Taxonomic Study of *Lambertella* (Rutstroemiaceae, Helotiales) and Allied Substratal Stroma Forming Fungi from Japan

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Contents

Chapter 1 Introduction

1–1 The genus *Lambertella* in Rutstroemiaceae

The phylum Ascomycota is the largest group of Fungi and is characterized by the production of ascospores within asci as a result of sexual production (Kirk et al. 2008). The number of fungal species is estimated to be 1.5 million and about two-thirds of these species are thought to be Ascomycota (Hawksworth 1991, 2001). The Ascomycota and other fungi play essential roles in most land-based ecosystems as decomposers (Deacon 2006). However, some Ascomycota also interact with other organisms. Most species of Ascomycota are lichen-forming fungi, some are saprotrophs or parasites, and a few have mycorrhizal associations (Wang et al. 2006).

Helotiales is one of the largest non-lichen-forming ascomycetes, which include some saprotrophic and parasitic species with highly divergent characters in their morphology, ecology, and biology (Wang et al. 2006). Morphological characters such as the shape and color of the apothecia have been useful for characterizing the families of Helotiales (Dennis 1968; Korf 1973; Wang et al. 2006). However, classifications in Helotiales based on the apothecial morphology are not always consistent with cellular, ultrastructural or molecular characters (Verkley 1994; Wang et al. 2005, 2006), thus further studies are needed to determine the taxonomy of Helotiales.

The stroma is a complex hyphal structure that characterizes the three major families in Helotiales: Helotiaceae, Sclerotiniaceae, and Rutstroemiaceae. The primary function of the stroma is thought to be food storage (Whetzel 1945), but it also enables the survival of fungi in stressful environments. Based on morphology, two types of

stroma are recognized in Helotiales: **sclerotial stroma** and **substratal stroma** (Fig. 1.1). Sclerotial stroma is a determinate, tough structure that is often pellet-shaped, which comprises the medulla with a hyphal structure that is covered by a cortex of pigmented cells known as the rind. The sclerotial stroma is readily distinguishable from the substrate. The substratal stroma is an indeterminate structure, where a portion of the substrate is found in the medulla, which is usually surrounded by a black rind of differentiated cells. The substratal stroma is visible on the surfaces of dead leaves, petioles, branches, and fruits as black patches or crusts, or as irregular areas delimited by an irregular, thin, black line.

Initially, most of the apothecial fungi with inoperculate asci and without remarkable structures such as hair were placed in the family Helotiaceae (Höhnel 1918). Subsequently, the family Sclerotiniaceae was separated from Helotiaceae to include species with stroma, either substratal or sclerotial (Whetzel 1945). With the development of molecular methods, members with substratal or sclerotial stroma were shown to be a different group based on studies of western hybridizations and molecular phylogenetic analyses (Carbone and Kohn 1993; Holst-Jensen et al. 1997; Kohn and Grenville 1989a, b). According to phylogenetic analyses, the family Rutstroemiaceae was separated from Sclerotiniaceae to include members with substratal stroma, whereas species with sclerotial stroma remained in the family Sclerotiniaceae (Holst-Jensen et al. 1997).

Sclerotiniaceae comprises 284 species from 47 genera (Kirk et al. 2008), including many economically important plant pathogens, such as *Sclerotinia*, *Botryotinia*, and *Monilia* (Holst-Jensen et al. 1997; Whetzel 1945). By contrast, most of the species in Rutstroemiaceae are saprotrophs, rarely necrotrophic or biotrophic parasites. Most

members of Rutstroemiaceae form small and dark-colored apothecia, thus there has been little taxonomic research concerning Rutstroemiaceae. At present, only seven genera and 233 species have been reported in Rutstroemiaceae (Kirk et al. 2008). Compared with other families in Helotiales, the number of genera is remarkably low, thereby suggesting possibly inappropriate generic taxonomy.

Lambertella is one of the largest groups in the family Rutstroemiaceae, with 63 species (Kirk et al. 2008). It was erected by von Höhnel (1918) for the single species *Lambertella corni-maris* Höhn., and it remained monotypic for a long time until Whetzel (1943) first monographed *Lambertella* with eight species. Dumont (1971) monographed the genus with 29 species based on a large number of specimens and a detailed, clear definition of *Lambertella* was provided. Korf and Zhuang (1985) followed Dumont's definition of *Lambertella* and constructed a synoptic key of the 47 species known by that time. Since then, 16 new species have been added by various authors, but there has been no comprehensive taxonomic research subsequently after Dumont (1971).

Definition of *Lambertella* provided by Dumont (1971) was:

Lambertella Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 375 [47 repr.] (1918)

Type species: *Lambertella corni-maris* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 375 [47 repr.] (1918)

Apothecia 1-8 mm wide, stipitate to substipitate; receptacle flat, cupulate or rarely convex. **Stroma** substratal, delimited by blackened lines (rind) which are appearing both on the surface of the substrate and culture, rind pattern in face view of epidermoid to irregular cells. **Ectal excipulum**: outer covering layer present or absent; outer ectal

excipulum predominantly composed of brick-shaped cells forming a well-developed textura prismatica or more rarely with subglobose to globose cells; inner ectal excipulum absent or present as a well-developed textura porrecta, with pigmentation and roughenings present or absent; hairs when present originating from the prismatic cells of the outer ectal excipulum or from the out covering layer. **Medullary excipulum** composed of interwoven, branched, septate, thin-walled to thick-walled hyphae which formed textura intricate. **Asci** inoperculate, 8-spored or 4-spored, with the pore turning blue or not in Melzer's reagent. **Ascospores** normally unicellular, uniseriate or biseriate, biguttulate or multiguttulate, smooth or punctate, often with one side thicker and darker, with the guttules frequently masked at maturity; spore walls becoming brown either within the ascus or after discharge, germination varies greatly among species. **Paraphyses** generally numerous, unpigmented, equaling or slightly exceeding the length of asci.

The species of *Lambertella* predominantly occur on leaves and fruits but also less commonly on roots, twigs, herbaceous stems, and the fruit bodies of other fungi. Apothecia usually occur during rainy seasons in tropical regions and in the spring, late summer, and fall in temperate regions.

Ecologically, most of the *Lambertella* species are saprophytic, and play an essential role in most land-based ecosystems as decomposer along with other fungi (Deacon 2006). On the other hands, some *Lambertella* species are parasitic and important for agricultural science in controlling the diseases of plants. An antibiotic called "lambertellin" was isolated from cultures of *Lambertella hicoriae* and *L. corni-maris* (Sproston 1963). A U.S. patent for the purified lambertellin as a fungicide and bactericide has already been filed, which has been claimed to help control plant diseases

(Dumont 1971). Mycoparasitism with pathogens such as *Monilinia fructigena* (a widespread pathogen that causes brown rot on some stone or pome fruits, thereby resulting in considerable economic losses) is known in *Lambertella* and metabolites called lambertellols A, B, C, sucroneolambertellin, and norneolambertellin, which are responsible for the mycoparasitism, were extracted (Murakami et al. 2005a, 2005b, 2008).

1–2 Taxonomic problems of *Lambertella*

Three major problems are indicated in the taxonomy of *Lambertella*.

1) Both the substratal stroma and pigmentation of ascospores (particularly when the pigmentation occurs only after discharge from asci) are easily overlooked.

The rind (black line) is the major evidence of the presence of substratal stroma in the field (Dumont 1971; Whetzel 1945). However, in some cases the rind is not easily observed. Other possible morphological evidence of the presence of stroma may be black patches or blackened basal portions on the stalk, both of which may be easily overlooked or unclearly recognized in the host substrate. The rind surface of *Lambertella* comprises an epidermoid to irregular cells, but the morphology of the structure is difficult to recognize in the host substrate alone. Culture study is an efficient method for observing the substratal stroma (Korf and Zhuang 1985; Whetzel 1943), but few studies have been conducted.

The pigmentation of ascospores in *Lambertella* occurs before or after discharge from the asci. In some species, the ascospores are discharged still hyaline, and the pigmentation occur immediately or in several days after discharge (Dumont 1971).

Because the color of discharged ascospores can only be observed when fresh materials are available, and the timing of pigmentation differs from species to species and the maturation of the apothecia, the pigmentation may be overlooked by researchers if appropriate specimens are not examined.

If these characters are overlooked, members that should be included in *Lambertella* may be allocated to allied genera. Thus, culture studies should be conducted to observe the formation of the substratal stroma and fresh samples should be collected for the observation of discharged ascospores.

2) Overstress of the pigmented ascospores as a diagnostic character may have resulted in the overlapping generic concept.

Ascospore pigmentation is a remarkable feature, so it has been used as a distinguishing character to delimit *Lambertella.* This is the major reason *Lambertella* is the only genus with the pigmented ascospores in Rutstroemiaceae. In the current definition of *Lambertella*, the typical structure of the ectal excipulum is composed of textura prismatica without gelatinized matrix. However, greater attention to the pigmented ascospores means that a wide diversity of ectal excipular structure has been recognized in *Lambertella*.

For example, *Moellerodiscus advenulus* (W. Phillips) Dumont was transferred to the genus *Lambertella* because the ascospores were found to turn brown after discharge from asci (Hosoya and Otani 1997), but its structure in the ectal excipulum comprises textura globulosa to angularis, which is very different from textura prismatica. Moreover, the ascospore of *M. advenula* is equipped with gelatinous appendages, which may be unique in *Lambertella*. Although the pigmentation of ascospores is a clear

criterion when it is known, its taxonomic value during generic delimitation should be examined carefully.

The classification of rutstroemiaceous genera allied with *Lambertella* mainly depends on ectal excipular structures (this classification will be explained in Chapter 1–3), so the circumscription of *Lambertella* overlaps with the morphology of allied genera. To clarify the circumscription of *Lambertella* from allied genera, morphological observations should pay attention to the ectal excipular structure.

3) Polyphyly of *Lambertella* has been suggested.

The polyphyly of *Lambertella* was first indicated by phylogenetic analysis conducted by Holst-Jensen et al. (1997), because *Lambertella langei* T. Schumach. & Holøs was situated out of Rutstoroemiaceae or Sclerotiniaceae.

To resolve the confusions in the taxonomy of *Lambertella*, morphological examination, culture studies, and phylogenetic analyses should be conducted. Because *Lambertella* appears to overlap with allied genera, the related genera of *Lambertella* should be included in the study. The allied genera are separately explained as follows.

1–3 Allied genera of *Lambertella*

Most genera with substratal stroma are considered to be allied with *Lambertella*. There are currently seven genera in Rutstroemiaceae (Kirk et al. 2008). The genus *Scleromitrula* S. Imai is characterized by the stipitate-capitate apothecia in gross morphology (Schumacher and Holst-Jensen 1997). Because of this character, *Scleromitrula* is easily distinguished from other genera of Rutstroemiaceae. Five other genera of Rutstoremiaceae are reviewed in the following text.

In addition to the genera belonging to Rutstroemiaceae, *Hymenoscyphus* Gray is suggested to be related to *Lambertella*, although *Hymenoscyphus* belongs to the family Helotiaceae. *Hymenoscyphus* in its original sense is not considered to have substratal stroma, but both substratal stroma and brown ascospores have been observed in *Hymenoscyphus pseudoalbidus* (Kowalski and Holdenrieder 2009). Some *Lambertella* have a similar apothecial appearance to *Hymenoscyphus* and prismatic ectal excipular structures, so it will be necessary to focus on *Hymenoscyphus* to determine the generic delimitation of *Lambertella*.

1. *Rutstroemia* P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 12, 105 (1871)

Type species: *Rutstroemia bulgarioides* P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 105 (1871)

Rutstroemia was erected by Karsten (1871) to include 7 species, but the type species was not designated. Honey (1928) selected *R. bulgarioides,* the first species listed by Karsten, as the type species. On the other hand, Clements and Shear (1931) followed by Nannfeldt (1932), White (1941), and Whetzel (1945), listed *Rutstroemia firma* (Pers.) P. Karst. as the type, because they considered that *R. firma* represent a typical morphology of the genus. However, Dumont and Korf (1971) followed Honey (1928), selected *R. bulgarioides* as the lectotype, and transferred *R. firma* to *Poculum* because of the apothecial structure.

Although the above discussion is not settled, we follow the treatment of Dumont and Korf (1971) for the type of *Rutstroemia* because their decision is based on earlier treatment. As a result, *Rutstroemia* become monotypic (based on *R. bulgarioides*).

Rutstroemia bulgarioides usually occurs on the cones of *Picea* in late spring or

early summer in temperate areas. It is distinguished by its greenish to black apothecia and the complicated ectal excipular structure with angular to irregular cells, also having globose cells in gelatinous matrix in the ectal excipulum.

2. *Poculum* Velen., Monogr. Discom. Bohem. (Prague): 221 (1934)

Type species: *Poculum ruborum* Velen., Monogr. Discom. Bohem. (Prague): 222 (1934)

Poculum was erected by Velenovsky (1934) to include two species, *P. juncorum* Velen. and *P. ruborum* Velen., neither of which was designated as type. Dumont (1972) found no material remains on *P. juncorum*, and therefore selected *P. ruborum* as lectotype.

The ectal excipulum is usually composed of interwoven hyphae and immersed in a gelatinous matrix (Dumont 1972; Spooner 1987). The presence of gelatinized matrix in ectal excipulum distinctly distinguishes *Poculum* from other genera in Rutstoremiaceae.

3. *Lanzia* Sacc., Bot. Zbl. 18: 218 (1884)

Type species: *Lanzia flavo-rufa* (Sacc.) Sacc. Centralbl. 18: 218(1884)

The genus *Lanzia* was proposed by Saccardo (1884) for a single species *Helotium flavo-rufum* Sacc., to accommodate species having stipitate apothecia with blackened base. The genus was neglected for a long time until Dumont (1972) examined the type material of *Lanzia flavo-rufa* and adopted it as the generic name for the portion of *Rutstroemia sensu* White (1941) containing species with brick-shaped cells in the ectal excipulum.

Lanzia includes both lignicolous and foliicolous species, characterized by the prismatic cells in the ectal excipulum (Dumont 1972), and frequently with granulated,

hair-like hyphae projecting above the surface to form a downy or tomentose covering layer (Spooner 1987). According to Spooner (1987), the figure provided by Saccardo (1877) for the type *L. flavo-rufa* suggested the presence of incrustation. However, not all the *Lanzia* species currently reported have covering layer.

4. *Moellerodiscus* Henn., Hedwigia 41: 33 (1902)

Type species: *Moellerodiscus brockesiae* Henn., Hedwigia 41: 33 (1902)

The genus *Moellerodiscus* is shown to be an old name for *Ciboriopsis* Dennis, which was described for seven foliicolous species of inoperculate discomycetes with uniformly small asci and ascospores and an ectal excipulum composed of isodiametric rounded cells (Dennis 1962). *Ciboriopsis* has been generally accepted by most researchers (Kar and Pal 1970; Korf 1973; Spevak and Korf 1966). Dumont (1976a) examined the type species of *Moellerodiscus* (*M. brockesiae*), and regarded *Ciboriopsis* is synonymous with *Moellerodiscus*. The generic name of *Moellerodiscus* was therefore adopted.

The diagnostic character of *Moellerodiscus* is the presence of angular to globose cells in the ectal excipulum (Dennis 1962; Dumont 1976a).

5. *Dicephalospora* Spooner, Biblthca Mycol. 116: 267 (1987)

Type species: *Dicephalospora calochroa* (Syd.) Spooner, *Biblthca Mycol.* 116: 269 (1987)

The genus *Dicephalospora* was erected by Spooner (1987) for two species. The diagnostic character of *Dicephalospora* is the large ascospores capped at the poles with a mucilaginous collar (Spooner 1987). This character clearly distinguishes

Dicephalospora from other rutstroemiaceous genera. However, it is suggested to be closely related to *Lanzia* because of the prismatic cells in the ectal excipulum, and the thickened, refractive walls of these cells also suggest a relationship with *Poculum* (Spooner 1987).

6. *Hymenoscyphus* Gray, Nat. Arr. Brit. Pl. (London) 1: 673 (1821)

Type species: *Hymenoscyphus fructigenus* (Bull.) Gray 1821

The generic name *Hymenoscyphus* was erected by Gray (1821), but the diagnosis failed to distinguish *Hymenoscyphus* from related genera. Dennis (1964) accepted *Hymenoscyphus* as a valid name for species placed mainly in the old genus *Helotium* and selected *H. fructigenus* as the type species. Then the genus has been adopted by many researchers (Dumont and Carpenter 1982; Korf 1973; Lizoň 1992; Thind and Sharma 1980; Zhang and Zhuang 2004; Zheng and Zhuang 2012).

The genus *Hymenoscyphus* is characterized by light colored apothecia, textura prismatica, sometimes mixed with textura angularis cells in ectal excipulum, and fusoid, ellipsoid to scutuloid ascospores (Dennis 1956; Lizoň 1992; Sharma 1991).

Key to *Lambertella* and related genera based on the current definition (including the genus *Scleromitrula*)

1 Apothecia without substratal stroma, with prismatic ectal excipulum ……*Hymenoscyphus* (Helotiaceae)

1 Apothecia arising from substratal stroma……...2 (Rutstoremiaceae)

2 Apothecia with a cylindric and capitate head…….*Scleromitrula*

2 Apothecia discoid……3

3 Apothecia occur on cones of *Picea*……..*Rutstroemia*

3 Apothecia not occur on cones of *Picea*……4

4 Ascospore equipped with a mucilaginous appendages……*Dicephalospora*

4 Ascospore without appendages……. 5

5 Pigmented ascospore present, occurring before or discharge from asci……*Lambertella*

5 Pigmented ascospore absent……6

6 Ectal excipulum gelatinized……*Poculum*

6 Ectal excipulum without gelatinization……..7

7 Ectal excipulum composed of prismatic cells…….....*Lanzia*

7 Ectal excipulum composed of glubose to angular cells……..*Moellerodiscus*

1–4 Objectives of the present research

Based on the above background, the aims of the present study are to re-circumscribe the genus *Lambertella* and to clarify the phylogenetic relationships among *Lambertella* and other allied genera.

Morphological examinations, culture studies, and phylogenetic analyses of *Lambertella* and allied genera were conducted. Fresh samples are required to observe the pigmentation of ascospores, particularly after discharge. Thus, fresh samples were collected in Japanese areas, helping to elucidate the Japanese mycobiota, which can be utilized as potential biological resources. Previous studies of the Japanese mycobiota including *Lambertella* and other substratal stroma-forming fungi are sparse, and only

eight *Lambertella* species have been reported (Dumont 1971; Hosoya and Otani 1997; Hosoya et al. 1993; Korf 1958; Korf and Zhuang 1985; Terui et al. 1969; Zhao et al. 2013).

