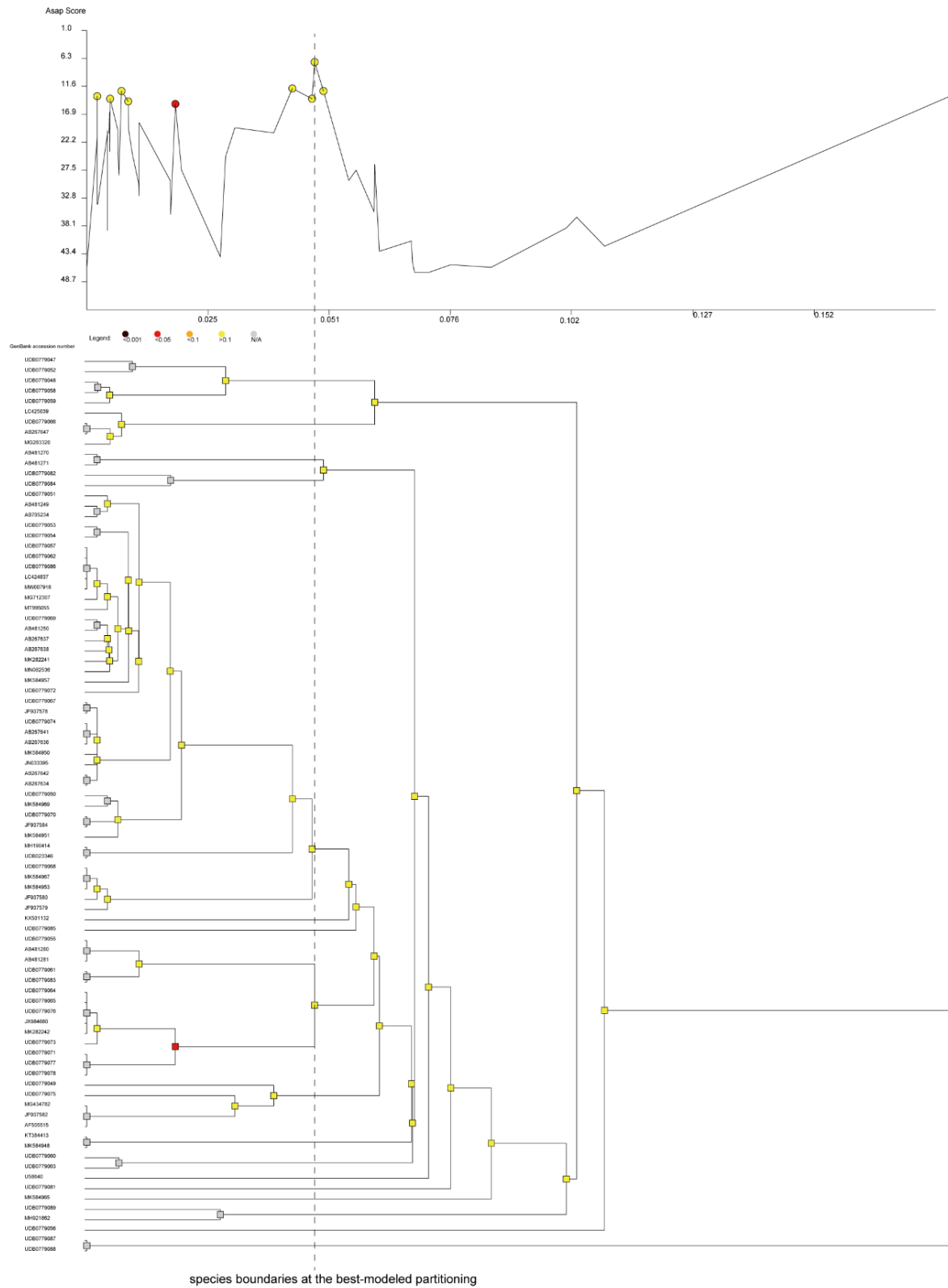
Appendix 1. ML trees based on ITS (A), LSU (B), mtSSU (C) and RPB2 (D) constructed using MEGA X. Bootstrap values > 50% are indicated on branches.