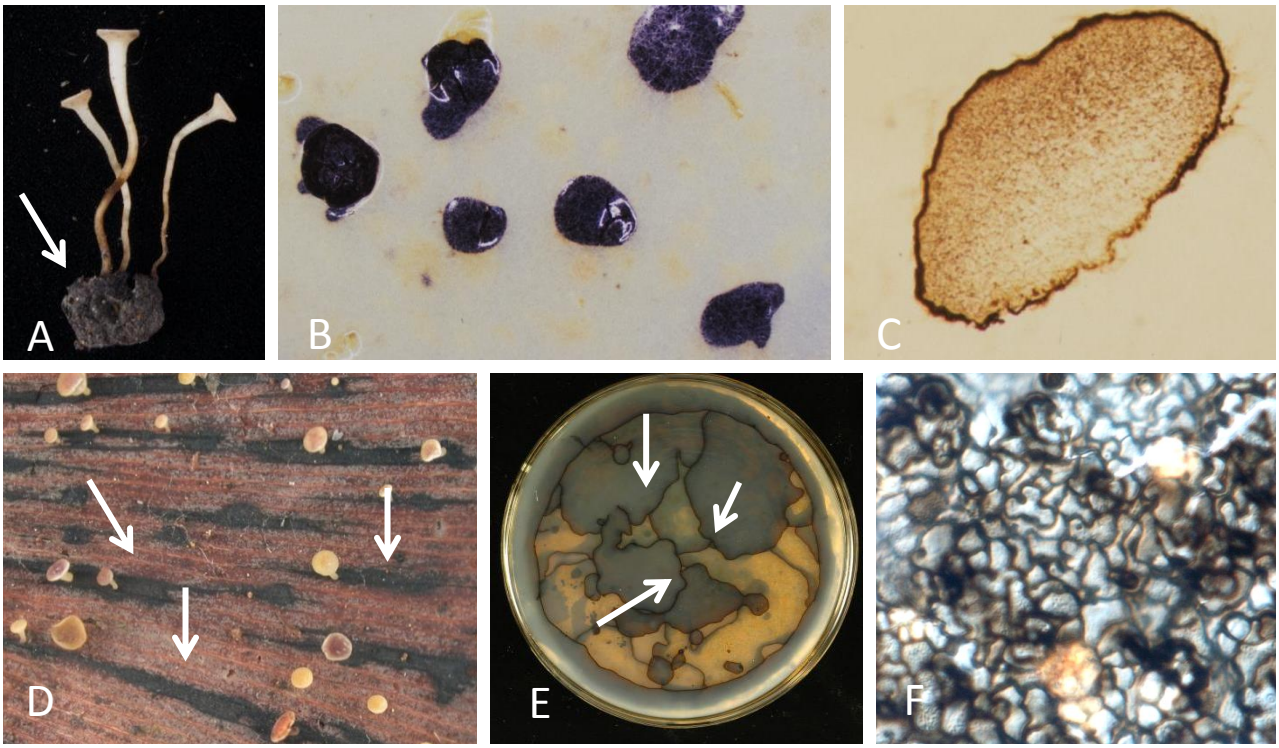


Fig. 1.1. Sclerotial stroma (A-C) and Substratal stroma (D-F). A. Sclerotial stroma in fresh specimen; B. Sclerotial stroma formed in PDA under culture; C. Hyphal structure in the medulla of sclerotial stroma, covered by a cortex of pigmented rind; D. Rind observed in the host substrate, indicating the substratal stroma; E. Rind formed in PDA culture; F. Surface view of the rind showing the epidermoid structure.

Fig. 1.2.1

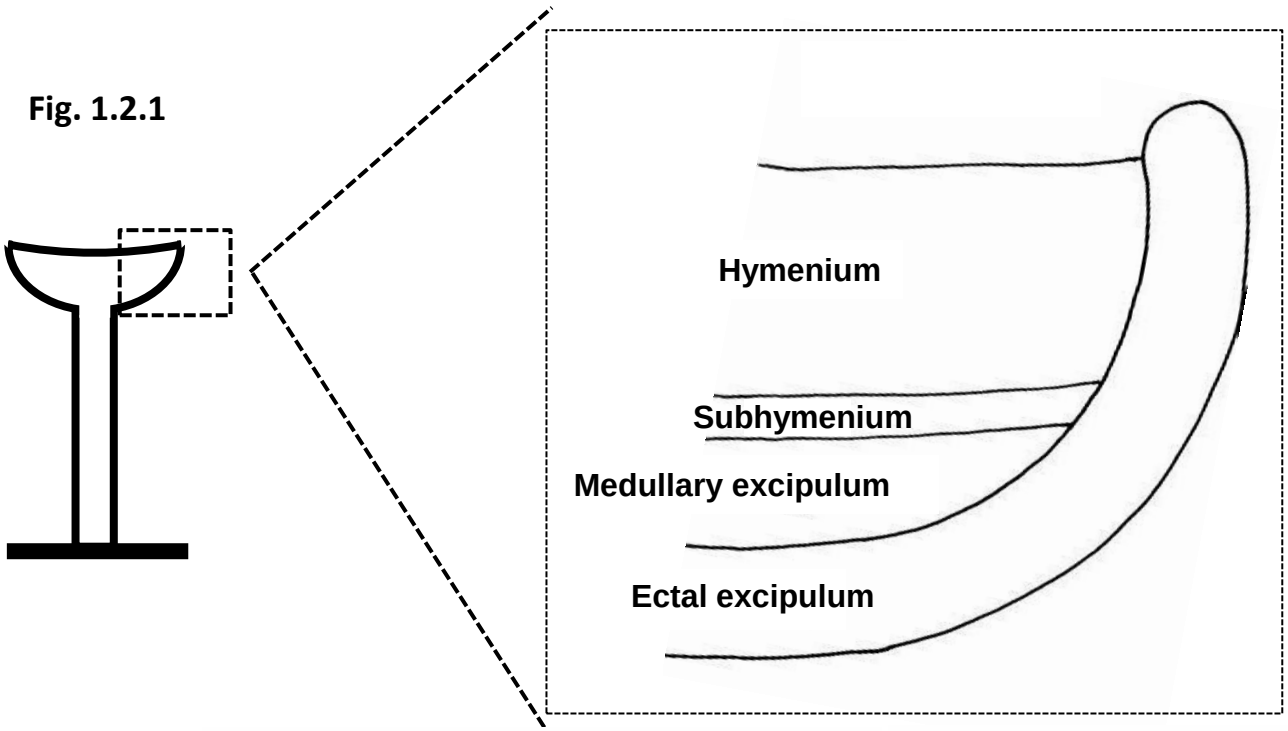


Fig. 1.2.2

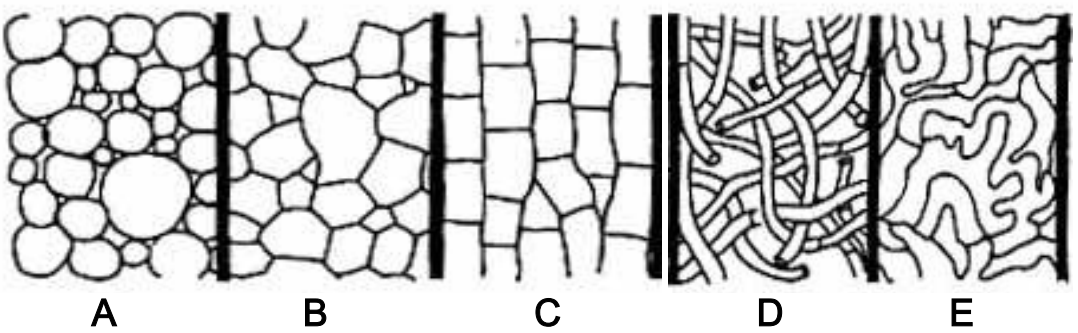


Fig. 1. 2. 1. Vertical median section of apothecium;

Fig. 1. 2. 2. Tissue types as viewed in section (Korf 1973). A. textura globulosa; B. textura angularis; C. textura prismatica; D. textura intricata; E. textura epidermoidea.

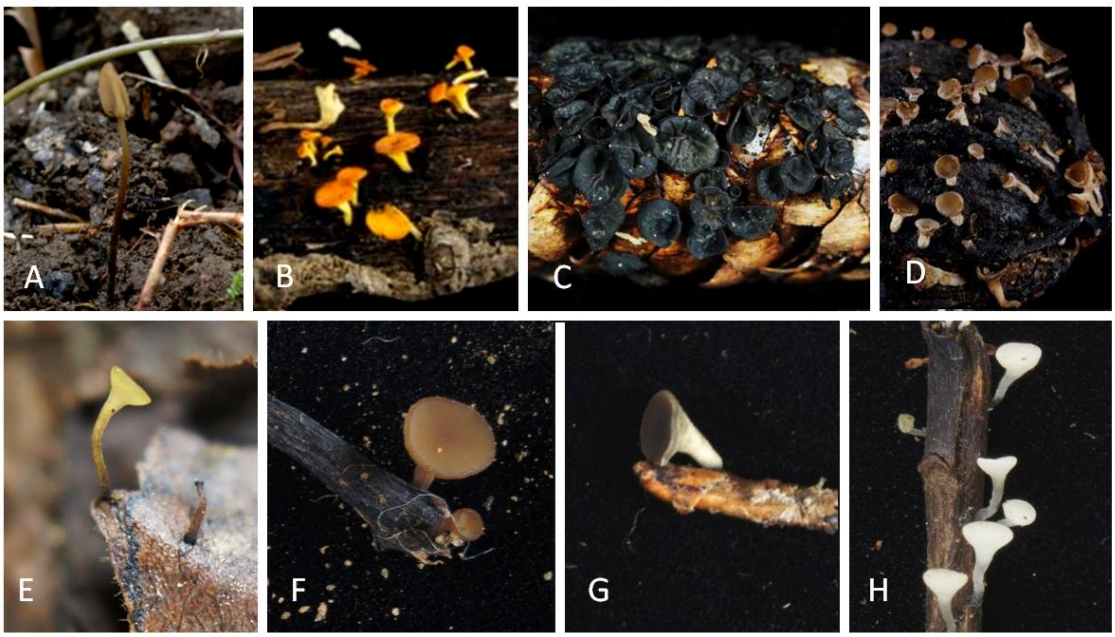


Fig. 1.3. Fresh apothecia. A. *Scleromitrula* sp.; B. *Dicephalospora rufocornea*; C. *Rutstroemia bulgarioides*; D. *Lambertella corni-maris*; E. *Lanzia* sp.; F. *Poculum sydowianum*. G. *Moellerodiscus pinicola*. H. *Hymenoscyphus microserotinus*.

Chapter 2 Materials and Methods

2–1 Collection and isolation

Fresh apothecia were collected from the decayed leaves, petioles, branches or herb stems. The apothecia attached to the substrate were brought to the laboratory in plastic bags. Single apothecium was removed from the substrate, affixed with a block of water agar to the inner surface of the lids of Petri dish, and left for 15–20 min for ascospores to be discharged onto the agar (PDA, Nissui, Tokyo, Japan) surface. After confirming the discharge of ascospores under ×10 objective lens, the apothecium was removed and dried as a voucher specimen. The plates with the discharged ascospores were kept at room temperature in the dark and ascospore morphology was observed under microscope after 12h, 24h, 32h or later until the spore germinated. In case spore germination was not recognized, the agar plate was kept up to 7 days.

To obtain culture, single ascospore isolation was carried out using Skerman's micromanipulator (Skerman 1968). The obtained culture was kept on potato dextrose agar (PDA) slants at 15℃.

The specimens used in the present study were deposited in the mycological herbarium of the National Museum of Nature and Science (TNS). Isolates were deposited in the National Institute of Technology and Evaluation, Biological Resource Center (NBRC) (Table 2.1).

2–2 Morphological examination

Both fresh and dried specimens were examined. The apothecium was embedded in

a drop of mucilage (Tissue Tek II; Miles laboratories, Inc., Naperville, Illinois, USA), and sliced at 15–25 μm thickness with a freezer microtome (FX-801, Japan). The sliced materials were mounted in plain lactic acid (LA) or Melzer's reagent (MLZ; 0.5 g of iodine, 1.5 g of KI, 20 g of chloral hydrate, 20 ml of distilled water) for observation. Color codes followed Pantone color code adopting CYMK color system (Anonymous, 2005). Line drawings were prepared using camera lucida for specimens mounted in LA or MLZ. The generic identification was based on the current definitions which have been reviewed in Chapter 1. The specific identification was based on the reported literatures.

2–3 Observation of cultural characteristics

To observe colony morphology and stroma formation, the single spore isolate was inoculated on PDA and revised Weizman and Silva Hutner's agar [revised WSH, 10 g oat flour (Nippon food manufacturer), 1 g $NaNO_3$, 1 g KH_2PO_4, 1 g $MgSO_4.7H_2O$, 20 g agar, 1000 ml distilled water], and all were incubated at 20°C for 3 months. After 1 month's and 3 months' incubation, the colony morphology was examined for the presence of stroma recognized by the formation of rind composed of black line or layer consisted by pigmented cells. The structure of rind was observed in vertical view and face view under the light microscope.

2–4 DNA extraction

Genomic DNA was extracted from the isolates or dried apothecia. Isolates were cultivated in 2 ml of 2% malt extract broth (20 g malt extract, 1000 ml distilled water)

for 2 weeks to obtain mycelium. Genomic DNA of isolates was obtained from about 50 mg of frozen mycelia using a DNeasy Plant Mini Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's instruction. Genomic DNA from apothecia was extracted using the modified cetyltrimethylammonium bromide (CTAB) extraction following glass milk purification methods as summarized by Hosaka (2009) and Hosaka & Castellano (2008). Briefly, samples were ground in liquid nitrogen using mortar and pestle, incubated in CTAB buffer (2% CTAB, 100 mM Tris pH 8.0, 20 mM EDTA, 1.4 M NaCl) at 65°C for 1 hour, and proteins were removed using the mixture of chloroform: isoamylalcohol (24: 1). The materials were further purified using 6M sodium iodine buffer (1M Tris pH 6.8, 2M Na_2SO_3) with glass milk, washed with ethanol/buffer solution (10 mM Tris pH 7.4, 1mM EDTA, 100 mM NaCl, 50% EtOH), and finally eluted in 100 µl Tris-EDTA buffer (TE, 10 mM Tris-HCl pH 8.0, 1 mM EDTA).

2–5 PCR and sequencing

The internal transcribed spacer regions (ITS-5.8S), the large subunit (28S) rDNA gene (LSU), and the second largest subunit of RNA polymerase II gene (RPB2) were sequenced using primer pairs ITS1F and ITS4 (Gardes and Bruns 1993; White et al. 1990), LROR and LR4 (Moncalvo et al. 1995; Vilgalys and Hester 1990), RPB2-P6F and RPB2-P7R (Hansen et al. 2005), respectively.

PCR reactions were performed using 10 µl reaction volumes each containing: 0.5 µl genomic DNA, 0.25 µl of each primer (10 µM), 0.05 µl (0.25 unit) of ExTaq DNA polymerase (TaKaRa, Tokyo, Japan), 5.0 µl deoxynucleotide triphosphate (dNTP)

mixture containing 2.5 mM each dNTP and ExTaq buffer containing 2 mM Mg_2^+ (adding 3.95 µl distilled water to get 10 µl reaction volumes). Amplifications were performed for ITS-5.8S and LSU region with preliminary denaturation at 94°C for 3 min, 35 amplification cycles (94°C for 1 min, 52°C for 30 s and 72°C for 1 min), followed by a final extension at 72°C for 7 min. For RPB2 gene, the annealing temperature was set at 54°C for 30s. PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. PCR products were then purified using an ExoSAP-IT purification kit (USB, Cleveland, OH, USA) following the manufacturer's instruction.

Sequencing was carried out using the Big Dye Terminator Cycle Sequencing Kit on the DNA auto sequencer 3130x (Applied Biosystems Inc., Norwalk, CT, USA), following the manufacturer's instructions. Sequences were assembled and edited by SeqMan (Lasergen v6 DNAstar), and congruent sequences obtained from both strands were saved. The sequences generated from this study were deposited in Sequence Database of the National Museum of Nature and Science (TNS) (Table 2.3).

2–6 Phylogenetic analyses

The obtained DNA sequences were aligned by Clustal W using the default parameters (Thompson et al. 1994) and edited manually when necessary using BioEdit ver. 7.0.5.2 (Hall 1999). Ambiguous regions having gaps were excluded from the analyses by checking the alignment in BioEdit. The obtained alignments of three individual datasets (ITS-5.8S, LSU, RPB2) were analyzed separately by Maximum parsimony (MP) method initially. For the ITS dataset analysis 23 sequences obtained

from GenBank were incorporated.

The individual dataset of LSU, RPB2 and combined dataset of LSU and RPB2 were also analyzed by maximum likelihood (ML) methods. The combination of MP and ML has been suggested as a supportive method when constructing a phylogenetic tree (Kolaczkowski and Thornton 2004). All the datasets were rooted with *Lachnum abnorme* and *Lachnum virgineum* (Hyaloscyphaceae) as outgroups.

The MP analyses were performed using PAUP version 4.0b10 (Swofford 2002). Heuristic searches were conducted with tree bisection–reconnection branch swapping algorithm (TBR), random sequence additions, and with Multrees option on. Bootstrap values (BP) of the most parsimonious trees were obtained from 1000 replications.

The ML analyses were conducted using GARLI version 0.951 (Zwickl 2006). The analyses were conducted using the GTR+G+I model (six general time reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariable sites), with model parameters estimated over the duration of specified runs. The tree topology with the highest likelihood was inferred from 10 independent runs from random starting trees. The "stopgen" parameters were set to 50,000,000 and other parameters were set to default values. Bootstrap analyses were done using 1000 replications with the same parameters as the initial tree search.

Chapter 3 Results and discussion

3–1 Identification of the collected materials

The collected specimens were identified to 44 taxa (Table 3.1), including 6 taxa belonging to Sclerotiniaceae, 22 taxa to Rutstroemiaceae, and 16 taxa to Helotiaceae.

3–2 Morphological characters of collected materials

Among the 44 taxa, substratal stroma was recognized by the presence of rind for 15 taxa (13 in Rutstroemiaceae, 2 in Helotiaceae), and the presence of substratal stroma was suggested by the blackened basal part of the stalk or the blackened areas of the substrate for other 10 taxa (9 in Rutstroemiaceae, 1 in Helotiaceae). Two *Hymenoscyphus* species (*H. ginkgonis* and *H. pseudoalbidus)* was included in the group with clear substratal stroma while the other group included *H. microserotinus*, all classified in Helotiaceae. Among these 25 species, 19 species (16 in Rutstroemiaceae, 3 in Helotiaceae) were successfully cultured and will be examined for the formation of stroma under culture (Table 3.2).

Among the 25 substratal stroma forming taxa, 2 were confirmed to have pigmented ascospores only before discharge, and 9 had the pigmented ascospores only after discharge. So, in total 11 taxa were found to have distinguishing character of *Lambertella*. (Names in bold, Table 3.2).

In these 11 species, the structure of ectal excipulum was found to be diverse, including textura prismatica, t. angularis, t. globulosa with or without gelatinous matrix,

and they were classified to *Lanzia*, *Moellerodiscus*, *Poculum* and *Lambertella* if the present generic concept based on the ectal expular structure was adopted (see Appendix). Thus, heterogeneity of *Lambertella* when solely based on the two distinguishing character was demonstrated (Table 3.2).

3–3 Substratal stroma formation in culture

Among the 19 species successfully cultured, clear rind was observed in10 species in nature on the host. For the remaining 9 species, rind was unclear or not observed in nature. The result of culture study showed that 18 species (9 with clear rind in nature, 9 without clear rind in nature) were successfully observed for the formation of substratal stroma recognized by the presence of rind. Stroma was not observed in one species (*Poculum sydowianum*) even in the prolonged cultivation up to six months, although the clear rind has been observed in nature on the host. Culture study was confirmed to be an efficient experimental method, to supplementary demonstrate the presence of substratal stroma in most cases.

Two types of substratal stroma formation were recognized in culture: one with the clear rind (10 species) through the whole culture (Table 3.2) and the other with less clear, having blackened area in the vertical section in culture (termed as diffused rind, 8 species, Table 3.2). In the species with the clear rind, t. globulosa, t. epidermoidea or t. angularis tissue were observed in rind surface (see Figs. 5.18, 5.50, 5.53, 5.56, 5.63, 5.66, 5.71, 5.74, 5.92, 5.101). In the species with diffused rind, no remarkable surface structure was observed except for the mass of blackened hyphae (see Figs. 5.3, 5.12, 5.21, 5.77, 5.84, 5.89, 5.95, 5.107). Such a diffused rind in culture has been reported in

Moellerodiscus tenuistipes (J. Schröt.) Dumont (as *Ciboriopsis bramley*i Dennis, Spevak and Korf 1966).

In the current definition, *Hymenoscyphus* has no stroma. However, substratal stroma was recognized for three *Hymenoscyphus* species (*H. pseudoalbidus*, *H. ginkgonis* and *H. microserotinus*). Therefore it seemed inappropriate to accommodate these species in *Hymenoscyphus*. To draw a decisive conclusion, phylogenetic analysis was required and the discussion concerning these three species will be provided in Chapter 3–2.

In the current definition of *Lambertella*, the epidermoid structure in rind surface was descripted as one of morphological characters (Dumont 1971; Korf and Zhuang 1985; Whetzel 1943). However, variations such as t. globulosa or t. angularis were observed in the present study. And the structure in rind surface also presented variations in genus *Lanzia*, *Poculum* and *Moellerodiscus*. From all these observation, the structure of rind surface was found to be less related with generic taxonomy.

3–4 Phylogenetic analysis

3–4–1 Phylogenetic analysis inferred from ITS-5.8S dataset

Because ITS-5.8S region was the most widely available for *Lambertella* and allied genera in public database (GenBank http://www.ncbi.nlm.nih.gov/ or AFTOL http://aftol.org/data.php), the phylogenetic analysis was conducted using ITS-5.8S to check the conspecificity of species. In total 101 ingroup operational taxonomic units (OTUs) were incorporated to the phylogenetic analysis, including 61 OTUs (44 taxa) of material collected for the present study, 17 OTUs (15 taxa) from culture collection

(Table 3.3) and 23 OTUs (21 taxa) from GenBank (Table 3.4). 78 ITS sequences were newly generated from present research.

The aligned ITS-5.8S rDNA dataset comprised of 507 bp, and 110 ambiguously aligned sites (site nos. 16–17, 22–191, 200, 209–211, 354, 383–389, 408, 419–440, 470, 491–492) were excluded from the analyses. Six equally parsimonious trees were obtained [tree length (TL): 496 steps, consistency index (CI): 0.3206, retention index (RI): 0.8044, rescaled consistency index (RC): 0.2579], one is shown in Fig. 3.1.

Morphologically identical species were grouped in one clade. The collected materials identified based on morphology was in good concordance with taxa from culture collection or GenBank, and the identification at the specific level was highly supported. However, neither the generic relationship nor support at the generic rank was well resolved or supported. Nevertheless, one clade with the sclerotial stroma was supported with 82% bootstrap values, suggesting Sclerotiniaceae is a phylogenetically supported group. Sequences of three taxa labeled as *Lanzia cuniculi*, "*Rutstroemia paludosa*", and *Poculum calopum* were shown to be identical and included into one clade with 80% bootstrap values, the close relationship or the taxonomic confusion of these three species was suggested. However, because they all were obtained from culture collection and the specimens are not available, I was unable to get the concrete conclusion. *Lambertella corni-mari*s, *Lambertella pruni* and *L. hicoriae* were included into one clade but with the >50% bootstrap values, suggesting the close relationship of these three species.

Although ITS-5.8S is currently used as a barcoding region for fungi (Schoch et al. 2012), it is also known that higher phylogenetic resolution is not obtained solely based on ITS-5.8S region due to the high evolutionary rate at this region. Further analysis

based on region with lower evolutionary rate was required to infer higher rank taxonomy.

3–4–2 Phylogenetic analysis inferred from individual dataset of LSU, RPB2, and the combined dataset of LSU and RPB2

For the conspecific OUTs proved by phylogenetic analysis inferred from ITS-5.8S region, only one OUT was selected for each taxon to conduct further analyses using the dataset of LSU, RPB2 and LSU+RPB2.

Totally 58 taxa were included in the LSU rDNA dataset, 45 taxa from Japan (including 2 outgroup) and 13 taxa from culture collection. The aligned LSU rDNA dataset comprised of 826 characters, and 5 ambiguously aligned sites (site nos. 54, 157, 309, 389, 733) were excluded from the analyses. RPB2 dataset included 55 taxa. The aligned RPB2 dataset comprised 545 characters, and 13 ambiguously aligned sites (site nos. 87, 291, 310–313, 343, 389, 486, 525, 535–536, 540) were excluded from the analyses.

The combined dataset of LSU and RPB2 included 58 taxa, including 4 taxa without RPB2 sequences treated as missing data (*Lanzia* sp.4, *Lanzia* sp.5, *Lanzia* sp.6, and *H. ginkgonis*). The dataset included 36 species with substratal stroma, 7 species with sclerotial stroma and 15 species without stroma (including 2 outgroups). The aligned dataset of LSU and RPB2 comprised 1371 characters, and 15 ambiguously aligned sites (site nos. 54, 157, 309, 389, 733, 913, 1117, 1139, 1169, 1215, 1312, 1351, 1361, 1362, 1366) were excluded from the analyses.

Although the branching orders and bootstrap confidence differed from each other in the above 3 dataset, the clades supported at >50% BP generated by LSU+RPB2 datasets had no conflict with those in LSU and RPB2 (Table 3.5, Fig. 3.2, Fig. 3.3, Fig. 3.4).

Because the number of highly supported clades in LSU+RPB2 exceeded that in the other two datasets (Table 3.5), the following discussion is mainly based on this dataset.

Two large monophyletic clades were recognized (Clades A and B). Clade A included all the species belong to Sclerotiniaceae and most of the rutstroemiaceous species. Clade B (not well supported in RPB2) included most of *Hymenoscyphus* species and two rutstroemiaceous members (*L. yunnanensis* and *D. rufocornea*). *Lambertella viburni* was situated next to Clade A in LSU, but it was excluded from neither Clade A nor B in the phylogenetic tree based on LSU+RPB2, suggesting the less closer phylogenetic relationship. Further analysis for this fungus was impossible in the current study because it was obtained only as an isolate and no specimens are available for morphological examination.

Polyphyly of Rutstroemiaceae

Because two rutstroemiaceous members (*L. yunnanensis* and *D. rufocornea*) were included into Clade B, and *L. viburni* was excluded from Clade A and B, the polyphyly of Rustroemiaceae in current definition was suggested. The formation of substratal stroma was also observed in Helotiaceae, which suggested that the substratal stroma may have evolved multiple times.

The type genus of Rutstroemiaceae, *Rutstroemia*, was included in the present research. However, the core clade of *Rutstroemia* (Clade f) which included the type (*R. bulgarioides*) has no other closely related clades or species except for *M. pinicola*. If Rutstroemiaceae is defined by this clade, it will narrowly circumscribe the family, and an enormous taxonomic confusion around other genera will be brought up. In addition, to re-circumscribe the family, more members of Rutstroemiaceae should be included. Moreover, it is beyond the scope of the present study. Therefore, I hesitate to

re-circumscribe the family, leaving further analysis for future studies at this moment.

Polyphyly of Lambertella and allied genera

Species with substratal stroma and pigmented ascospores mainly distributed in the subclades a, c, e, and g, and four lineages each with only one species (*Lambertella* sp.1, *Lanzia longipes*, *L. advenula* and *L. viburni*). The polyphyly of *Lambertella* was confirmed again.

The species of *Lanzia*, *Moellerodiscus*, *Poculum* and *Hymenoscyphus*, are separated into different clades and the polyphyly was indicated. Because the present analyses failed to include the type species of *Lanzia*, *Moellerodiscus* and *Poculum*, further taxonomic work is needed and the current definitions of rutstroemiaceous genera need to be reconsidered.

The stroma forming species were mainly distributed in 7 subclades (Clades a-g), they will be discussed in the following text.

Clade a

Clade a included several genera, *Poculum* (3 spp.), *Lanzia* (4 spp.), *Moellerodiscus* (2 spp.), along with one species identified as *Lambertella* sp. 2. In this clade, no common morphological characters were observed except for the substratal stroma. None of the genera included in this clade formed the highly supported monophyletic clade. In the analysis generated from RPB2 dataset, *Lambertella* sp. 2 was excluded from clade a (Fig. 3.3), suggesting the less close relationship of *Lambertella* sp. 2 to other members in clade a. Because *Lambertella* sp. 2 had completely different morphological characters compared with other rutstroemiaceous genera in current definition, a new genus should be proposed.

Clade b

Clade b included all the sclerotial stroma forming species with a strong support, the monophyly of Sclerotiniaceae was confirmed. The ITS analysis also provided the same result (Fig. 3.1). Holst-Jensen (1997) included 20 species with sclerotial stroma and all of them were included into one highly supported clade and the monophyly of Sclerotiniaceae has been suggested. The close relationship of Sclerotiniaceae and Rutstroemiaceae was also confirmed in the present research.

Clade c

Clade c included five *Lanzia* species and *Lambertella subrenispora* Korf & W.Y. Zhuang, being well supported in LSU+RPB2 tree using the MP method, not well supported in ML method and in RPB2 tree. With the morphological examination on *Lanzia* sp. 1, *Lanzia* sp. 2, *Lanzia* sp. 3 and *Lanzia* sp. 4, and the data from the literatures of *Lanzia allantospora* (Dennis) Spooner (Spooner 1987) and *L. subrenispora* (Korf and Zhuang 1985), all the species in this clade have three layers in ectal excipulum, the outer coving layer mainly brownish and granulated, the medium layer smooth and brownish, the inner layer brownish and granulate. Spooner (1987) indicated *Lanzia* species including the type *Lanzia flavo-rufa* are frequently with a granulated covering layer. It is suggested that the type *Lanzia flavo-rufa* may be included in this clade if analyzed. However, *Lanzia flavo-rufa* was not included in the present phylogenetic analyses, and not all the reported *Lanzia* species have covering layer based on the previous descriptions (Dumont 1976b; Sharma and Sharda1985), it would be too early to consider it as “core” group of *Lanzia*.

Clade c also included *Lambertella subrenispora*, but I was not able to examine the specimen. However, *L. subrenispora* should be excluded from *Lambertella* because it was separated from the type of *Lambertella* as described in the Clade e.

Clade d

Clade d included three species from culture collection. They were currently identified as three different species in different genera with different morphological characters (Dumont 1975; Groves and Elliott1961; Sharma 1986), but in phylogenetic analysis they always grouped into one highly supported clade and with the identical sequences in ITS and LSU region, having slight differences in RPB2. Because I was unable to examine the herbarium specimens of these species, it is not appropriate to get the concrete conclusion but close phylogenetic relationship for these species are suggested.

Clade e

Clade e included 5 *Lambertella* species (*L. corni-maris, L. hicoriae, L. himalayaensis, L. pyrolae, and L. pruni*) including the type (*L. corni-maris*) with strongly support. Besides two species collected from Japan (*L. corni-maris* and *L. pyrolae*), I examined the type specimens of *L. hicoriae* [CUP 27881, treated as one of synonymous species of *L. corni-maris* by Dumont (1971)].Taking the descriptions (Dumont 1971) of *L. himalayaensis*, *L. pruni* into consideration, the common characters for this clade were found: the combination of pigmented ascospores before discharge and a brownish ectal excipulum of t. prismatica (Figs. 5.51–5.56) (Dumont 1971; Zhao et al. 2013; Table 3.2). Because the combination of these morphological characters is unique when compared with other rutstroemiaceous members, the combination defines the "core" group of *Lambertella*, or *Lambertella sensu stricto* (emended definition given in Chapter 3–4).

Clade f

Clade f included the type species of *Rutstroemia* and *Moellerodiscus pinicola*,

being well supported in LSU+RPB2 tree and moderately supported in LSU and RPB2 tree. Both of them occur on conifers. Morphologically, they have the dark-brownish and irregular-shaped cells in ectal excipulum arranged almost vertical to the surface (Figs. 5.87–5.89, 5.105–5.107). I therefore transfer *Moellerodiscus pinicola* as a new combination of *Rutstroemia*.

Clade g

Clade g was well supported in RPB2 tree, and moderately supported in LSU+RPB2 tree, not support in LSU tree. Clade g included the type species of *Hymenoscyphus* (*H. fructigenus*) along with several *Hymenoscyphus* species and *L. yunnanensis*. Although redefinition of *Hymenoscyphus* may be beyond the scope of present study, this clade may be regarded as *Hymenoscyphus s. s.*

In this clade *Lambertella yunnanensis* is included, and I confirmed the pigmentation of ascospores occur after discharge. Combination of the bright colored apothecia, thick-walled ectal excipulum, larger ascospores and negative reaction in MLZ, distinguishes *L. yunnanensis* from other species of *Lambertella*, but, these characters are obviously different from *Lambertella* species. Because the phylogenetic analysis clearly showed the relationship with *Hymenoscyphus*, it should be transferred to *Hymenoscyphus* from *Lambertella*.

Two *Hymenoscyphus* species (*H. pseudoalbidus* and *H. ginkgonis*) have the substratal stroma and pigmented ascospores after discharge (Han and Shin 2008; Hosoya, personal observation; Zhao et al. 2012). Because pigmented ascospores after discharge was excluded from definition of *Lambertella*, these two species should not be disposed to *Lambertella*. As they have some common morphological characters with other *Hymenoscyphus* spp. such as the light colored apothecia, narrow prismatic cells in

ectal excipulum (Han and Shin 2008; Kowalski and Holdenrieder 2009), they should be retained in *Hymenoscyphus*.

Hymenoscyphus microserotinus was found to have a subtratal stroma from culture study (Fig. 5.18). It was firstly reported as *Lanzia* species because the black base of the stipe, considered to substratal stroma (Zhuang 1996). Later it was transferred to *Hymenoscyphus* based on phylogenetic analysis (Zhuang and Liu 2007). The present phylogenetic analyses confirmed the treatment of Zhuang and Liu (2007), but the substratal stroma formation was observed in culture. Ignoring the stroma, the apothecial characters of *H. microserotinus* are more related with other *Hymenoscyphus* species (Zhuang 1996). Thus we retained it in *Hymenoscyphus* at present.

3–5 Taxonomy

3–5–1 Delimitation of *Lambertella sensus stricto*

Based on the discussion provided previously (in Chapter 3–4), *Lambertella* is now limited based on the Clade e, and re-circumscribed as follows:

***Lambertella sensus stricto* Höhn. emend. Y.-J. Zhao**

Type species – *Lambertella corni-maris* Höhn.

Apothecia stipitate to substipitate; receptacle flat, cupulate or rarely convex. **Stroma** substratal. **Ectal excipulum**: outer covering layer absent or present; outer ectal excipulum composed of brick-shaped cells forming a well-developed textura prismatica, brownish; inner ectal excipulum absent or obscure; hairs absent or present. **Medullary excipulum** composed of interwoven, branched, septate, thin-walled to thick-walled

hyphae which formed textura intricata. **Asci** inoperculate, 8-spored or 4-spored, with the pore turning blue or not in Melzer's Reagent. **Ascospores** normally unicellular, uniseriate or biseriate, biguttulate or multiguttulate, smooth or punctate; spore walls becoming brown within the ascus. **Paraphyses** generally numerous, unpigmented, equaling or slightly exceeding the length of asci.

Notes – In the delimitation of *Lambertella s. s.* "epidermoid cells in the surface of rind" was not included because the rind was not examined in the *L. hicoriae* and *L. pruni*. Further studies are need about the structure of substratal stroma.

The structure in ectal excipulum was provided more in detail compared with the previous definition, such as the brownish cells in outer ectal excipulum. The most important character of *Lambertella s. s.* is the pigmented ascospores only occurring before discharge.

3–5–2 *Crassitunica* Y.-J. Zhao & T. Hosoya

Species with pigmented ascospores after discharge were proved to be polyphyletic, and some of them were observed to have distinct morphological characters. *Lambertella* sp. 2 was included into Clade a which also included species identified as *Moellerodiscus*, *Poculum* and *Lanzia*, but no common morphological characters were recognized. Because *Lambertella* sp. 2 had completely different morphological characters compared with other rutstroemiaceous genera in current definition, a new genus is proposed.

***Crassitunica tsubakii* Y.-J. Zhao & Hosoya gen. et sp. nov. Figs. 5.61–5.63**

Holotype – On decaying leaves of *Aucuba japonica* Thunb.: TNS-F-44021 (Culture

FC-2834), Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo, (35º40'25.56"N, 139º42'2.79"E), 18 Nov. 2011, Col. T. Hosoya.

Etymology – The specific epithet refers to the name of a Japanese researcher who firstly found this species.

Stroma substratal, visible as blackened zones on leaf veins and petioles of host. **Apothecia** superficial, solitary or gregarious, short-stipitate, occurring mainly on leaves, sometimes on petioles and branches of the host; disc flat to convex, 0.5–2.5 mm in diameter when dry; hymenium beige to pale brown (Pantone 7502PC = C0 M8 Y33 K10) when fresh, becoming paler when dry; receptacle cream to off white (Pantone warm gray 2C = R213 G210 B202), floccose; stipe concolorous with receptacle, becoming black towards the base, furfuraceous. **Ectal excipulum** textura prismatica, pale brown, composed of thick-walled, brick-shaped cells, 3–6×6–10 μm, tightly packed and arranged almost perpendicular to the surface, ending up to hyphal protrusions (hairs) in the outermost layer; at the upper portion of the margin, cells becoming thinner walled, arranged almost parallel to the surface. **Medullary excipulum** textura intricata, hyaline, smooth, loosely interwoven, separate hyphae of 2.5–3.5 μm wide. **Hairs** mostly cylindrical, sometimes tapered to the apex, thin-walled, 1–2 septate, 4 μm thick at the base, 6–15 μm long; apical cells mostly blunt, sometimes irregularly protruding. **Asci** 85–132.5×8.8–12 μm, cylindrical clavate, 8-spored, arising from croziers; apex flattened, thickened; pore faintly stained by Melzer's reagent, becoming more clearly stained after KOH pretreatment. **Ascospores** 11.3–13.8×4.5–6.3 μm (12.2 ± 0.7×5.4 ± 0.5 μm on average ± SD, n= 33), uniseriate or irregularly biseriate, ellipsoid, aseptate,

mostly with 1–2 large guttules; when germinated, spores becoming light brown, 1-septate and thick walled, one cell of ascosporus cell sometimes darker than the other, or one cell remaining hyaline. **Paraphyses** equal to or slightly exceeding the asci by 7.5–15 μm, filiform, septate, simple or branched near the base, frequently expanded at the apex of 2.5–3.5 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, initially beige, becoming grayish to pale brown in age; Aerial mycelium gray, cottony, partly developed; Margins entire; Reverse brown, filamentous; Rind becoming distinct after 8 weeks incubation, delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, epidermoid in face view.

Other speciemens examined – On decaying leaves of *A. japonica*: TNS-F-190417, Government Forest Station, Asakawa, Tokyo, Honshu, 8 Dec. 1957, Col. K. Aoshima, K. Tubaki et al.; TNS-F-30018 (Culture FC-1985), The Fukiage Gardens in the Imperial Palace Grounds, Chiyoda-ku, Tokyo (35º41'10.7"N,139º45'8"E), 7 Oct. 2009, Col. T. Hosoya; TNS-F-31157 (Culture FC-1190), Iryuda Maruyama, Odawara-shi, Kanagawa Pref., 18 Aug. 2005, Col. Y. Degawa; TNS-F-40099, Meiji Jingu, Chiyoda-ku, Tokyo (35º40'11.4"N,139º42'20"E), 24 Nov. 2011**,** Col. Y. Zhao; TNS-F-44244 (Culture FC-2839), Iryuda Odawara, Kanagawa Pref. (35º14'30.02"N,139º7'11.87"E), 12 Nov. 2011, Col. T. Hosoya; TNS-F-44262 (Culture FC-2843), Kokyo, Chiyoda-ku, Tokyo (35º41'14.34"N,139º44'55.2"E), 21 Nov. 2011, Col. T. Hosoya;

On petioles of *Fatsia japonica* (Thunb.) Decne. & Planch.: TNS-F-36994 (Culture

FC-2645), Fukiagegyoen, Kokyo, Chiyoda-ku, Tokyo (35º41'9.73"N,139º45'3.79"E), 5 Oct. 2010, Col. T. Hosoya.