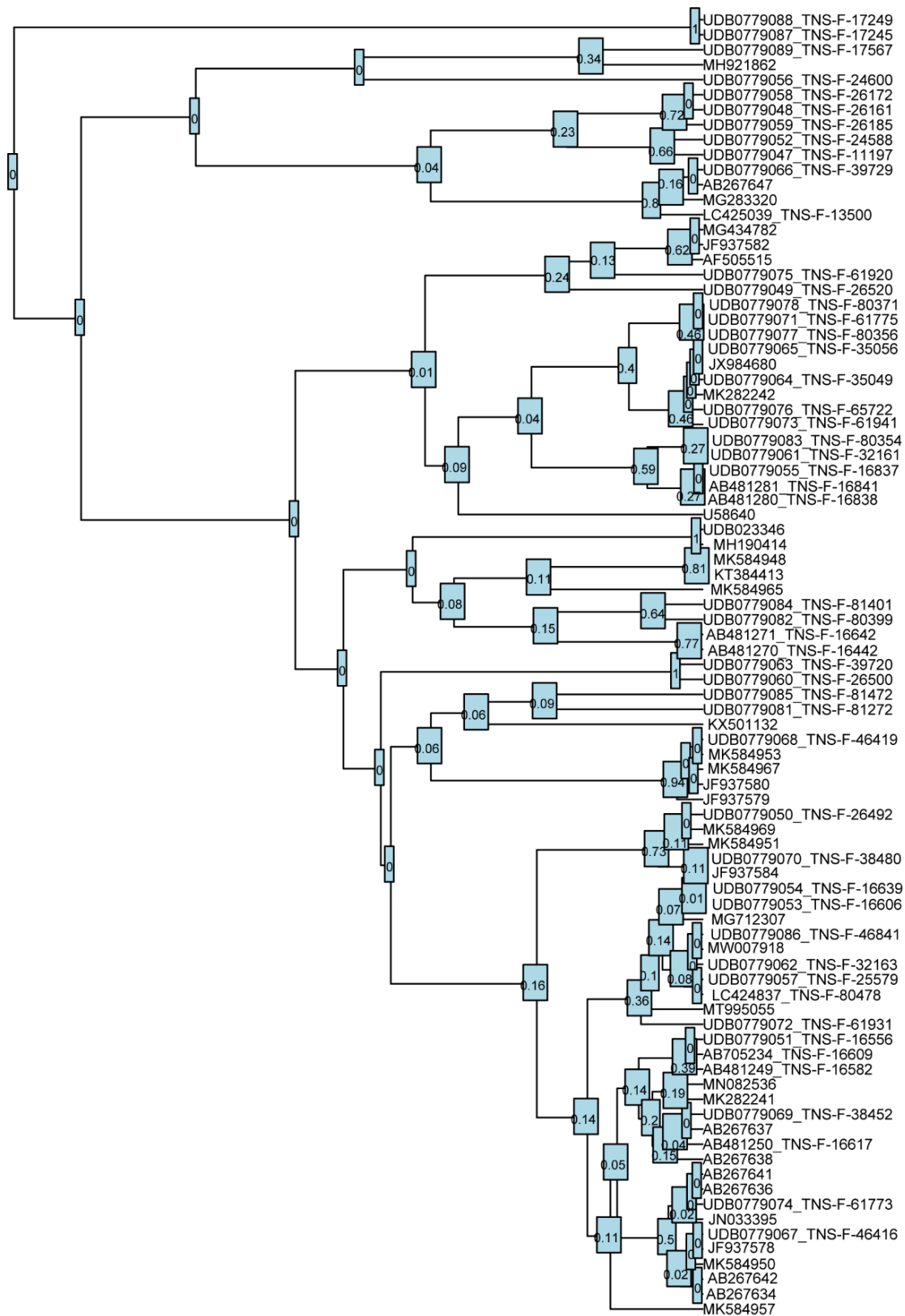
Appendix 2. Hair apices of members of Clade A. **A** *Erioscyphella abnormis* TNS-F-32163 **B** *E. abnormis* TNS-F-61773 **C** *E. brasiliensis* TNS-F-46419 **D** *E. sclerotii* TNS-F-26492 **E** '*Lachnum*' *mapirianum* TNS-F-17245 **F** '*Lachnum*' *palmae* TNS-F-17567 **F1** Hair with resinous matters **F2** Hair with apical amorphous material **G** '*Lachnum*' *palmae* TNS-F-24600 **G1** Hair with a resinous matter **G2** Hair with apical amorphous materials **H** *E. hainanensis* TNS-F-80371 **I** *E. sinensis* TNS-F-80354. Mounted in CB/LA. Scale bars: 10 mm. Arrowheads show hair apical materials.