Notes – Korf (1958) firstly collected this fungus from dying young shoot and leaves of *A. japonica* which is endemic in Japan, and identified it as *Lambertella brunneola* (Pat.) Le Gal. Dumont (1971) re-checked the Korf's collection, and mentioned that it is clearly distinct from other *Lambertella* species in its unique apothecial structure. He suggested exclude it from the genus. However, Dumont (1971) also considered that "it would be premature to erect a new genus" at that time and since then no further studies were done.

As Dumont (1971) suggested, the most remarkable distinguishing character of the present fungus is the structure of ectal excipulum, which is composed of narrow, thick-walled, vertically oriented to the surface and tightly compressed cells.

3–5–3 *Brunneimargo* Y.-J. Zhao & T. Hosoya

Lambertella sp. 1 was definitely separated from other rutstroemiaceous genera based on the result of phylogenetic analyses. Because it is distinguishable from current definition of other rutstroemiaceous genera in morphology, a new genus is proposed for *Lambertella* sp. 1.

Brunneimargo camelliae Y.-J. Zhao & Hosoya gen. et sp. nov. Figs. 5.59–5.60

Holotype – On decaying leaves of *Camellia japonica*: TNS-F-40027, Hirasuna Residence Hall, University of Tsukuba, Tsukuba City, Ibaraki Pref., 20 June 2011, Col.

Y.-J. Zhao.

Etymology – The specific epithet refers to the genus of host plant, "*Camellia"*

Stroma substratal, visible as clearly, irregular, black lines delimiting blackened zones on substrate. **Apothecia** stipitate, occurring on decaying leaves; disc flat to cupulate, 1.3–3 mm in diameter in dried specimen; hymenium beige or brown when fresh, becoming buff (1205 PC= C0 M5 Y35 K0), dark-gray (cool gray 11PC= C48 M36 Y24 K66) or dark brown to black when dry; receptacle concolorous with hymenium, hairy; stipe concolorous with the receptacle, 0.2–1 mm long when dry, hairy, some with mass of brown hyphae attached on the surface. **Ectal excipulum** two layered: outer layer textura prismatica, composed of slightly thick-walled, brick-shaped to somewhat globose cells of 5–25 × 5–15 μm, becoming brown towards the margin; inner layer thin-walled or slightly thick-walled, subhyaline to pale brown, separate hyphae of 2.5–5 μm wide. **Hairs** arising from the outermost layers of the ectal excipulum, cylindrical, 1–2 septate, 20–35 μm long, subhyaline to pale brown, apex expanded up to 4–6 μm. **Medullary excipulum** textura intricata, composed of slightly thick-walled, hyaline, smooth, loosely interwoven hyphae of 3–5 μm wide. **Asci** 90–115×5–10 μm, clavate, 8-spored, arising from simple septa; apex rounded to truncate, 4–5 μm thick; pore stained blue in Melzer's reagent. **Ascospores** 8–12×4–6 μm (10.5 ± 1.2×5.0 ± 0.5 μm on average ± SD, n= 23), uniseriate, ellipsoid, non-septate, hyaline or pale brown; hyaline spores smooth, eguttulate; pale brown spores rarely seen, with one large, central guttules or eguttulate; some germinated spores were observed in specimen, thick-walled, dark brown, no germination was observed in PDA. **Paraphyses** two types: one of

filiform, septate, hyaline, simple or branched near the base, 1.5–2.5 μm wide; the other of clavate, septate, slightly expanded at the apex up to 3–5 μm wide.

Other speciemens examined – On decaying leaves of *C. japonica*: TNS-F-40145, Fukiage Garden, Kokyo, Chiyoda-ku, Tokyo (26m, 35º41'9.29"N, 139º45'4.39"E), 23 July 2012, Col. K. Matsukura.

Notes – The most remarkable character of this fungus is the structure in ectal excipulum, which is composed with two layers, the outer ectal excipulum composed of thick-walled, brick-shaped to somewhat globose cells, becoming brown towards the margin and the inner layer of thin-walled or slightly thick-walled, subhyaline to pale brown.

3–5–4 *Luteidiscella* Y.-J. Zhao & T. Hosoya

Lambertella advenula (W. Phillips) Hosoya & Y. Otani was excluded from the *Lambertella s. s*. based on the phylogenetic analyses and formed its own lineage. Morphologically it has the different characters compared with other rutstroemiaceous genera.

The fungus was thought to be a member of *Moellerodiscus* (Dumont 1976). He emphasised the presence of globose cells in ectal excipulum, but Dumont also mentioned that the shape and apex of asci seems different from other *Moellerodiscus* species. Based on the discovery of ascospores pigmentation prior to germination, Hosoya and Otani (1997) transferred it to *Lambertella*. However, this species completely differs from other *Lambertella* species in the structure of ectal excipulum, and also differs from *Moellerodiscus* species with the subglobose to subcuboid cells in

ectal excipulum. They noticed the presence of appendages at the both poles of the ascospores, but did not take it as a character to establish a new genus. A new genus should be proposed to dispose this fungus with special structure ectal excipulum and asci.

***Luteidiscella advenula* (W. Phillips) Y.-J. Zhao & Hosoya, gen. et comb. nov.**

Figs. 5.48–5.50

Helotium advenulum W. Phillips, Grevillea 6: 24 (1877)

Hymenoscyphus advenula (W. Phillips) W. Phillips [as '*Hymenoscypha*'], Man. Brit. Discomyc. (London): 133 (1887)

Phialea advenula (W. Phillips) Sacc., Syll. fung. (Abellini) 8: 256 (1889)

Ciboriopsis advenula (W. Phillips) Dennis, Kew Bull. 16: 319 (1962)

Moellerodiscus advenulus (W. Phillips) Dumont, Mycologia 68: 235 (1976)

Lambertella advenula (W. Phillips) Hosoya & Y. Otani, Mycoscience 38: 303 (1997)

Stroma substratal, visible as the single black rind encircling the leaf. **Apothecia** stipitate, minute, occurring on the leaves of *Larix*; disc flat or cupulate when fresh, becoming slightly concave when dry, 0.2–0.6 mm in diameter in dried specimen; hymenium white to yellowish-white (1215 PC=R 250 G221 B128) when fresh, becoming off-white when dry; receptacle concolorous with the hymenium when fresh, becoming flesh to dull orange when dry; stipe short, ca. 1mm high, concolorous with receptacle when fresh, smooth, drying off-white, darkish toward the base. **Ectal excipulum** two layered: outer layer composed of hyaline, slightly refractive, subglobose, subcuboid, ovoid to elongate cells, 7–12 μm wide toward the margin, 8–15 μm wide

toward the stipe; inner layer composed of 1–2 layers of narrow, hyaline, thin-walled, smooth hyphae parallel to the surface of the apothecium and continuing into the margin; **Medullary excipulum** textura intricata, hyphae 1.5–3 μm broad, hyaline to subhyaline, smooth, tightly interwoven. **Asci** 8-spored, clavate, 64–75×6–7 μm, produced from croziers; apex slightly conical, ca. 1 μm thick, pore stained blue in Melzer's reagent surrounding by the diffuse blueing. **Ascospores** 6–9×3–4 μm (8.2 ± 0.8×3.1 ± 0.2 μm, on average ± SD, n= 26), uniseriate or biseriate, ellipsoid, smooth, eguttulate or with two polar guttules; In germination on PDA, the spores becoming rectangle-shaped, pale brown to brown, 1–2 septa, with coarse surface. **Paraphyses** filiform or clavate, septate, branch, hyaline, frequently expanded at the apex, 1.5–5 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, attaining ca. 43 mm in diameter after 4 weeks incubation, floccose, initially white to pallid, becoming darker in age, forming the blackened area around central; Aerial mycelium white to gray, cottony, little developed; Margins entire; Reverse gray to pale brown, with the blackened areas diffusing from center; Surface of culture becoming granular to glandular, darker colored after 3 mo. incubation; Rind vertically poorly developed, becoming distinct after 6 mo. incubation, delimiting irregular portions of the agar, ca. 25μm thick, textura globulosa to angularis in face view.

Other speciemens examined – On decaying *Larix* sp. needles: TNS-F-181723, Sugadaira, Nagano Pref., 20 July 1996, Col. I. Tanaka; TNS-F-11194 (Culture FC-1007), Wako-hara, Kuni-mura, Agatsuma, Gunma Pref., 15 May 2004, Col. T. Hosoya; TNS-F-18115, Sugadaira, Ueda-shi, Nagano Pref., 31 May 2007, Col. T. Hosoya;

TNS-F-16822, Sugadaira, Ueda City, Nagano Pref., 10 June 2007, Col. R. Sasagawa; TNS-F-40015 (Culture FC-2718), Sugadaira, Ueda City, Nagano Pref. (36º31'9.78"N, 138º21'11.23"E, Alt. 1346m), 17 May 2011, Col. Y.-J. Zhao; TNS-F-40019 (Culture FC-2722), Sugadaira, Ueda City, Nagano Pref. (36º31'9.78"N, 138º21'11.23"E, Alt. 1346m), 17 May 2011, Col. Y.-J. Zhao; TNS-F-40030, Sugadaira, Ueda City, Nagano Pref. (36º31'32.1"N, 138º20'52"E, Alt. 1340m), 1 July 2011, Col. Y.-J. Zhao; TNS-F-39510, Tashiro, Shikazawa Hot Spring, Tsumagoi-mura, Agatuma-gun, Gunma Pref. (36º27'47.82"N, 138º26'13.9"E, Alt. 1332m), 1 July 2011, Col. T. Hosoya; TNS-F-40130, Sugadaira, Ueda City, Nagano Pref. (36º31'30"N, 138º20'51.2"E, Alt. 1349m), 23 June 2012, Col.Y.-J. Zhao.

Notes – The distinguishing characters of this genus is the two layered ectal excipulum and the ascal apex. Outer ectal excipulum composed of hyaline, subglobose, subcuboid, ovoid to elongate cells, inner layer composed of 1–2 layers of narrow, hyaline, thin-walled, smooth hyphae. The pore of ascal apex stained blue in Melzer's reagent surrounding by the diffuse bluing reaction.

3–5–5 *Hymenoscyphus yunnanense* (S.H. Ou) Y.-J. Zhao & T. Hosoya

Phylogenetically *Lambertella yunnanensis* was included in Clade g which supposed as *Hymenoscyphus s. s.*, and morphologically it have some common characters with *Hymeoscyphus* spp. such as the bright colored apothecia, narrow prismatic cells in ectal excipulum. A new combination of *Hymenoscyphus* was recommended.

***Hymenoscyphus yunnanense* (S.H. Ou) Y.-J. Zhao & Hosoya, comb. nov.**

Figs. 5.57–5.58

Helotium yunnanense S.H. Ou, Sinensia 7: 674 (1936)

Lambertella yunnanensis (S.H. Ou) W.Y. Zhuang & Yan H. Zhang, Taxon 51: 769 (2002)

Stroma obscure, with irregular black spots at the base of some apothecia in substrate. **Apothecia** short-stipitate, solitary to gregarious, occurring on branches; disc flat to discoid, 0.2–1.5 mm in diameter when dry; hymenium yellow to orange (Pantone 1375C= R255 G160 B47) when fresh, becoming dark brown to black when dry; receptacle whitish yellow to buff, pruinose; stipe lighter than receptacle, buff to cream white, with pruinose surface, 0.4–1 mm long when dry. **Ectal excipulum** textura prismatica, 20–80 μm thick, composed of thick-walled, hyaline to subhyaline, tightly arranged prismatic cells, 8–20×3–6 μm, becoming slightly brownish towards outer layer; covering layer lacked; inner layer obscure or lacked. **Medullary excipulum** textura intricata, loosely interwoven, hyaline, smooth, separate hyphae of 1–3 μm wide. **Asci** 137.5–170×17–20 μm, cylindrical clavate to saccate, 8-spored, arising from croziers; apex slightly flattened, pore not stained blue by Melzer's reagent with or without 3% KOH pretreatment. **Ascospores** 22–29×10–14 μm (25.4 ± 1.8×12.1± 1.1 μm on average ± SD, n= 20), uniseriate or irregularly biseriate, ellipsoid; hyaline spores aseptate, thick-walled, with 1-2 large guttules when young, some with points at both end; brown colored ascospores were observed merged in the apothecium structure, slightly thicken-walled, with or without one-septate in the middle; the germination of ascospores not observed. **Paraphyses** equal or slightly exceeding the asci, filiform, septate, hyaline to subhyaline, some containing the yellowish to brownish guttules or

granules especially at the upper part, giving the yellowish to pale brown tint to the hymenium, simple or branched near the base, frequently expanded at the apex, up to 1.5–2.5 μm wide.

Specimens examined – On decayed branches of unknown plant: TNS-F-40028, TNS-F-40035, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt.1340m), 1 July 2011, Col. Y.-J. Zhao.

Notes – Zhuang and Zhang (2002) mentioned that *L. yunnanensis* has a brown hymenium and considered the pigmentation was caused by the discharged brown ascospores. However, I carefully observed the epitype specimen (HMAS 33719) of *L. yunnanensis* and Japanese specimens, and found that the pigmentation in the hymenium was not caused by the pigmented ascospores, but by the paraphyses containing brown pigmentation.

3–5–6 *Rutstroemia pinicola* (Y. Otani) Y.-J. Zhao & T. Hosoya

Clade f included the type species of *Rutstroemia* and *M. pinicola*. They all occur on the conifers and with some common morphological characters. *Moellerodiscus pinicola* was proposed as a new combination of *Rutstroemia*.

***Rutstroemia pinicola* (Y. Otani) Y.-J. Zhao & Hosoya, comb. nov.**

Figs. 5.87–5.89

Moellerodiscus pinicola Y. Otani, Bull. natn. Sci. Mus., Tokyo, B 5: 51 (1979)

Stroma obscure on substrate. **Apothecia** stipitate, occurring on fallen needle leaves; disc flat to cupulate, 0.5–1 mm in diameter in dried specimen; hymenium olive greenish to beige when fresh, becoming grayish (427PC=C7 M3 Y4 K8) when dry; receptacle pallid to grayish when fresh, becoming dark gray when dry, flocculose; stipe concolorous with receptacle when fresh, becoming black when dry, 0.2–0.6 mm long when dry, flocculose, darkish towards the base. **Ectal excipulum** two layered: covering layer absent; outer ectal excipulum composed of prismatic to angularis cells, also merged with somewhat globosa cells, extremely thick-walled, smooth, subhyaline, becoming pale brown towards margin, individual cells 2.5–15×4–10 μm; inner layer obviously in margin and base of stipe, originated from hyphae of medullary excipulum, thin walled, pale brown to brown, slightly granulate, separate hyphae of ca. 3μm wide; protrusions originated from the outmost layer of ectal excipulum, brownish, rounded in apex, slightly granulate, 0–2 septate, 2–3 μm wide. **Medullary excipulum** textura intricata, pale brown, slightly granulate, separate hypha of 2–4 μm wide. **Asci** 73–92×6–9 μm, cylindric, 8-spored, crozier absent or obscure; apex rounded to truncate, 1–2 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 8.5–12.5×3–4 μm (10.7 ± 1.1×3.4 ± 0.4 μm on average ± SD, n= 21), uniseriate to irregular biseriate above, ellipsoid to subfusoid, obtuse, non-septate, hyaline, mostly eguttulate, some with 1–2 guttules. **Paraphyses** filiform, septate, hyaline, expanded at the apex up to 1.5–2.5 μm wide.

Cultural characteristics – Colonies on PDA grows fast, nearly covering the whole plate after 4 weeks incubation, circular, cottony, initially grayish (Warm gray 2C = R213

G210 B202), becoming darker in age; Aerial mycelium gray, partly developed; Margins entire; Reverse dark brown, filamentous, margin grayish; Rind not observed even after 12 months incubation.

Specimens examined – On fallen leaves of *Pinus thunbergii* Parlat.: TNS-F-50211, Ooarai Kaigan, Ibaraki Pref., 15 May 1978, Col. Y. Otani; TNS-F-50212, Shonan kaigan, Kanagawa Pref., 17 May 1978, Col. Y. Otani; TNS-F-18413, Yanagi-kawa, Kamisu-shi, Ibaraki Pref., 23 April 2008, Col. T. Hosoya;

On fallen leaves of *Pinus luchuensis*: TNS-F-40105 (Culture FC-2981), Kenmin-no-mori, Onnna-son, Kunigami-gun, Okinawa Pref., 28 April 2012, Col. Y.-J. Zhao;

On fallen leaves of *Pinus densiflora* Sieb. & Zucc.: TNS-F-40115 (Culture FC-2988), Takayama-shi, Gifu Pref. (35º50'49.00"N, 137º1'50.40"E, Alt. 422m), 28 May 2012, Col. Y.-J. Zhao; TNS-F-40117 (2 June 2012), Ueda-shi, Nagano Pref., Col. Y. Degawa; TNS-F-40118 (Culture FC-2989), Ueda-shi, Nagano Pref., Col. Y. Degawa,; TNS-F-40131 (Culture FC-2995), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'30.00"N, 138º20'51.20"E, Alt. 1350m), 23 June 2012, Col. Y.-J. Zhao.

Notes – *Moellerodiscus pinicola* is widely distributed in Japan, and it is host specific on *Pinus* spp. (Otani 1979). Otani focused on the globose cells in ectal excipulum, and considered it as a species of *Moellerodiscus.* In addition, the diagnostic characters of *M. pinicola* are the greenish apothecia, irregular cells in ectal excipulum, the brown protrusions and the brownish, granulate hyphae in medullary excipulum (Otani 1979).

Table 3.1. List of *Lambertella* and its allies collected from Japan in the present study

No.	Name	Substrate	Locality	Collected date (Y/M/D)	Specimen number (TNS-F-)	Culture number*** (FC-)
Rutstroemiaceae						
1	*Dicephalospora rufocornea* (Berk. & Broome) Spooner	Unknown branch	Otomi, Iriomote Isl., Okinawa	2011/6/12	40024	2730
2	*Lambertella advenula* (W. Phillips) Hosoya & Y. Otani	*Larix* needle	SMRC*, Ueda-shi, Nagano	2011/5/17	40019	2722
3	*Lambertella corni-maris* Höhn.	*Mallotus japonicus* leaf	Sanshiro-ike, Tokyo University, Hongo, Bunkyo-ku, Tokyo	2009/12/7	30402	2389
		Torreya nucifera fruit	TBG**, Tsukuba City, Ibaraki	2011/10/14	40083	2821
4	*Lambertella pyrolae* Y.-J. Zhao & T. Hosoya	*Pyrola incarnata* leaf and petiole	SMRC*, Ueda-shi, Nagano	2012/6/23	40132	2996
5	*Lambertella yunnanensis* (S.H. Ou) W.Y. Zhuang & Yan H. Zhang	Unknown branch	SMRC*, Ueda-shi, Nagano	2011/7/2	40035	-
6	*Lambertella* sp. 1	*Camellia japonica* leaf	SMRC*, Ueda-shi, Nagano	2011/6/20	40027	-
7	*Lambertella* sp. 2	*Aucuba japonica* leaf and petiole	Iryuda Maruyama, Odawara-shi, Kanagawa	2005/8/18	31157	1190
		Fatsia japonica petiole	Fukiage Garden, Kokyo, Chiyoda-ku, Tokyo	2010/10/5	36994	2645
		Aucuba japonica leaf and petiole	Fukiage Garden, Kokyo, Chiyoda-ku, Tokyo	2012/6/20	40126	2992
8	*Lanzia longipes* (Cooke & Peck) Dumont & Korf	Unknown petiole	TBG**, Tsukuba City, Ibaraki	2011/10/14	40082	-
			TBG**, Tsukuba City, Ibaraki	2011/11/7	40097	2832
			SMRC*, Ueda-shi, Nagano	2012/9/15	40148	-
			Hanazono Shrine, Kitaibaraki-shi, Ibaraki	2012/9/26	40160	-
			TBG**, Tsukuba City, Ibaraki	2012/10/15	40177	5098
9	*Lanzia pruni-serotinae* (Whetzel & W.L. White) M.P. Sharma & R.M. Sharma	*Prunus grayana* leaf	TBG**, Tsukuba City, Ibaraki	2012/6/13	40119	-
10	*Lanzia* sp. 1	Unknown leaf	SMRC*, Ueda-shi, Nagano	2011/7/13	40038	2790
11	*Lanzia* sp. 2	*Quercus* sp. fruit	University of Tsukuba, Tsukuba City, Ibaraki	2011/10/13	40081	2820
12	*Lanzia* sp. 3	*Castanea crenata* involucre and spine	Yichinoya, Tsukuba City, Ibaraki	2011/10/20	40095	2830
			TBG**, Tsukuba City, Ibaraki	2011/10/27	40096	2831
			Yuhira Hot Spring, Yuhuin-cho, Yuhu-shi, Oita	2012/11/6	40192	5109

Table 3.1. List of *Lambertella* and its allies collected from Japan in the present study **(Continued)**

No.	Name	Substrate	Locality	Collected date (Y/M/D)	Specimen number (TNS-F-)	Culture number (FC-)
13	*Lanzia* sp. 4	*Swida controversa* leaf	TBG**, Tsukuba City, Ibaraki	2012/6/13	40120	-
14	*Lanzia* sp. 5	*Pyrus pyrifolia* leaf	Yatsugatake, Kawakami forests, University of Tsukuba.	2012/7/20	40139	-
		Prunus leaf	Yatsugatake, Kawakami forests, University of Tsukuba.	2012/7/20	40144	-
15	*Lanzia* sp. 6	Herb stem	Kokenodomon Gulley, Shikotsu Toya National Park, Chitose-shi, Hokkaido	2011/9/14	40055	2802
16	*Lanzia* sp. 7	Unknown leaf	SMRC*, Ueda City, Nagano	2011/7/3	40036	2789
17	*Moellerodiscus pinicola* Y. Otani	*Pinus luchuensis* leaf	Kenmin-no-mori, Onnna-son, Kunigami-gun, Okinawa	2012/4/28	40105	2981
		Pinus densiflora leaf	Takayama-shi, Gifu	2012/5/28	40115	2988
		Pinus densiflora leaf	Ueda-shi, Nagano	2012/6/2	40118	2989
18	*Moellerodiscus* sp. 1	Unknown petiole	SMRC*, Ueda-shi, Nagano	2011/10/17	40085	2823
19	*Moellerodiscus* sp. 2	Unknown branch	Kokonoe-machi, Kuzu-gun, Oita	2012/11/6	40188	5105
20	*Poculum sydowianum* (Rehm) Dumont	*Quercus crispula* petiole	SMRC*, Ueda-shi, Nagano	2011/9/28	40071	2813
		Quercus serrata petiole	Takayama-mura, Gunma	2012/10/2	40163	5091
		Quercus crispula petiole	Green tunnel, Tomakomai-shi, Hokkaido	2011/9/13	42316	2766
21	*Poculum* sp.	Unknown branch	Midorinotonneru, Tomakomai-shi, Hokkaido	2011/9/13	40048	2796
22	*Rutstroemia bulgarioides* P. Karst	*Picea jegoensis* fruit	Kamikawa-cho, Hokkaido	2011/4/23	40005	2715
Sclerotiniaceae						
23	*Ciboria batschiana* (Zopf) N.F. Buchw.	Acorns of *Quercus* species	Iryuda Odawara, Kanagawa	2011/11/12	44241	2836
24	*Ciboria* sp.	Unknown	Mt. Mikamo, Tochigi	2011/5/16	40014	2787
25	*Ciborinia camelliae* L.M. Kohn	Soil under *Camellia japonica*	Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo	2012/3/22	40102	2976
26	*Dumontinia tuberose* (Bull.) L.M. Kohn	Soil under *Sambucus racemosa*	Meiho, Gujo-shi, Gifu	2012/5/28	40114	2987
27	*Sclerotinia sclerotiorum* (Lib.) de Bary	Decayed part of *Anemone* sp.	Karasawa water fall, Sugadaira, Ueda City, Nagano	2011/5/18	40021	2724
28	*Stromatinia cryptomeriae* Kubono & Hosoya	*Cryptomeria japonica* branch	Kanagawa	2012/3/20	40104	2977

Table 3.1. List of *Lambertella* and its allies collected from Japan in the present study **(Continued)**

No.	Name	Substrate	Locality	Collected date (Y/M/D)	Specimen number (TNS-F-)	Culture number (FC-)
Helotiaceae						
29	*Hymenoscyphus caudatus* (P. Karst.) Dennis	*Alnus* fruit	Shikotsu Toya National Park, Chitose-shi, Hokkaido	2011/9/14	40056	2803
30	*Hymenoscyphus fructigenus* (Bull.) Gray	Unknown acorns	Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo	2011/11/8	44644	2855
31	*Hymenoscyphus ginkgonis* J.G. Han & H.D. Shin	*Ginkgo biloba* leaf	Fuchizawa Horyo, Towada-ko-machi, Aomori	2004/8/25	11208	1093
32	*Hymenoscyphus menthae* (W. Phillips) Baral	*Hydrangea* flower	Shikotsu lake, Chitose-shi, Hokkaido	2011/9/14	40052	2800
33	*Hymenoscyphus microserotinus* (W.Y. Zhuang) W.Y. Zhuang	*Aesculus turbinata* branch	SMRC*, Ueda-shi, Nagano	2011/9/27	40067	2811
34	*Hymenoscyphus pseudoalbidus* Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr.	*Fraxinus mandshurica* petiole	SMRC*, Ueda-shi, Nagano	2006/9/11	12761	1445
			Nishikionuma Park, Tomakomai-shi, Hokkaido	2011/9/12	40043	2793
			Hukkonomori, Shikotsu lake, Chitose-shi, Hokkaido	2011/9/14	40051	2799
35	*Hymenoscyphus scutula* (Pers.) W. Phillips	Unknown petiole	Alnus forest, Minoto, Chino City, Nagano	2004/10/14	17507	1080
36	*Hymenoscyphus varicosporoides* Tubaki	Herb stem	Yukiiri, Chiyoda-machi, Kasumigaura-shi, Ibaraki	2005/5/5	16472	2038
37	*Hymenoscyphus* sp. 1	Unknown petiole	University of Tsukuba, Tsukuba City, Ibaraki	2011/10/13	40079	2818
38	*Hymenoscyphus* sp. 2	Herb stem	Yuhira Hot Spring, Yuhuin-cho, Yuhu-shi, Oita	2012/11/6	40193	5110
39	*Phaeohelotium epiphyllum* (Pers.) Hengstm. (*Hymenoscyphus epiphyllus* (Pers.) Rehm ex Kauffman)	*Populus maximowiczii* leaf	Nishikionuma Park, Tomakomai-shi, Hokkaido	2011/9/12	40042	2792
40	"*Hymenoscyphus*" sp. 1	Unknow leaf	Hanazono Shrine, Kitaibaraki-shi, Ibaraki	2012/9/26	40156	5090
41	"*Hymenoscyphus*" sp. 2	Unknown acorns	Ohara, Nakayama, Koriyama-shi, Gunma	2012/10/2	40168	-
42	"*Hymenoscyphus*" sp. 3	Unknown fruit	Mt. Buko-san, Chichibu-shi, Saitama	2012/10/13	40173	5096
43	"*Hymenoscyphus*" sp. 4	Unknown acorns	Mt. Takao, Oita-shi, Oita	2012/11/4	40179	5101
44	"*Hymenoscyphus*" sp. 5	Unknown branch	Kokonoe-machi, Kuzu-gun, Oita	2012/11/6	40186	5104

*SMRC: Sugadaira Montane Research Center, Tsukuba University; **TBG: Tsukuba Botanical Garden

*** The isolates used in the present study partially have already been deposited to NBRC and the registered numbers are given, and the rest of isolates will be registered. In the present table, however, registration numbers (FC numbers) for the present study is shown.

Table 3. 2. Morphological characters and substratal stroma formation of *Lambertella* and allied specie

No.	Name*	Pigmentation of ascospores***	Structure in ectal excipulum	Substratal stroma in nature	Isolation	Substratal stroma in culture	Surface structure of the rind**
1	*Dicephalospora rufocornea*	-	t. prismatica	Unclear	Y	Diffused rind	-
2	***Hymenoscyphus ginkgonis***	after discharge	t. prismatica	Clear rind	Y	Diffused rind	-
3	*Hymenoscyphus microserotinus*	-	t. prismatica	Unclear	Y	Clear rind	t. globulosa
4	***Hymenoscyphus pseudoalbidus***	after discharge	t. prismatica	Clear rind	Y	Diffused rind	-
5	***Lambertella advenula***	after discharge	t. angularis	Clear rind	Y	Clear rind	t. globulosa to t. angularis
6	***Lambertella corni-maris***	before discharge	t. prismatica	Clear rind	Y	Clear rind	t. epidermoidea
7	***Lambertella pyrolae***	before discharge	t. prismatica	Clear rind	Y	Clear rind	t. epidermoided
8	***Lambertella yunnanensis***	after discharge	t. prismatica, thick-walled or with gelatinous matrix	Unclear	N	-	-
9	***Lambertella* sp. 1**	after discharge	t. angularis to t. prismatica, with globosa cells	Clear rind	N	-	-
10	***Lambertella* sp. 2**	after discharge	t. prismatica to irregular, thick walled	Unclear	Y	Clear rind	t. epidermoidea
11	***Lanzia longipes***	after discharge	t. prismatica	Unclear	Y	Clear rind	t. epidermoidea to t. irregularis
12	*Lanzia pruni-serotinae*	-	t. prismatica	Clear rind	N	-	-
13	*Lanzia* sp. 1	-	t. prismatica	Clear rind	Y	Diffused rind	t. globulosa ?

Table 3. 2. Morphological characters and substratal stroma formation of *Lambertella* and allied specie **(Continued)**

No.	Name*	Pigmentation of ascospores***	Structure in ectal excipulum	Substratal stroma in nature	Isolation	Substratal stroma in culture	Surface structure of the rind**
14	*Lanzia* sp. 2	-	t. prismatica	Unclear	Y	Clear rind	t. globulosa to irregular
15	*Lanzia* sp. 3	-	t. prismatica	Clear rind	Y	Clear rind	t. globulosa to irregularis
16	*Lanzia* sp. 4	-	t. prismatica	Clear rind	N	-	-
17	*Lanzia* sp. 5	-	t. prismatica	Clear rind	N	-	-
18	*Lanzia* sp. 6	-	t. prismatica	Clear rind	Y	Diffused rind	-
19	*Lanzia* sp. 7	-	t. prismatica	Clear rind	N	-	-
20	*Moellerodiscus pinicola*	-	t. prismatica, t. angularis	Clear rind	Y	Diffused rind	-
21	***Moellerodiscus* sp. 1**	after discharge	t. globulosa	Unclear	Y	Clear rind	t. globulosa to irregularis
22	*Moellerodiscus* sp. 2	-	t. globulosa to t. angularis	Unclear	Y	Diffused rind	-
23	***Poculum sydowianum***	after discharge	t. prismatica, with gelatinous matrix	Clear rind	Y	Not observed	-
24	*Poculum* sp.	-	t. prismatica, with gelatinous matrix	Unclear	Y	Clear rind	t. globulosa
25	*Rutstroemia bulgarioides*	-	t. globulosa to t. angularis	Unclear	Y	Diffused rind	-

* Species given in **bold** have both stroma and pigmented ascospores

** "-": not observed.

*** "-": no change.

Table 3.3. List of taxa used in the phylogenetic analyses

Name	Culture (FC-no.)	Specimen (TNS-F- no.)	ITS*	LSU*	RPB2*	LSU+ RPB2
Taxa from Japan						
Rutstroemiaceae						
Dicephalospora rufocornea	2730	40024	**2808**	**5570**	**3818**	○
Lambertella advenula	2722	40019	**2790**	**5571**	**3678**	○
Lambertella corni-maris	2389	30402	**2802**	-	3672	-
Lambertella corni-maris	2821	40083	**3338**	**5589**	**3686**	○
Lambertella pyrolae	2996	40132	**3534**	**5617**	**3848**	○
Lambertella yunnanensis	-	40035	**2797**	**5572**	**3819**	○
Lambertella sp. 1	-	40027	**2812**	**5573**	**3679**	○
Lambertella sp. 2	1190	31157	**2207**	-	-	-
Lambertella sp. 2	2645	36994	**3018**	-	-	-
Lambertella sp. 2	2992	40126	**3531**	**5616**	**3847**	○
Lanzia longipes	-	40082	**3545**	-	-	-
Lanzia longipes	2832	40097	**3349**	**5592**	**3689**	○
Lanzia longipes	-	40148	**5272**	-	-	-
Lanzia longipes	-	40160	**5274**	-	-	-
Lanzia longipes	5098	40177	**5292**	-	-	-
Lanzia pruni-serotinae	-	40119	**3547**	**5835**	**5831**	○
Lanzia sp. 1	2790	40038	**3312**	**5577**	**3682**	○
Lanzia sp. 2	2820	40081	**3337**	**5588**	**3685**	○
Lanzia sp. 3	2830	40095	**3347**	-	-	-
Lanzia sp. 3	2831	40096	**3348**	**5591**	**3688**	○
Lanzia sp. 3	5109	40192	**5302**	-	-	-
Lanzia sp. 4	-	40120	**3548**	**5619**	-	○
Lanzia sp. 4	-	40136	**3549**	-	**3850**	-
Lanzia sp. 5	-	40139	**3550**	-	-	-
Lanzia sp. 5	-	40144	**3551**	**5620**	-	○
Lanzia sp. 6	2802	40055	**3320**	**5581**	-	○
Lanzia sp. 7	2789	40036	**3311**	**5576**	**3681**	○
Moellerodiscus pinicola	2981	40105	**3520**	-	3844	-
Moellerodiscus pinicola	2988	40115	**3527**	**5614**	**3698**	○
Moellerodiscus pinicola	2989	40118	**3528**	-	-	-

Table 3.3. List of taxa used in the phylogenetic analyses **(Continued)**

Name	Culture (FC-no.)	Specimen (TNS-F- no.)	ITS*	LSU*	RPB2*	LSU+ RPB2
Moellerodiscus sp. 1	2823	40085	**3340**	**5590**	**3687**	○
Moellerodiscus sp. 2	5105	40188	**5299**	**5626**	**5827**	○
Poculum sydowianum	2813	40071	**3330**	**5585**	**3684**	○
Poculum sydowianum	5091	40163	**5285**	-	-	-
Poculum sydowianum	2766	42316	**3296**	-	-	-
Poculum sp.	2796	40048	**3315**	**5579**	**5755**	○
Rutstroemia bulgarioides	2715	40005	**2785**	**5569**	**3676**	○
Sclerotiniacea						
Ciboria batschiana	2836	44241	**3298**	**5594**	**3690**	○
Ciboria sp.	2787	40014	**3310**	**5575**	**3680**	○
Ciborinia camelliae	2976	40102	**3516**	**5611**	**3843**	○
Dumontinia tuberosa	2987	40114	**3526**	**5613**	**5758**	○
Sclerotinia sclerotiorum	2724	40021	**2792**	5568	3675	-
Stromatinia cryptomeriae	2977	40104	**3517**	**5612**	**3696**	○
Helotiaceae						
Hymenoscyphus caudatus	2803	40056	**3321**	**5582**	**3820**	○
Hymenoscyphus fructigenus	2855	44644	**3308**	**5595**	**3692**	○
Hymenoscyphus ginkgonis	1093	11208	**2333**	**5563**	-	○
Hymenoscyphus menthae	2800	40052	**3318**	**5580**	**5093**	○
Hymenoscyphus microserotinus	2811	40067	**3328**	**5583**	**5094**	○
Hymenoscyphus pseudoalbidus	1445	12761	**444**	-	-	-
Hymenoscyphus pseudoalbidus	2793	40043	**4898**	-	-	-
Hymenoscyphus pseudoalbidus	2799	40051	**3160**	**5596**	**3693**	○
Hymenoscyphus scutula	1080	17507	**5832**	**5564**	**3669**	○
Hymenoscyphus varicosporoides	2038	16472	**2302**	**5567**	**1403**	○
Hymenoscyphus sp. 1	2818	40079	**3335**	**5587**	**3823**	○
Hymenoscyphus sp. 2	5110	40193	**5303**	**5627**	**5762**	○
Phaeohelotium epiphyllum (*Hymenoscyphus epiphyllus*)	2792	40042	**3313**	**5578**	**5820**	○
"Hymenoscyphus" sp. 1	5090	40156	**5284**	**5622**	**5761**	○
"Hymenoscyphus" sp. 2	-	40168	**5276**	**5621**	**5760**	○
"Hymenoscyphus" sp. 3	5096	40173	**5833**	**5623**	**5824**	○

Table 3.3. List of taxa used in the phylogenetic analyses **(Continued)**

Name	Culture (FC-no.)	Specimen (TNS-F- no.)	ITS*	LSU*	RPB2*	LSU+ RPB2
"Hymenoscyphus" sp. 4	5101	40179	**5295**	**5624**	**5825**	○
"Hymenoscyphus" sp. 5	5104	40186	**5834**	**5836**	**5826**	○
Hyaloscyphaceae (Outgroup)						
Lachnum abnorme	2172	16617	**1251**	**5566**	**1420**	○
Lachnum virgineum	2137	16583	**1245**	**5565**	**1417**	○
Taxa from culture collection						
Rutstroemiaceae						
Lambertella corni-maris	CBS 774.95	-	**3596**	-	-	-
Lambertella corni-maris	CBS 184.93	-	**3597**	-	-	-
Lambertella corni-maris	CBS 197.47	-	**3598**	-	-	-
Lambertella hicoriae	CBS 198.47	-	**3600**	**5601**	**3835**	○
Lambertella himalayensis	CBS 230.77	-	**3601**	**5602**	**3836**	○
Lambertella pruni	CBS 199.47	-	**3602**	**5603**	**3837**	○
Lambertella subrenispora	CBS 811.85	-	**3603**	**5604**	**5821**	○
Lambertella viburni	CBS 200.47	-	**3604**	**5605**	**3838**	○
Lanzia allantospora	CBS 1243.34	-	**3605**	**5606**	**5822**	○
Lanzia cuniculi	NBRC 9671	-	**3591**	**5598**	**3829**	○
Poculum calopum	CBS 854.97	-	**3606**	**5607**	**5095**	○
Poculum firmum	CBS 341.62	-	**3609**	**5609**	**3840**	○
Poculum sydowianum	CBS 1159.75	-	**3611**	-	-	-
Rutstroemia paludosa	CBS 464.73	-	**3610**	**5610**	**3841**	○
Scleromitrula shiraiana	NBRC 30255	-	**3593**	**5599**	**3830**	○
Sclerotiniaceae						
Ciboria conformata	CBS 518.75	-	**3607**	**5608**	**3839**	○
Sclerotinia sclerotiorum	CBS 537.77	-	**3595**	**5600**	**3831**	○

* Sequences are conserved in TN-S- database; Sequences no. **in bold**: used in the analysis;

"-": not available; "○": combination sequences of LSU and RPB2 was included in the analysis.