Appendix 3. Species delimitation analyses using ITS sequences of *Erioscyphella* and its potential members. Clustering results by UNITE SH and VSEARCH at 3% and 1.5% thresholds are shown. Schematic phylogenetic relationships are shown using the ultrametric tree constructed for the GMYC analysis. The taxon names shown on the tree branches follow the results of the present study.

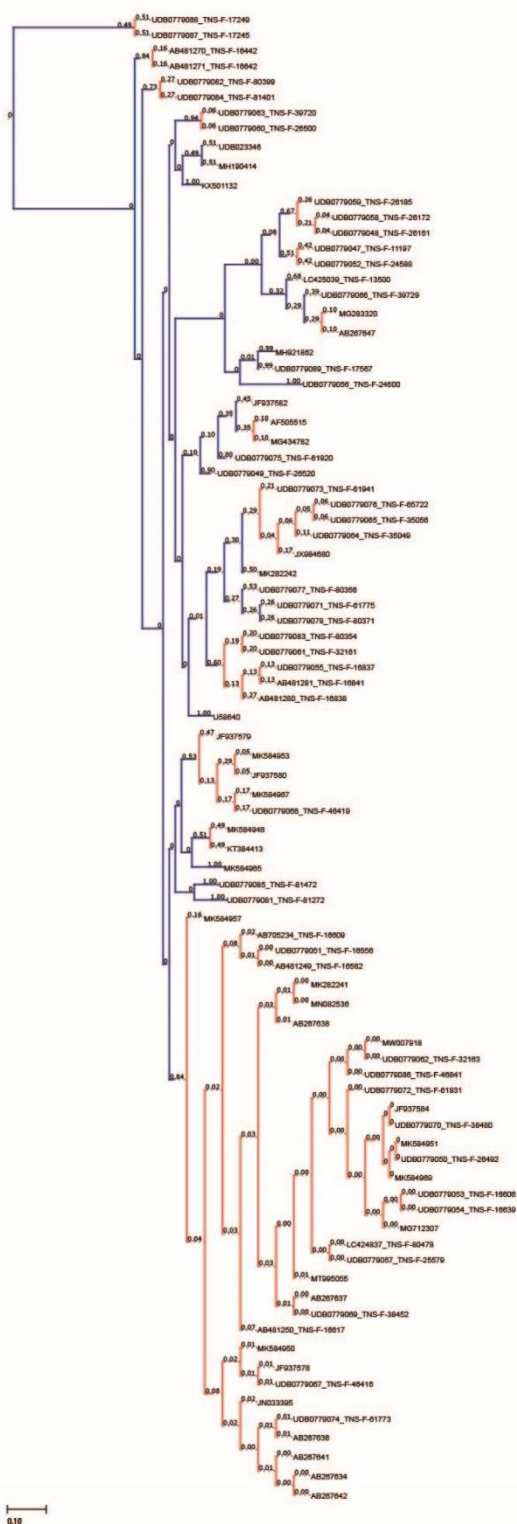


Appendix 4. Result of the ASAP species delimitation analysis. The graph shows the distribution of ASAP scores according to partitioning results, and the phylogenetic tree shows the way of partitioning.