Table 3.4. ITS-5.8S sequences downloaded from GenBank or AFTOL used in the present research

Name	GenBank no.
Dicephalospora rufocornea	**DQ986480**
Hymenoscyphus cf. *menthae*	**AY348588**
Hymenoscyphus caudatus	**AY348577**
Hymenoscyphus epiphyllus	**AY348580**
Hymenoscyphus epiphyllus	**AY348581**
Hymenoscyphus fructigenus	**AJ430396**
Hymenoscyphus microserotinus	**DQ986481**
Hymenoscyphus scutula	**AY348591**
Hymenoscyphus serotinus	**AY348592**
Hymenoscyphus rhodoleucus	**AJ430395**
Lambertella pruni	**DQ335471**
Rutstroemia bolaris	**Z80894**
Lambertella tubulosa	**EF029195**
Lambertella langei	**Z81435**
Lanzia allantospora	**AY755334**
Lanzia berggrenii	**KC164647**
Lanzia griseliniae	**AY755333**
Lanzia griseliniae	**FR667970**
Lanzia huangshanica	**DQ986485**
Lanzia luteovirescens	**KC533545**
Moellerodiscus coprosmae	**EU599575**
Poculum firmum	**Z80893**
Poculum sydowianum	**AY853238**

Table 3.5. Bootstrap probability of clades generated in the phylogenetic analysis based on LSU, RPB2 and LSU+RPB2 datasets suggesting the reliability

Clade	LSU + RPB2		LSU		RPB2	
	MP	**ML**	**MP**	**ML**	**MP**	**ML**
A	100	100	98	94	89	97
A'	83	93	81	69	-	60
B	57	80	53	59	-	-
a	78	97	89	95	-	-
a'	89	89	77		89	94
b	100	100	100	99	97	96
c	73	95	81	82	-	-
c'	75	96	68	62	-	83
d	100	100	100	96	100	100
e	100	100	100	100	96	99
f	91	94	57	73	76	72
g	58	84	-	-	96	100
g'	100	100	80	87	100	100

* "-": BP≧50% or not supported.

Fig. 3.1. One of six most parsimonious trees based on ITS rDNA sequences. Numbers above the branches are bootstrap values (BP) in maximum parsimony analysis (MP). [tree length (TL): 496 steps, consistency index (CI): 0.3206, retention index (RI): 0.8044, rescaled consistency index (RC): 0.2579]; BP ≤50% not shown. Sequences from GenBank or AFTOL all with the original sequence number before the species name.

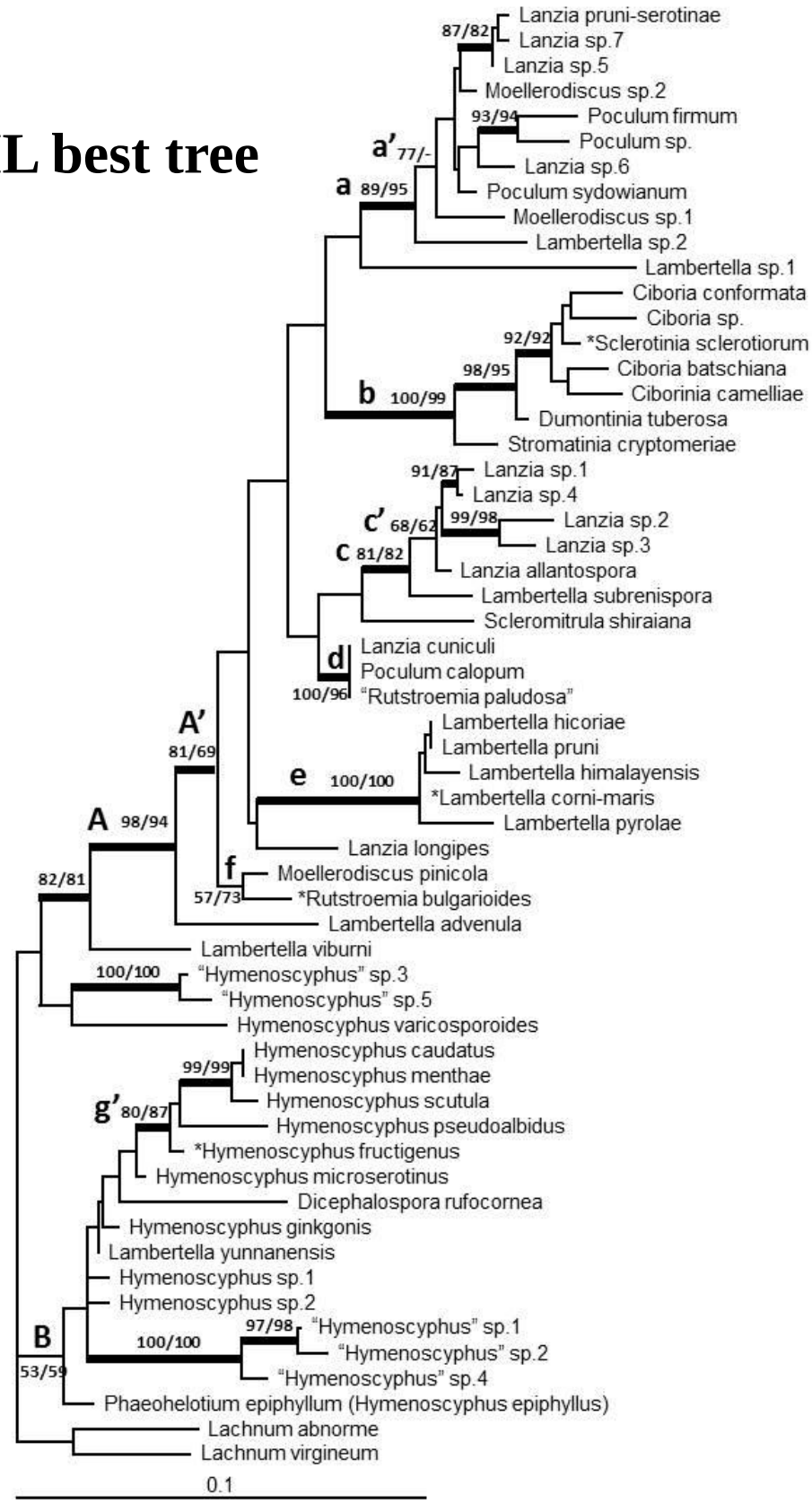


Fig. 3.2. Maximum likelihood (ML) best tree inferred from LSU sequences. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. "–": BP ≤50%.

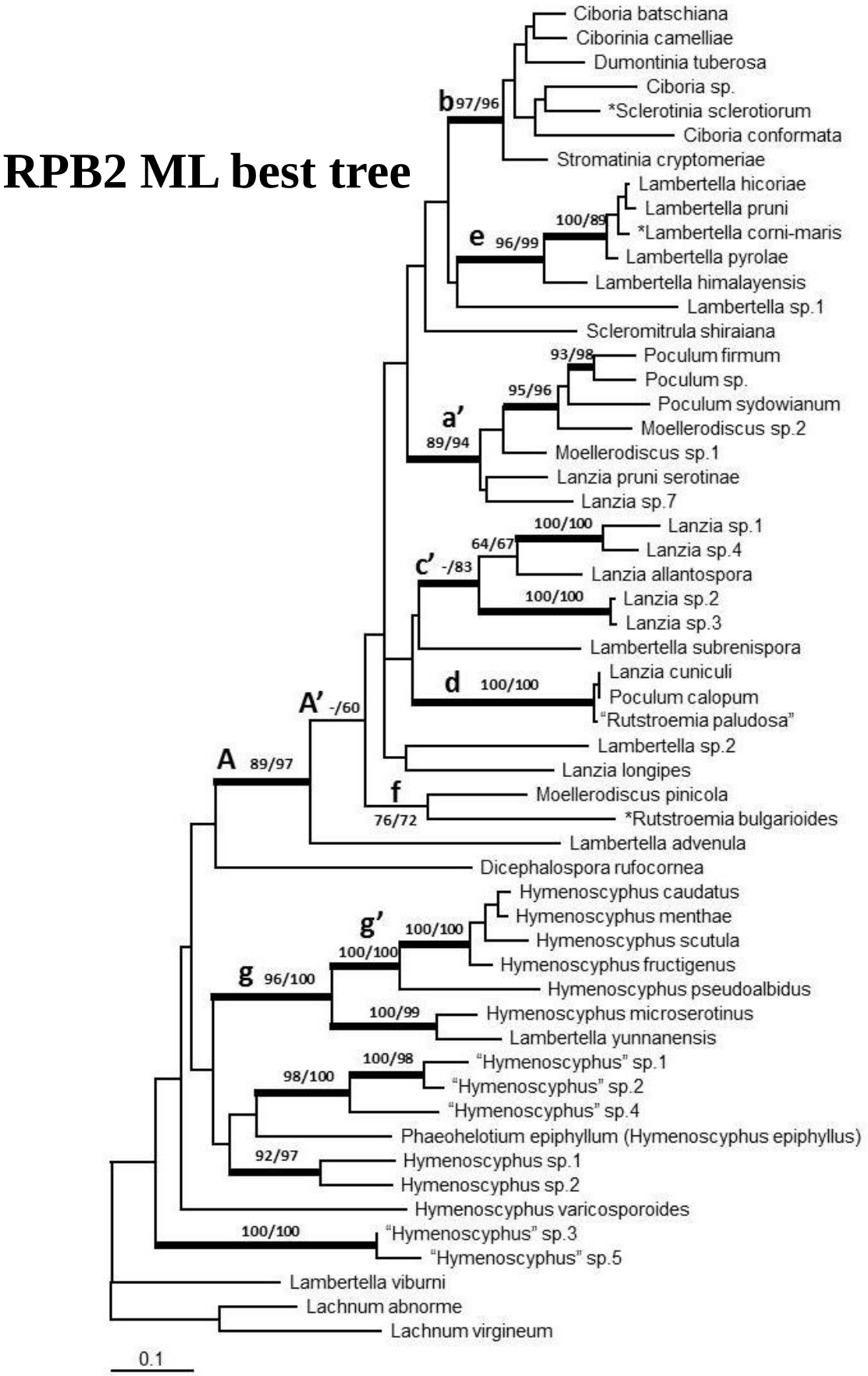
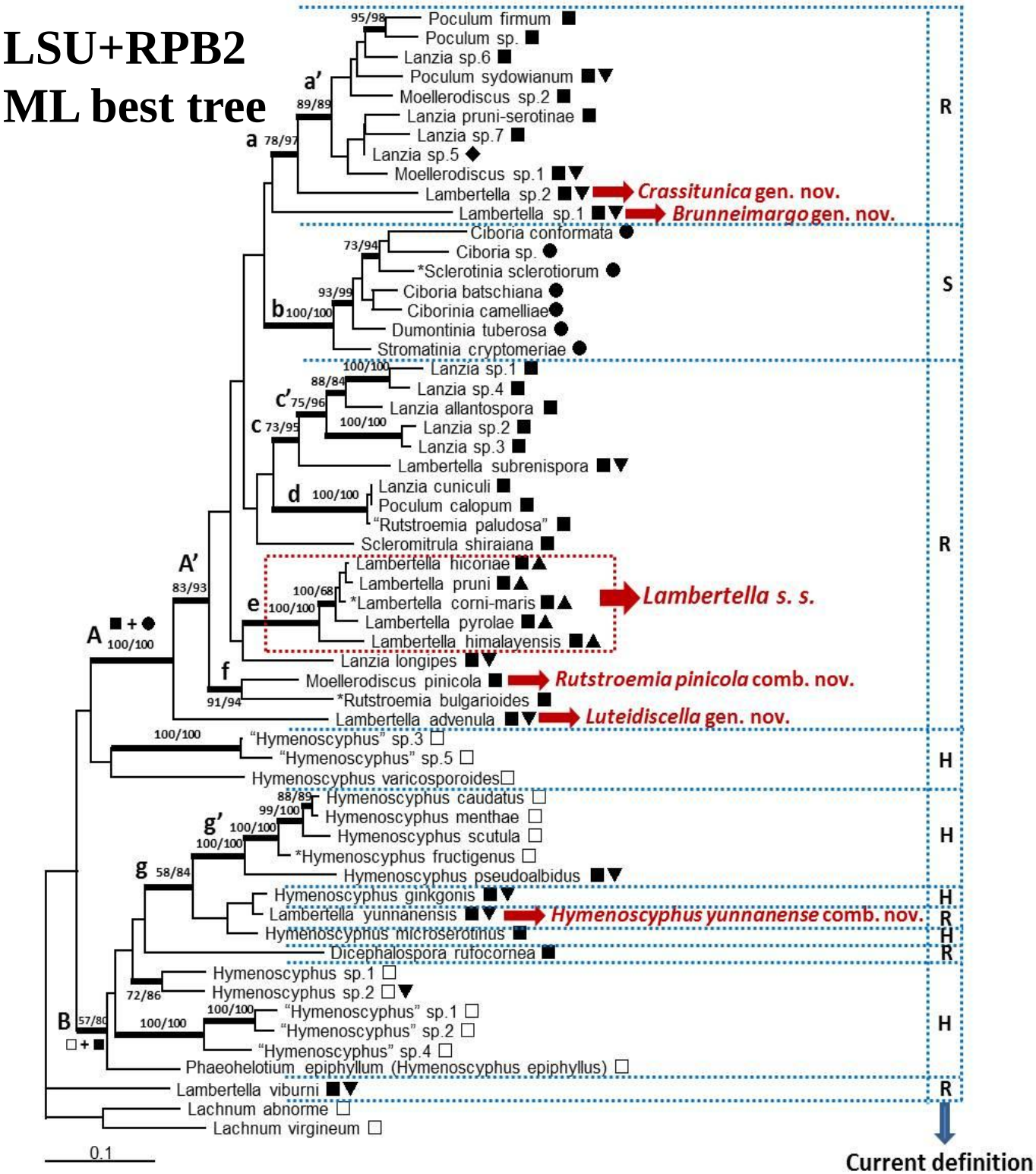


Fig. 3.3. Maximum likelihood (ML) best tree inferred from RPB2 sequences. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. "–": BP ≤50%.



● with sclerotial stroma; ■ with substratal stroma; □ without stroma;

▲ spore pigmented before discharge;▼ spore pigmented after discharge; *Type species

S: Sclerotiniaceae R: Rutstroemiaceae H: Helotiacea

Fig. 3.4. Maximum likelihood (ML) best tree inferred from combined dataset of LSU and RPB2 sequences. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. "–": BP ≤50%.

Chapter 4 General discussion

Evolution of the substratal stroma and sclerotial stroma

The presence of stroma is considered to be a remarkable feature that characterizes the families in Helotiales. The close relationship between Rutstroemiaceae and Sclerotiniaceae in the order Helotiales, both characterized by the presence of stroma, was suggested by a molecular phylogeny that incorporated a wide range of Helotiales members (Wang et al. 2006), whereas Holst-Jensen et al. (1997) focused on more members with stroma. However, these studies did not include members of Helotiaceae. Han and Shin (2008) demonstrated the possibility that stroma-forming members may be included in Helotiaceae by describing a new species, H. ginkgonis. The same possibility was also demonstrated when H. pseudoalbidus was described (Kowalski and Holdenrieder 2009). However, the phylogenetic analyses were limited in these studies. Zhao et al. (2012) clearly showed that stroma-forming species are included in Helotiaceae. The present study confirmed the analysis by Zhao et al. (2012) based on a wider range of members, by demonstrating that stroma formation evolved more than once in different clades via convergent evolution (Fig. 3.4). In fact, structures that correspond to substratal and sclerotial stroma have been observed in other fungal groups, which are also phylogenetically isolated. The former structure is included in Sarcoscyphaceae and Sarcosomataceae (Pezizales) whereas the latter structure is also known in Typhula (Basidiomycota, Corner 1950).

A focus on clade A, which includes most of the Rutstroemiaceae (Fig. 3.4), indicates that the stroma is a synapomorphic character because the outgroup does not have stroma, and the sclerotial stroma is a synapomorphic character within this clade.

Willets (1997) suggested that the sclerotia evolved later because it has a more organized structure with a well-differentiated medulla covered by cortex (rind) and a definite, tight structure composed of morphologically differentiated hyphae. The phylogenetic analysis supports this suggestion.

The evolutionary traits of the stroma can be elucidated on the basis of closer observations of stroma in nature and in culture. In substratal stroma-forming members, the rind structure often comprises well-differentiated hyphae (e.g., Fig. 5.53), which comprise dark pigmented cells that are tightly combined together, although some have a more loosely entangled hyphal structure that gives rise to a dark-colored area (e.g., Fig. 5.71). In the case of H. microserotinus, stroma formation is much clearer in culture than in nature. It is speculated that the stroma defined by loosely entangled, dark hyphae is the transitional form between non-stroma and clearly defined substratal stroma.

Relevance of pigmented ascospores

Pigmentation, particularly melanization, occurs frequently in various fungi. Melanization occurs during certain developmental stages of the mycelium, such as sporulation or defensive reactions to wounding. Melanization gives mechanical strength to the cell wall and makes the cells more stress-resistant in unfavorable environmental conditions, such as drought, osmotic pressure, pH, and temperature (Deacon 2006). Thus, melanin acts as a strengthening component and may physically protect spores from the action of enzymes produced by other microbes (Isaac 1994). In addition, the melanins of pigmented ascospores can absorb radiation and dissipate energy, protecting the spore membrane (Isaac 1994). For example, the domination of melanized fungal genera such as *Alternaria* and *Cladosporium* was observed in soil communities affected

by long-living radionuclides emitted at the site of the Chernobyl accident (Zhdanova et al. 1994).

Melanin plays an essential role in the virulence of plant and human pathogenic fungi (Taylor et al. 1985; Langfelder et al. 2003). The human pathogenic fungi include many melanotic fungi with increasing clinical importance (Silveira and Nucci 2001; Revankar et al. 2002), such as *Cryptococcus neoformans* (Nosanchuk et al. 2000), *Sporothrix schenckii* (Romero-Martinez et al. 2000) and *Aspergillus* spp. (Rosas et al. 2000; Tsai et al. 2001). In some plant pathogenic fungi, such as *Pyricularia oryzae* (pathogen of rice blast), melanin was identified as a pathogenicity factor (Woloshuk et al. 1980). In *Brunneimargo camelliae,* the emerging germ tube of ascospores forms an appressorium-like structure (Fig. 5.59). This structure may also be involved in the infection of other hosts.

In the present phylogenetic analysis, all the species with ascospores that melanized before discharge were grouped into one highly supported clade and characterized as *Lambertella sensu stricto* (*s. s*). By contrast, the pigmentation of ascospores after discharge occurred in several independent clades. Therefore, melanization after ascospore discharge is regarded as a result of convergent evolution.

Ecologically, species with pigmented ascospores have no clear preference in their host, substrate, or season. Instead, spore pigmentation may be correlated with stroma formation (Fig. 3.4) because most of the pigmented ascospores are accompanied by stroma. Therefore, it is suggested that the metabolisms of melanization may be more developed in these fungi than others, and may be advanced during stress tolerance.

Septation is usually accompanied by spore pigmentation (e.g. Fig. 5.58, 5.62), which is suggested to provide an additional function during the early stage of spore

germination, but the actual role is unknown.