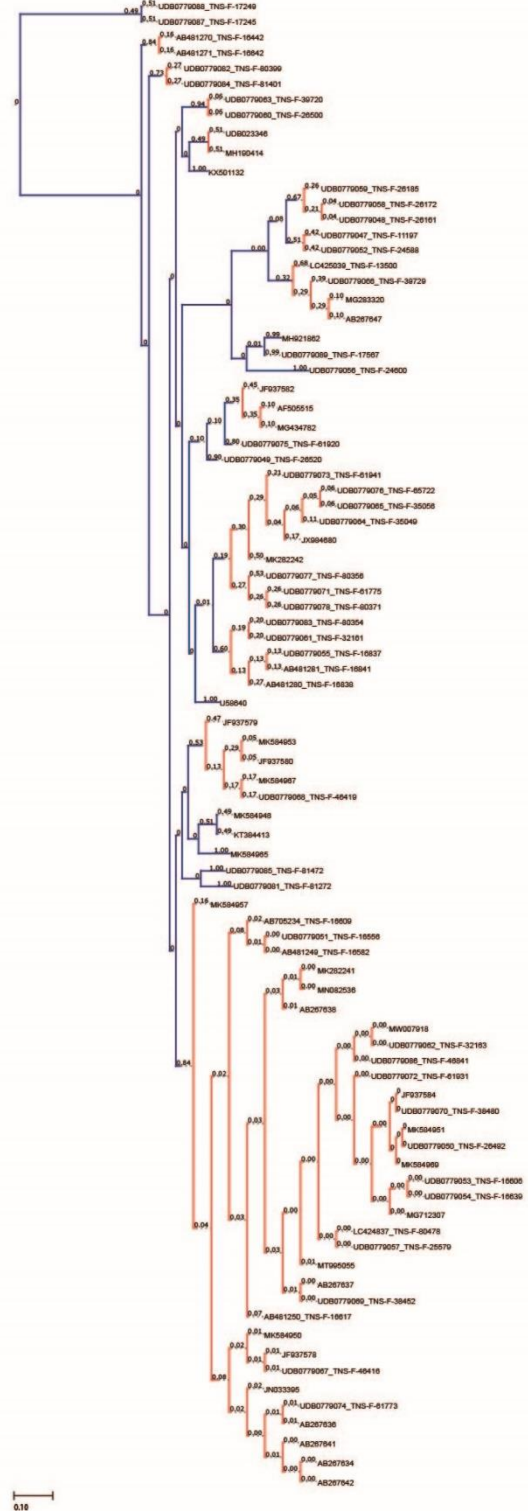


Appendix 5. Result of the GMYC species delimitation analysis. Number with each node shows the support value that each cluster is an independent species.

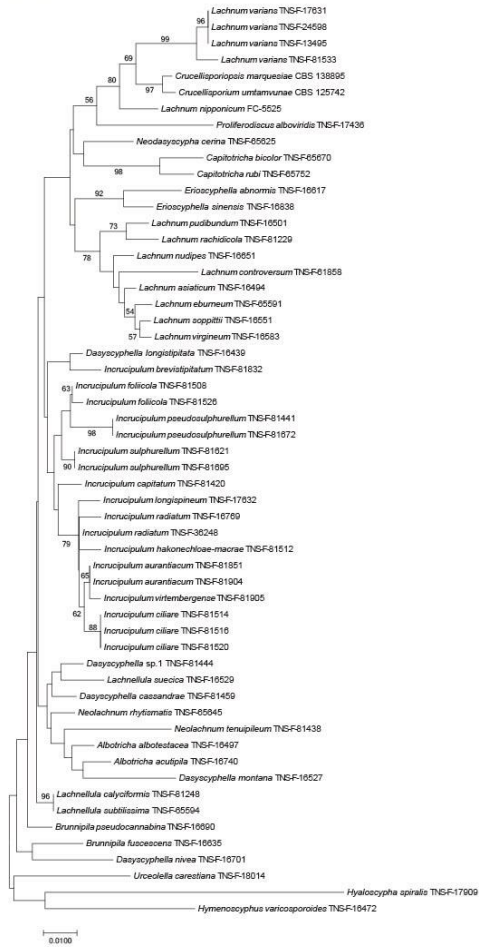
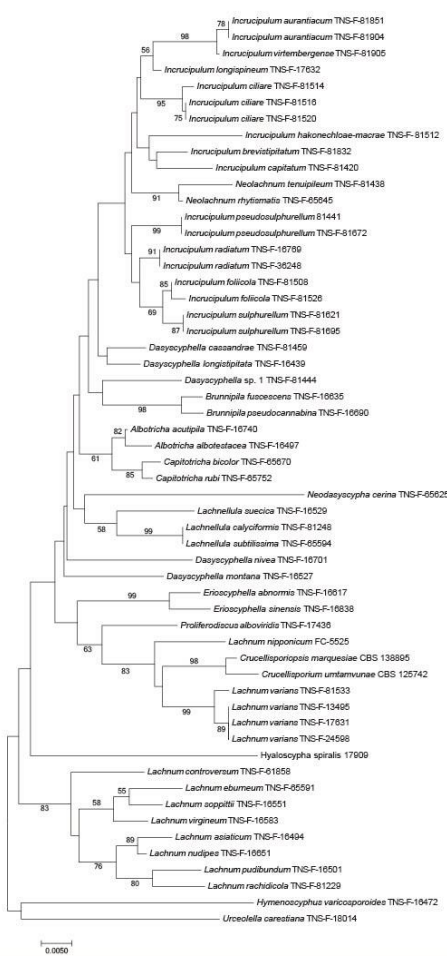
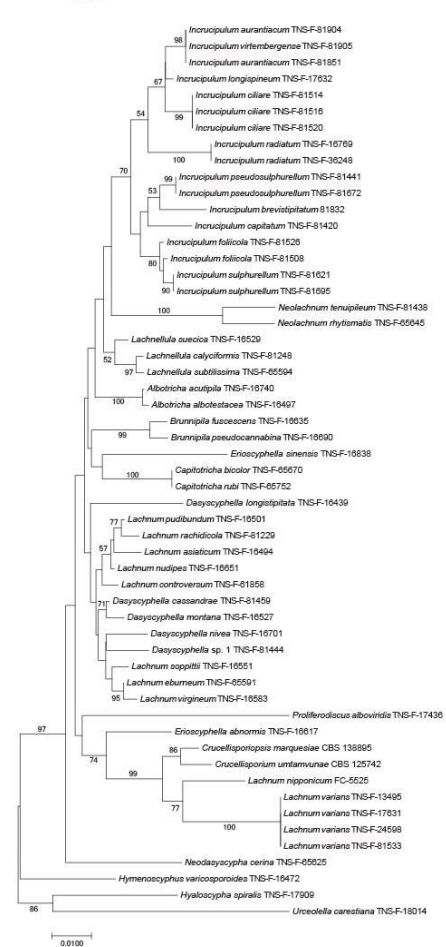
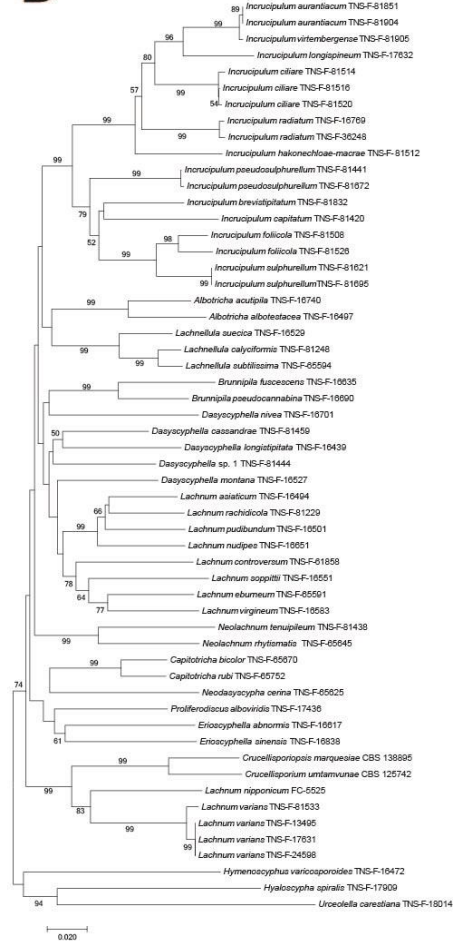
Bayesian support