Further issues related to taxonomic research in Lambertella

The present study clearly shows that *Lambertella s. s.* is defined by ascospore pigmentation before discharge and by pigmented ectal excipulum composed of textura prismatica. In previous studies, this combination of characters is recognized in 25 known species (40%) of *Lambertella* (Dumont 1971; Korf and Zhuang 1985), thereby suggesting the presence of a well-defined monophyletic core group in the genus. However, it also suggests the diversity of the previous concept of *Lambertella*. The remaining *Lambertella* species requires careful allocation based on molecular phylogeny and morphological examinations that incorporate other genera, as well as taking into consideration revisions of the concept of other genera.

The present study illustrated the heterogeneity of the major groups of Helotiales: Rutstroemiaceae and Helotiaceae. As previously suggested (Wang et al. 2006), Helotiales is one of the most heterogeneous and diverse groups in ascomycetes. Most of the rutstroemiaceaeous members and a monophyletic group of Sclerotiniaceae were included in one highly supported clade (Clade A, Fig. 3.4). Merging Sclerotiniaceae is one possible solution to make Rutstroemiaceae more homogeneous, but it will result in a larger group that is equally defined by the previous concept (Whetzel 1945). In addition, the newly established group needs to be defined by extra characters other than stroma, because we now know that stroma is a result of convergent evolution. If the sclerotiniaceous group in subclade B (Fig. 3.4) is retained as a family, other small monophyletic families should also be established on the basis of the subclades (Fig. 3.4), and the taxonomy at the familial rank will be highly ambiguous. At

present, I indicate that merging Sclerotiniaceae in Rutstroemiaceae may be more realistic, but more species from the sclerotial members should be included to draw a more definite conclusion.

Possible application of taxonomy to agricultural science

Taxonomy provides the fundamental basis for understanding the components of biodiversity. It is also the basis of exploiting natural resources, because taxonomy provides knowledge about how natural groups (species and higher taxa) are arranged in a hierarchical manner. In agricultural science, taxonomy is the basic tool used for pathogen or pest detection and identification, thereby facilitating the control of diseases. Taxonomic information also plays essential roles in minimizing the risk of transferring pathogens or pests, which may cause economic losses and ecosystem damage. To explore further applications, sound taxonomy is required. The present study highlights how a sound taxonomy can be obtained on the basis of a comprehensive analysis of morphology, phylogeny, and the evaluation of various criteria using cultures.

According to the new concept for *Lambertella s. s.* in the present study, species having both substratal stroma and ascospores that are pigmented only after discharge, such as *H. pseudoalbidus*, are excluded from *Lambertella*. *Hymenoscyphus pseudoalbidus* is the emerging pathogen of ash dieback in Europe since 1992 and causes enormous damage to common ash (*Fraxinus excelsior*) (Bakys et al. 2009; Kowalski 2006). *Hymenoscyphus pseudoalbidus* was once confused with *Hymenoscyphus albidus*, which was previously accommodated in *Lambertella* based on the pigmentation of its ascospores. In Japan, *H. pseudoalbidus* occurs on the petioles of *Fraxinus mandshurica* but exhibits no pathogenicity. The pathogen of ash dieback is now clearly excluded

from *Lambertella s. s.* (Zhao et al., 2012), and *Lambertella s. s.* is currently characterized as including no pathogenic species. This treatment may help the correct diagnosis and characterization of this pathogen, which could improve the prevention of this disease.

Taxonomic knowledge could be also utilized in discovery and research of useful metabolites. After a specific useful compound is discovered in one taxon, other derivative compounds are usually found in taxonomically closely related groups (Omura 1986). A famous example is the isolation of penicillin and related compounds (β-lactams) from *Penicillium* spp. (Kavanagh 2013). Therefore, the classification and identification of microorganisms is fundamental in industry when searching for bioactive compounds. Thus reliable taxonomies and accurate identification procedures can improve the effectiveness of discovery (Bull et al. 1992). Sproston (1963) isolated an antibiotic compound (lambertellin) from *L. corni-maris* and *L. hicoriae*, and both species are now recognized as belonging to *Lambertella s. s.* according to the present study. Lambertellin has not yet been applied to agriculture, but other functions may be discovered in the future or derivative compounds may be exploited. To increase the possibility of discovery, it is more appropriate to work with taxonomically related groups than random screening. Focusing on *Lambertella s. s.* may result in the discovery of lambertellin derivatives with industrial value.

Table 4.1. Possible *Lambertella s. s.* species.

No.	Name	Literature
1	*Lambertella agaricicola* (Berk. & Broome) Dumont	Dumont 1971
2	*Lambertella aurantiaca* V.P. Tewari & D.C. Pant	Tewari and Pant 1967 Dumont 1971
3	*Lambertella berberidis* M.E. Elliott & M.P. Sharma	Dumont 1976b
4	*Lambertella bonahawensis* Dumont	Dumont 1971
5	*Lambertella cephalanthi* Whetzel & Dumont	Dumont 1971
6	*Lambertella corni-maris* Höhn	Dumont 1971
7	*Lambertella crystallina* S.K. Gautam	Gautam et al. 1982
8	*Lambertella fructicola* Dumont	Dumont 1971
9	*Lambertella garryae* (Cash) Dumont	Dumont 1971
10	*Lambertella hicoriae* Whetzel	Whetzel 1943
11	*Lambertella himalayensis* V.P. Tewari & D.C. Pant	Tewari and Pant 1967 Dumont 1971
12	*Lambertella jasmini* Seaver, Whetzel & Dumont	Dumont 1971
13	*Lambertella kumaonica* V.P. Tewari & Ram N. Singh	Tewari and Singh 1972
14	*Lambertella malesiana* Dumont	Dumont 1971
15	*Lambertella mexicana* Dumont	Dumont 1971
16	*Lambertella minutula* (Seaver) Dumont	Dumont 1975
17	*Lambertella phaeoparaphysata* Dumont	Dumont 1971
18	*Lambertella pruni* Whetzel, Zeller & Dumont	Dumont 1971
19	*Lambertella pseudostriata* Dumont	Dumont 1971
20	*Lambertella pyrolae* Y.-J. Zhao & T. Hosoya	Zhao et al. 2013
21	*Lambertella rhamnicola* (L.R. Batra) Dumont	Dumont 1971
22	*Lambertella venezuelensis* Dumont	Dumont 1971
23	*Lambertella verrucosispora* W.Y. Zhuang	Zhuang 1990

Summary

Helotiales is one of the largest groups of apothecial ascomycetes, having highly divergent characters in terms of their morphology, ecology, and biology. Morphological characters such as the shape and color of the apothecia have been useful for characterizing the families in Helotiales. The stroma is a complex hyphal structure that characterizes three closely related families with similar apothecial characters in this order: the family Helotiaceae has no stroma whereas Rutstroemiaceae has a substratal stroma and Sclerotiniaceae has a sclerotial stroma. There has been little taxonomic research concerning Rutstroemiaceae and only seven genera are known, most of which are defined by differences in the structure of the outer layer of the apothecia (ectal excipulum).

Lambertella is one of the largest genera in Rutstroemiaceae with 63 species. It is an important group in agricultural sciences because some species are known to produce antibiotics, as well as to be involved in mycoparasitism with plant pathogens. *Lambertella* is distinguished from other genera by the pigmentation of its ascospores, which may occur before or after discharge of ascospores from the asci. The polyphyly of *Lambertella* was suggested by a previous study but only a limited number of members were included in the analysis. Moreover, the pigmentation of ascospores has been regarded as a unique feature of Rutstoremiaceae, but it may be easily overlooked if inappropriate specimens are observed. In addition, the current concept about *Lambertella* includes diverse ectal excipular structures, whereas it originally comprised only prismatic cells. Although there is an overlap with other genera, a comprehensive taxonomic reexamination of *Lambertella* and allied genera has not been conducted. Therefore, the objectives of the present study were to re-circumscribe *Lambertella* and

to clarify the phylogenetic relationships among *Lambertella* and other allied genera in Rutstroemiaceae, Sclerotiniaceae, and Helotiaceae.

Fresh apothecia were collected from Japan to observe the morphological characters, particularly the color of the ascospores after discharge. Cultures were obtained from single ascospores to observe the formation of stroma. DNA samples were extracted from the apothecia or cultures and the sequences of three regions (ITS-5.8S, LSU, and RPB2) were used in the phylogenetic analysis. In total, 58 taxa were examined using fresh apothecia, isolates from fresh apothecia, and strains from a culture collection (35 from Rustroemiacea, 7 from Sclerotiniaceae, and16 from Helotiaceae).

Two major clades (Clade A and B) were generated with high reliability by the maximum-parsimony and maximum-likelihood analyses based on the combined LSU and RPB2 sequences. All of the sclerotiniaceous members were included in a highly supported subclade in Clade A. Most of the rutstroemiaceous members were included in Clade A but two rutstroemiaceous members were included in Clade B. Most of the helotiaceous members were included in Clade B and three helotiaceous members were excluded from Clade A or B. The monophyly of Sclerotiniaceae was confirmed and the polyphyly of Rutstroemiaceae and Helotiaceae was suggested. In the culture studies, three species of *Hymenoscyphus* (Helotiaceae) in Clade B were found to have substratal stroma, thereby suggesting that the substratal stroma is a convergent character.

Species with substratal stroma and pigmented ascospores were distributed in the four subclades and four independent lineages. The polyphyly of *Lambertella* according to the current definition was confirmed again. A highly supported clade of five species of *Lambertella*, including the type species, was recognized. Morphologically, they were exclusively supported by the pigmentation of the ascospores before discharge and by the

brownish, thin-walled, typical prismatic cells in ectal excipulum. This clade was regarded as *Lambertella sensu stricto* (*s. s.*). Pigmentation of the ascospores was also recognized in non-*Lambertella* genera (e.g., *Hymenoscyphus* and *Poculum*), but only after discharge. *Lambertella* sp. 2 and species of *Lanzia*, *Moellerodiscus*, and *Poculum* were included in one highly supported clade, but no common morphological characters were recognized. A new genus *Crassitunica* was proposed because *Lambertella* sp. 2 has clearly different characters compared to other genera. Both *Lambertella* sp. 1 and *L. advenula* formed a lineage of their own, and were morphologically different from other allied genera, therefore two new genera were proposed, *Brunneimargo* and *Luteidiscella*, respectively. *Lambertella yunnanensis* was included in a highly supported clade that contained the type species of *Hymenoscyphus* and a new combination was proposed.

Based on the present phylogenic and morphological studies, the phylogenetic relationships of *Lambertella s. l.* and allied genera were clarified and the important morphological characters for taxonomic criteria were re-evaluated. In conclusion, *Lambertella* was re-circumscribed and three new genera in Rutstroemiaceae were described. The present study also demonstrated the need for further comprehensive analyses incorporating the helotiaceous members.

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Appendix

In the present study, I collected 44 species of Rutstroemiaceae, Helotiaceae, and Sclerotiniaceae from the field. The present appendix provides descriptions and illustrations for 38 rutstroemiaceous and helotiaceous members (Table 5.1). The collection from the field greatly expanded the previous knowledge of mycobiota and contributed to the fungal inventory in Japan. The collected taxa includes 7 newly reported species, 2 new combinations, 3 new species (see text in Chapter 3–5) (Table 5.1).

As discussed previously in Chapter 3, *Hymenoscyphus*, *Lanzia*, *Moellerodiscus* and *Poculum* have taxonomic uncertainty, but they were listed based on the conventional concept. The species are listed based on the conventional concept (see Table 3.1), and the taxonomic treatment based on the present study is also given (Table 5.1).

Table 5.1. Species from Japan with descriptions and illustrations in appendix

No.	Name	Substrate	Taxonomic status or treatment
1	*Dicephalospora rufocornea* (Berk. & Broome) Spooner	Unknown branch	
2	*Hymenoscyphus caudatus* (P. Karst.) Dennis	*Alnus* fruit	
3	*Hymenoscyphus fructigenus* (Bull.) Gray	Unknown acorns	
4	*Hymenoscyphus ginkgonis* J.G. Han & H.D. Shin	*Ginkgo biloba* leaf	
5	*Hymenoscyphus menthae* (W. Phillips) Baral	*Hydrangea* flower	New report to Japan
6	*Hymenoscyphus microserotinus* (W.Y. Zhuang) W.Y. Zhuang	*Aesculus turbinata* branch	New report to Japan
7	*Hymenoscyphus pseudoalbidus* Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr.	*Fraxinus mandshurica* petiole	
8	*Hymenoscyphus scutula* (Pers.) W. Phillips	Unknown petiole	New report to Japan
9	*Hymenoscyphus varicosporoides* Tubaki	Herb stem	
10	*Hymenoscyphus* sp. 1	Unknown petiole	
11	*Hymenoscyphus* sp. 2	Herb stem	
12	*"Hymenoscyphus"* sp. 1	Unknow leaf	
13	*"Hymenoscyphus"* sp. 2	Unknown acorns	
14	*"Hymenoscyphus"* sp. 3	Unknown fruit	
15	*"Hymenoscyphus"* sp. 4	Unknown acorns	
16	*"Hymenoscyphus"* sp. 5	Unknown branch	
17	*Lambertella advenula* (W. Phillips) Hosoya & Y. Otani	*Larix* needle	*Luteidiscella advenula* (W. Phillips) Y.-J. Zhao & T. Hosoya. comb. nov.
18	*Lambertella corni-maris* Höhn.	*Mallotus japonicus* leaf *Torreya nucifera* fruit	New hosts
19	*Lambertella pyrolae* Y.-J. Zhao & T. Hosoya	*Pyrola incarnata* leaf and petiole	
20	*Lambertella yunnanensis* (S.H. Ou) W.Y. Zhuang & Yan H. Zhang	Unknown branch	*Hymenoscyphus yunnanense* (S.H. Ou) Y.-J. Zhao & T. Hosoya, comb. nov.
21	*Lambertella* sp. 1	*Camellia japonica* leaf	*Brunneimargo camelliae* Y.-J. Zhao & T. Hosoya sp. nov.

Table 5.1. Species from Japan with descriptions and illustrations in appendix **(Continued)**

No.	Name	Substrate	Taxonomic status or treatment
22	*Lambertella* sp. 2	*Aucuba japonica* leaf and petiole, *Fatsia japonica* petiole	*Crassitunica tsubakii* Y.-J. Zhao & T. Hosoya sp. nov.
23	*Lanzia longipes* (Cooke & Peck) Dumont & Korf	Unknown petiole	New report to Japan
24	*Lanzia pruni-serotinae* (Whetzel & W.L. White) M.P. Sharma & R.M. Sharma	*Prunus grayana* leaf	New report to Japan
25	*Lanzia* sp. 1	Unknown leaf	
26	*Lanzia* sp. 2	*Fagus crenata* fruit	
27	*Lanzia* sp. 3	*Castanea crenata* involucre and spine	
28	*Lanzia* sp. 4	*Swida controversa* leaf	
29	*Lanzia* sp. 5	*Pyrus pyrifolia* leaf *Prunus* leaf	
30	*Lanzia* sp. 6	Herb stem	
31	*Lanzia* sp. 7	Unknown leaf	
32	*Moellerodiscus pinicola* Y. Otani	*Pinus luchuensis* leaf *Pinus densiflora* leaf	*Rutstroemia pinicola* (Y. Otani) Y.-J. Zhao & T. Hosoya, comb. nov.
33	*Moellerodiscus* sp. 1	Unknown plant petiole	
34	*Moellerodiscus* sp. 2	Unknown branch	
35	*Phaeohelotium epiphyllum* (Pers.) Hengstm.	*Populus maximowiczii* leaf	New report to Japan
36	*Poculum* sp.	Unknown branch	
37	*Poculum sydowianum* (Rehm) Dumont	*Quercus crispula* petiole *Quercus serrata* petiole	New hosts New report to Japan
38	*Rutstroemia bulgarioides* P. Karst	*Picea jegoensis* fruit	

1. *Dicephalospora rufocornea* (Berkeley & Broome) Spooner **Figs. 5.1–5.3**

Biblthca Mycol. 116: 272 (1987)

Helotium rufocorneum Berk. & Broome, J. Linn. Soc., Bot. 14: 108 (1873) [1875]

Hymenoscyphus rufocorneus (Berk. & Broome) Dennis, Persoonia 3: 62 (1964)

Lanzia rufocornea (Berk. & Broome) Dumont, Mycotaxon 12: 272 (1980)

Stroma substratal, visible as blackened areas on the substrate. **Apothecia** stipitate, occurring on decaying branches; disc concave or convex, 1–2 mm in diameter in dried specimen; hymenium yellowish-orange to orange (151C= R255 G121 B0); receptacle smooth, pale yellow to yellow; stipe concolorous with the receptacle, 0.6–1.8 mm long when dry, smooth, becoming black toward the base. **Ectal excipulum** textura prismatica, composed of slightly thick-walled, narrow, prismatic cells of 4–7×10–25 μm in flank and 2–4×3–12 μm in margin, smooth, pale brown to subhyaline. **Medullary excipulum** textura intricata, hyaline, smooth, thin walled, separate hypha of 2–3 μm wide. **Asci** 138–157×8–12 μm, cylindric-clavate, short stipitate, 8-spored, arising from simple septa; apex obtusely conical or broadly papillate, 3–4 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment, some surrounded by a diffuse blueing. **Ascospores** 28–49×3–5 μm (40.1 ± 5.0×4.2 ± 0.5 μm on average ± SD, n= 20), biseriate or biseriate above, uniseriate below in asci, fusoid or clavate-fusoid, capped with a small, obconical gelatinous collar, hyaline, non-septate, eguttulate or with a row of large guttules. **Paraphyses** filiform, obtuse, septate, hyaline, exlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 40 mm after 4 weeks incubation, irregular, flocculent, zonate, initially white to pale brown, becoming brown in age; Aerial mycelium whitish to pale brown, little developed; Margins undulate, pale brown; Reverse zonate, brown in the center, becoming paler towards margin. Rind diffused, vertically not well developed.

Specimens examined – On decayed wood or branches: TNS-F-40013, Mt. Mikamo, Tochigi Pref., 16 May 2011, Col. Y.-J. Zhao; TNS-F-40024 (Culture FC-2730), Otomi, Iriomote Isl., Okinawa Pref., 12 June 2011, Col. T. Hosoya; TNS-F-40094,Yichinoya, Tsukuba City, Ibaraki Pref., 20 Oct. 2011, Col. Y.-J. Zhao; TNS-F-40107, Kenmin-no-mori, Onnna-son, Kunigami-gun, Okinawa Pref., 28 Apr. 2012, Col. Y.-J. Zhao; TNS-F-40122, Bannna Park, Ishigaki-shi, Okinawa Pref., 15 June 2012, Col. T. Hosoya; TNS-F-40125, Fukiage Garden, Kokyo, Chiyoda-ku, Tokyo, 20-6-2012.Col. T. Hosoya; TNS-F-40124, Yichinoya Residence Hall, University of Tsukuba, Tsukuba City, Ibaraki Pref., 18 June 2012, Col. Y.-J. Zhao; TNS-F-40123, Bannna Park, Ishigaki-shi, Okinawa Pref., 15 June 2012, Col. T. Hosoya; TNS-F-40155, Hanazono Shrine, Kitaibaraki-shi, Ibaraki Pref., 26 Sep. 2012, Col. Y.-J. Zhao; TNS-F-48454, Shiroyama Park, Saeki-shi, Oita Pref., 5 Nov. 2012, Col. T. Hosoya; TNS-F-48890, Kantou, Higashiarita, Hita-shi, Oita Pref., 27 Nov. 2012, Col. Y. Murakami; TNS-F-48447, Mt. Takao, Oita-shi, Oita Pref., 4 Nov. 2012, Col. T. Hosoya; TNS-F-40185, Shiroyama Koen Park, Saeki-shi, Oita Pref., 5 Nov. 2012. Col. Y.-J. Zhao.