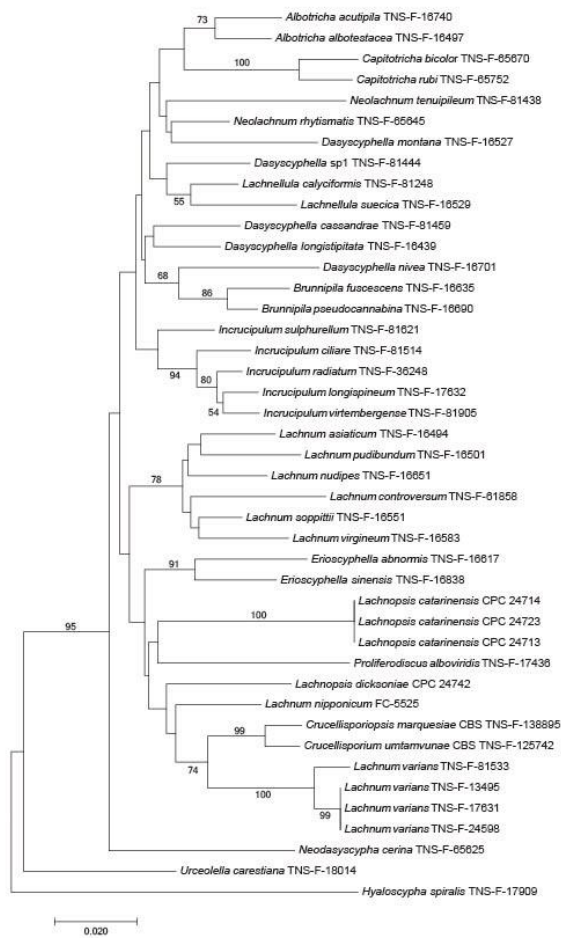
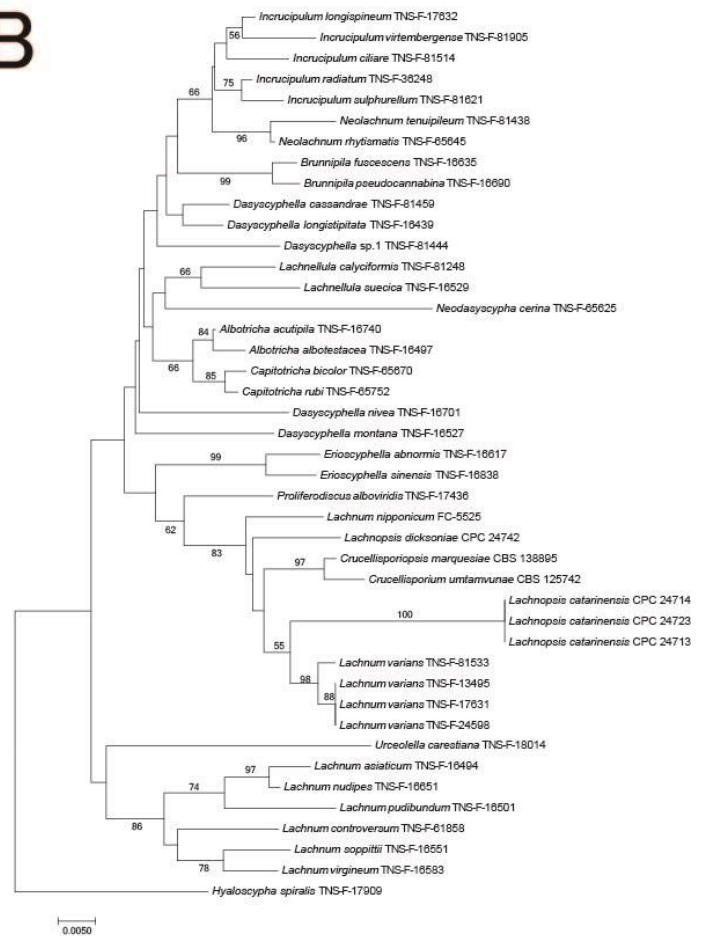
ML support



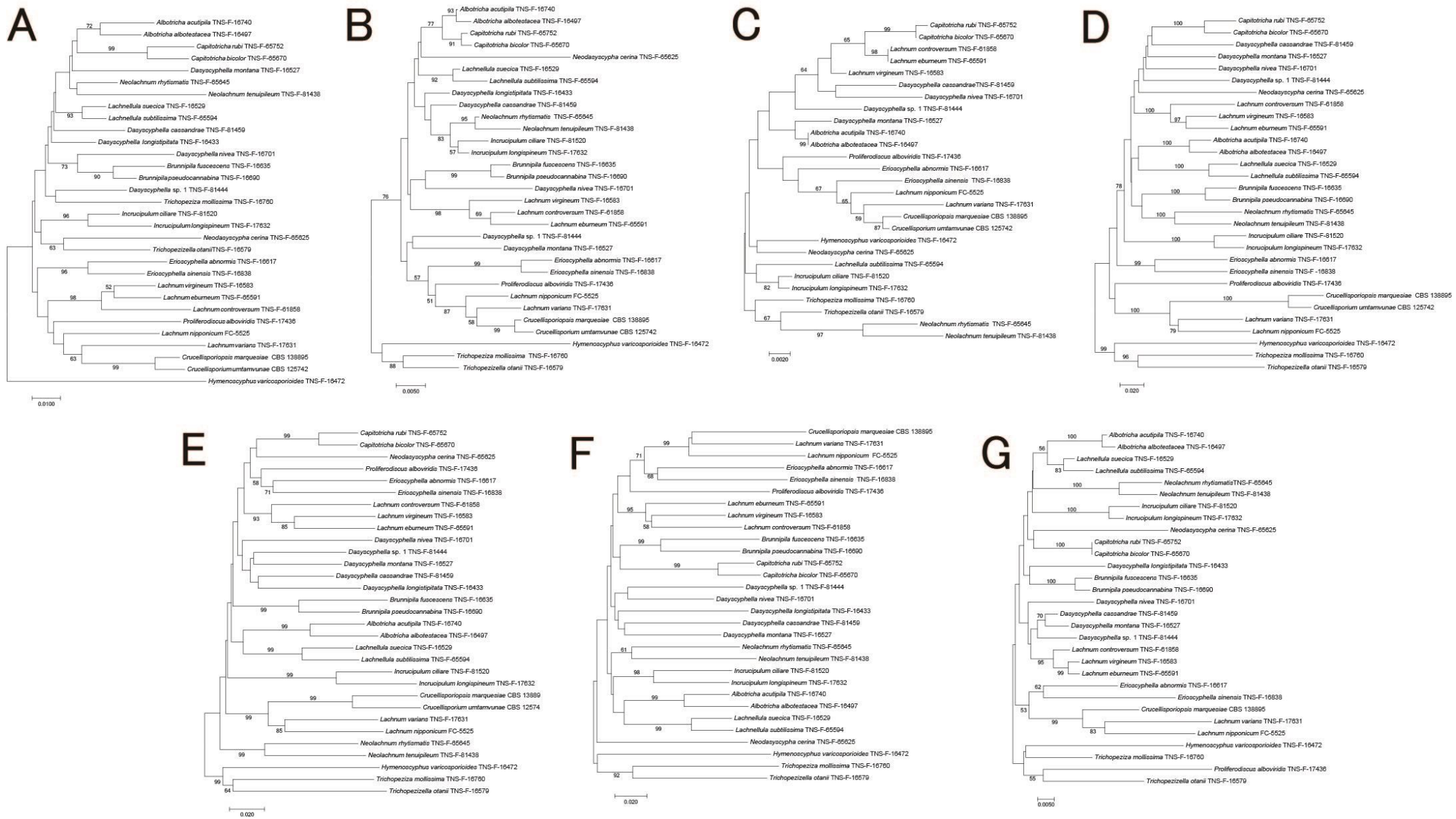
Appendix 6. Results of PTP species delimitation analyses. Number with each node shows the probability of the likelihood that each cluster is an independent species. Clusters showed by red branches are regarded as species.

A**B****C****D**

Appendix 7. ML trees based on ITS (A), LSU (B), mtSSU (C) and RPB2 (D) constructed using MEGA X. Bootstrap values > 50% are indicated on branches.

A**B**

Appendix 8. ML trees based on ITS (**A**) and LSU (**B**) constructed using MEGA X. Bootstrap values > 50% are indicated on branches.



Appendix 9. ML trees based on ITS (A), LSU (B), SSU (C), RPB1 (D), RPB2 (E), MCM7 (F) and mtSSU (G) constructed using MEGA X. Bootstrap values > 50% are indicated on branches.