Notes – The morphological features are well agreed with the description of *D. rufocornea* (Dennis 1964; Dumont 1980; Spooner 1987). The ascospores of Japanese

collections are slightly longer than those stated in the previous descriptions (Spooner 1987), but the other features are perfectly agreed. *Dicephalospora rufocornea* mainly occurs on woody and herbaceous plant parts (Spooner 1987). It is widely distributed throughout Japan.

2. *Hymenoscyphus caudatus* (P. Karst.) Dennis **Figs. 5.4–5.6**

Persoonia 3: 76 (1964)

Peziza caudata P. Karst., Fungi Fenniae Exsiccati, Fasc. 6: no. 547 (1866)

Peziza caudata P. Karst., Not. Sällsk. Fauna et Fl. Fenn. Förh. 10: 144 (1869)

Helotium caudatum (P. Karst.) Velen., Monogr. Discom. Bohem. (Prague) 1: 206 (1934)

Apothecia stipitate, occurring on fallen fruits of *Alnus*; disc flat to cupulate, 0.3–0.7 mm in diameter in dried specimen; hymenium whitish yellow when fresh, becoming yellowish to ochraceous (1215C= R250 G221 B128) when dry; receptacle smooth, whitish to whitish yellow; stipe concolorous with the receptacle, 1–1.6 mm long when dry, smooth. **Ectal excipulum** two layered: outer layer textura porrecta or prismatica, 37.5–62.5 μm thick, composed of smooth, hyaline or subhyaline, thin-walled or slightly thick-walled, brick-shaped cells, 2–5.5 μm wide, outmost layer with slightly pale brown; inner layer 12.5–20 μm thick, composed of slightly thick-walled, subhyaline to hyaline, smooth, separate hypha of ca.2.5 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of 2.5–4 μm wide. **Asci** 65–80×7–9 μm, cylindric-clavate, 8-spored, croziers absent or obscure; apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment.

Ascospores 14.5–21×3–4 μm (18.1 ± 1.6×3.7 ± 0.4 μm on average ± SD, n= 20), uniseriate or irregularly biseriate above, elongate, clavate-ellipsoid or with apically hooked, obtuse at the proximal end and pointed towards the distal end, with 0–3 guttules, non-septate. **Paraphyses** filiform, apex obtuse, septate, hyaline, enlarged at the apex up to 2.5–3.5 μm wide.

Cultural characteristics – Colonies on PDA grows normal, reach about 60 cm after 4 weeks incubation, irregular, cottony, pallid in the margin, grayish in the central; Aerial mycelium gray, partial developed; Margins undulate, pallid; Reverse pale brown in the central, with black pigmentation, margin pallid.

Specimens examined – On fallen fruits of *Alnus* sp.: TNS-F-40056 (Culture FC-2803), Kokenodomon Gulley, Shikotsu Toya National Park, chitose-shi, Hokkaido (42º42'45.60"N, 141º19'11.80"E, Alt. 448m), 14 Sep. 2011, Col. Y.-J. Zhao.

Notes – The morphological characters of present fungus are concordant with the descriptions of *H. caudatus* (Dennis 1964; Lizoň 1992; White 1943) which has never been reported in Japan. The asci of Japanese collections are slightly shorter than those stated in the previous description (85–105 μm), but other features perfectly agreed. The pigmentation of ascospores prior to germination and the *Idriella* anamorph (Kimbrough and Atkinson 1972) were not observed in Japanese collection.

Hymenoscyphus caudatus was considered to be a foliicolous species mainly occurring on fallen leaves of broad-leaved trees (Lizoň 1992; White 1943). It is the first time to be reported from fallen fruits. *Hymenoscyphus scutula* (Pers.) W. Phillips and

Hymenoscyphus serotinus (Pers.) W. Phillips are comparable taxa with scutuloid ascospores, but they all have the longer asci, darker apothecia and the textura porrecta in medullary excipulum (Lizoň 1992; White 1943).

3. *Hymenoscyphus fructigenus* (Bull.) Gray **Figs. 5.7–5.9**

British Plants 1: 673 (1821)

Helotium fructigenum (Bull.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 314 (1870) [1869–70]

Phialea fructigena (Bull.) Gillet, Champignons de France, Discom. (4): 99 (1881) [1879]

Helotium virgultorum var. *fructigenum* (Bull.) Rehm, in Winter, Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.3 (lief. 39): 783 (1893) [1896]

Ciboria fructigena (Bull.) Killerm., Kryptogamenflora Forsch. Bayer. Bot. Ges. Erforsch Leim. Flora 2: 278 (1935)

Hymenoscyphus fructigenus var. *coryli* (Feuilleaub.) Hengstm., Persoonia 12: 489 (1985)

Apothecia stipitate, occurring on fallen acorns of broad-leaved trees; disc cupulate or saucer-shaped, 0.6–1.1 mm in diameter in dried specimen; hymenium whitish to ochre-whitish when fresh, becoming pale brown (148PC = C0 M17 Y42 K0) when dry; receptacle smooth, whitish to pale brown, concolorous with hymenium; stipe concolorous with the receptacle, 0.3–2.8 mm long when dry, smooth. **Ectal excipulum** two layered: outer layer textura prismatica, composed of smooth, hyaline, thin-walled,

brick-shaped cells, 2–8×7–1.6 μm in the margin; inner layer composed of thin walled, hyaline, smooth, separate hypha of 2.5–3 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of 2–4 μm wide. **Asci** 85–110×6–8 μm, cylindric-clavate, 8-spored, arising from simple septa; apex conical, 2.5–3.5 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 15.5–18.5×3–4 μm (16.9 ± 1.0×3.6 ± 0.4 μm on average ± SD, n= 20), uniseriate or irregularly biseriate, cylindric-fusoid or slightly inequilateral, rounded at the proximal end and pointed towards the distal end, 0-multi guttules, non-septate or rarely 1-septate, becoming 1-septae and fusoid when germinated. **Paraphyses** filiform, apex obtuse, septate, hyaline, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows normal, reach about 50 cm after 4 weeks incubation, circular, cottony, grayish to pale brown, curled; Aerial mycelium gray, partly developed; Margins entire; Reverse curled, gray to brown, with brown pigmentation, margin gray.

Specimens examined – On fallen acorns of *Quercus* sp.: TNS-F-40058 (Culture FC-2805), Tomakomai Experimental Forest, Hokkaido University, Tomakomai-shi, Hokkaido (42º40'21.70"N, 141º36'26.60"E, Alt. 22m), 15 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40062 (Culture FC-2807), Tyouju no mori, Sugadaira Montane, Ueda City, Nagano Pref. (36º31'35.00"N, 138º20'26.00"E, Alt. 1268m), 27 Sep. 2011, Col. Y.-J. Zhao,; TNS-F-44644 (Culture FC-2855), Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo (35º40'36.15"N, 139º42'3.12"E, Alt. 29m), 8 Nov. 2011, Col. T. Hosoya,; TNS-F-40164 (Culture FC-5092), Takayama-mura, Gunma Pref. (36º35'19.30"N, 138º57'36.50"E, Alt.

242m), 2 Oct. 2012, Col. Y.-J. Zhao.

Notes – *Hymenoscyphus fructigenus* mainly occurrs on pericarps, acorns and nuts of broad-leaved tree, and also on wood and twigs (Lizoň 1992). It is widely distributed from Europe to Asia. The diagnostic characters of *H. frutigenus* are the long-stipitate and whitish apothecia, and the cylindric-fusoid ascospores. The Japanese materials showed somewhat shorter asci (85–110 μm) than Europe and North American materials (with 200 μm long, Lizoň 1992).

4. *Hymenoscyphus ginkgonis* J.G. Han & H.D. Shin Figs. 5.10–5.12

Mycotaxon 103: 192 (2008)

Apothecia stipitate, occurring on fallen trees; disc cupulate, 0.2–0.6 mm in diameter in dried specimen; hymenium beige to grayish orange when fresh, becoming black when dry; receptacle smooth, whitish yellow to beige when fresh, becoming brown to dark brown when dry; stipe concolorous with the receptacle, 0.6–2 mm long when dry, smooth. **Ectal excipulum** textura prismatica, composed of smooth, hyaline, thick-walled, brick-shaped cells, 15–30×7–12 μm, external cells sometimes elongating to form hairs. **Hairs** cylindrical, smooth, aseptate to few-septate, 3–5 μm thick, hyaline or with dark purple pigments in the upper. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of 2–4 μm wide. **Asci** 75–97×7–9 μm, cylindric-clavate, 8-spored, arising from simple septa; apex conical, 2.5–3.5 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 13–18×4–6 μm (16.4 ± 1.5×5.3 ± 0.52 μm on average ± SD, n= 25), uniseriate or

irregularly biseriate, subelliptic to elliptic with acute lower end, 2-multi guttules, non-septate or 1-septate, hyaline; becoming brown and 1-septae when germinated. **Paraphyses** cylindrical, apex obtuse, septate, hyaline or with dark purple pigments in the upper, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 15 cm after 4 weeks incubation, circular, orange, tough; Aerial mycelium white, little developed; Margins entire; Reverse filiform, brown. Rind inconspicuous, diffused and blackened, merged in the hyphal structure.

Specimens examined – On leaves of *Ginkgo biloba*: TNS-F-11208 (Culture FC-1093), Fuchizawa horyo, Towada-ko-machi, Aomori, 25 Aug. 2004, Col. T. Hosoya; TNS-F-13249, Green house in Hirosaki University, Hirosaki-shi, Aomori, 22 July 2006, Col. Y. Harada.

Notes – The morphological features well agreed with the previous description of *H. ginkgonis* (Han and Shin 2008). The diagnostic character of *H. ginkgonis* is the dark purple pigments in hairs and paraphyses. The pigmentation of ascospores in germination was firstly observed by Hosoya (personal comment) used the same Japanes material.

5. *Hymenoscyphus menthae* (W. Phillips) Baral **Figs. 5.13–5.15**

Baral & Krieglsteiner, Beih. Z. Mykol. 6: 131 (1985)

Helotium menthae W. Phillips, Elv. Brit: no. 188 (1877)

Hymenoscyphus scutula var. *menthae* W. Phillips, Man. Brit. Discomyc. (London): 137

(1887)

Phialea scutula var. *menthae* (W. Phillips) Sacc., Syll. fung. (Abellini) 8: 266 (1889)

Helotium scutula var. *menthae* (W. Phillips) Boud., Hist. Class. Discom. Eur. (Paris): 114 (1907)

Apothecia stipitate, occurring on fallen fruits of *Hydrangea* sp.; disc flat to cupulate, 0.2–0.6 mm in diameter in dried specimen; hymenium whitish yellow when fresh, becoming yellowish (1215PC = C0 M8 Y48 K0) to pale brown when dry; receptacle smooth, concolorous with hymenium or brownish; stipe concolorous with the receptacle or slightly paler than receptacle, 0.2–0.7 mm long when dry, smooth. **Ectal excipulum** 50–85 μm thick, two layered: outer layer textura porrecta or prismatica, composed of smooth, hyaline or subhyaline, thin-walled or slightly thick-walled, brick-shaped cells, 4–5.5 μm wide, outmost layer with slightly pale brown; inner layer composed of slightly thick-walled, hyaline, smooth, separate hypha of 2.5–3 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of ca. 2.5 μm wide. **Asci** 80–95×5–9 μm, cylindric-clavate, 8-spored, arising from croziers but obscure; apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 15–21.5×3–4.5 μm (18 ± 2.1×3.8 ± 0.4 μm on average ± SD, n= 20), uniseriate or irregularly biseriate, clavate-ellipsoid or clavate-fusoid, rounded at the proximal end and pointed towards the distal end, with 0–2, globosa or irregular guttules, non-septate. **Paraphyses** filiform, apex obtuse, septate, hyaline, enlarged at the apex up to 2.5–4 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 43 cm after 4

weeks incubation, irregular, cottony, grayish to pale brown, curled, dark in the central; Aerial mycelium gray, little developed; Margins undulate, pallid; Reverse pale brown in the central, with black pigmentation, margin pallid.

Specimens examined – On fallen fruits of *Hydrangea* sp.: TNS-F-40052 (Culture FC-2800), Hukkonomori, Shikotsu lake, chitose-shi, Hokkaido (42º45'35.10"N, 141º26'23.10"E, Alt. 243m), 14 Sep. 2011, Col. Y.-J. Zhao.

Note – This is the first report of *Hymenoscyphus menthae* from Japan. It is also the first time to be reported from *Hydrangea*. *Hymenoscyphus menthae* is distinguished by its whitish yellow apothecia and small ascospores (Baral and Krieglsteiner 1985). *Hymenoscyphus caudatus* is similar with *H. menthae* in many aspects of microscopic characters, but the former have apically hooked ascospores (Dennis 1964; Lizoň 1992; White 1943).

6. *Hymenoscyphus microserotinus* (W.Y. Zhuang) W.Y. Zhuang Figs. 5.16–5.18

Zhuang & Liu, Mycotaxon 99: 128 (2007)

Lanzia microserotina W.Y. Zhuang, Mycosystema 8–9: 32 (1996)

Stroma substratal, rind not obviously, some apothecia with black rings in the base. **Apothecia** stipitate, mainly occurring on decaying petioles, midribs, rarely on leaves; disc discoid to cupulate, 0.4–0.9 mm in diameter in dried specimen; hymenium white to pale yellow (7401 PC = C0 M5 Y25 K0) when fresh, the color nearly unchanged when

dry; receptacle smooth, slightly paler or concolorous with hymenium when fresh or dry; stipe paler or concolorous with the receptacle, 0.5–2.5 mm long when dry, smooth, blackish towards the base. **Ectal excipulum** two layered: outer layer textura prismatica to angularis, composed of thin-walled cells of 6–25×3–10 μm, smooth, pale brown, becoming brownish towards the margin; inner layer composed of thin walled, subhyaline to pale brown, smooth, separate hypha of ca. 3 μm wide. **Medullary excipulum** textura porrecta, subhyaline to pale brown, smooth, brick-shaped cells of 8–12×3–5 μm. **Asci** 67–78×6–7 μm, cylindric-clavate, 8-spored, arising from simple septa; apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 15–22×2–4 μm (17.6 ± 1.8×3.3 ± 0.6 μm on average ± SD, n= 20), biseriate in asci, scutuloid, non-septate, mostly with two guttules, some of eguttulate or with 3–4 guttules. **Paraphyses** filiform, obtuse, septate, hyaline, arising from cells of medullary excipulum, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, irregular, cottony, initially whitish to yellowish orange (Pantone 1375C= R255 G160 B47), becoming dark brown in age, forming the orange, irregular shaped hyphae plate; Aerial mycelium white or yellowish orange, little developed; Margins undulate; Reverse orange, irregular hyphae plate visible as forming zonatly; Rind vertically not well developed but clear, textura globulosa in face view.

Specimens examined – On decaying petioles, midribs and leaves of *Aesculus turbinata* Blume, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt.1340m), Col. Y.-J. Zhao: TNS-F-40067

(Culture FC-2811), 27 Sep. 2011; TNS-F-40149 (Culture FC-5084), 15 Sep. 2012.

Notes – *Hymenoscyphus microserotinus* was firstly reported as *Lanzia* species because it was considered to have substratal stroma based on the blackish in stipe base (Zhuang 1996). However, in the phylogenetic analysis, it closely related with *Hymenoscyphus* species, and it was proposed to include in *Hymenoscyphus* (Zhuang and Liu 2007). The phylogenetic analysis conducted in the present research confirmed the result of Zhuang and Liu (2007), but the substratal stroma was clearly discovered in culture.

The present fungus was closely related with *Lambertellinia scutuloides* Korf & Lizoň in having the same sized scutuloid ascospores and textura porrecta structure in medullary excipulum (Korf and Lizoň 1994). *Lambertellinia scutuloides* also occurring on *Aesculus turbinate*, and its holotype was collected from Japan (Korf and Lizoň 1994). However, the present fungus lacks the brown ascospores and gelatinous structure in ectal excipulum.

7. *Hymenoscyphus pseudoalbidus* Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr. Figs. 5.19–5.21

For. Path. 41: 140 (2011)

Stroma substratal, visible as the blacked areas in substrate. **Apothecia** stipitate, occurring on fallen petioles; disc flat or convex, 0.3–2 mm in diameter in dried specimen; hymenium white when fresh, becoming pale brown to brown (1215C= R250 G221 B128) when dry; receptacle smooth, concolorous with hymenium; stipe concolorous with the receptacle, 1–3 mm long when dry, smooth, becoming pale brown

to brown toward base. **Ectal excipulum** three layered: outer covering layer lack or obscure in margin, obviously in flanks and stipe, subhyaline to pale brown, slightly granulate, thin-walled, composed 2–5 layers, 2–5.5 μm wide hyphae; outer ectal excipulum textura prismatica, composed of smooth, hyaline or subhyaline, thin-walled or slightly thick-walled, brick-shaped cells, 7.5–12.5×17.5–37.5 μm; inner layer thin-walled, hyaline, smooth, separate hypha of 3–5 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of ca. 5 μm wide. **Asci** 80–116×8–10 μm, cylindric-clavate, 8-spored, arising from croziers; apex conical, 3–4 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 13.5–19×3–4.5 μm (17 ± 1.5×3.8 ± 0.4 μm on average ± SD, n= 30), uniseriate or biseriate, elongate, clavate-ellipsoid or with apically hooked, obtuse at the proximal end and pointed towards the distal end, some with 1–2 large, irregular shaped guttules or with multi small guttules, non-septate; when germinated on PDA, ascospores becoming brown, multi-guttulate, slightly thick-walled and with 1-septate in the central. **Paraphyses** cylindric, septate, hyaline, 3–4 μm thick.

Cultural characteristics – Colonies on PDA grows normal, reach about 75 cm after 4 weeks incubation, irregular, cottony, white to pale brown,becoming darker in age; Aerial mycelium gray, partial developed; Margins undulate, pallid to pale brown; Reverse pale brown, margin pallid; Rind diffused, blackish, merged in the hyaline hyphae structure. **Spermatia and Spermatiophores** produced abundantly on PDA; Spermatia hyaline, bacilliform or globosa, 2–3.5 μm in diameter, clustered in mass at the tipe of spermatiophores; Spermatiophores flask-shaped with long collar, septate or asceptate, 10–17 μm long, pale brown.

Specimens examined – On fallen petioles of *Fraxinus mandshurica* Rupr.: TNS-F-52060, Tomakomai-shi, Hokkaido, July 1990, Col. T. Hosoya; TNS-F-52061, July 1990, Taki-no-ue Park, Yubari-shi, Hokkaido, Col. T. Hosoya; TNS-F-52062, Sugadaira Montane Research Center, Sugadaira, Sanada-machi, Nagano Pref., June 1992, Col. T. Hosoya; TNS-F-17817, Tsukuba University Sugadaira Montane Research Center, Ueda-shi, Nagano Pref., 7 Sep. 2005, Col. T. Shirouzu; TNS-F-12503, Tsukuba University Sugadaira Montane Research Center, Ueda-shi, Nagano Pref., 10 Sep. 2006, Col. T. Hosoya; TNS-F-12761 (Culture FC-1445), Tsukuba University Sugadaira Montane Research Center, Ueda-shi, Nagano Pref. (36º31'20.60"N, 138º20'56.70"E, Alt. 1346m) , 11 Sep. 2006, Col. T. Hosoya; TNS-F-40043 (Culture FC-2793), Nishikionuma Park, Tomakomai-shi, Hokkaido (42º36'39.60"N, 141º27'10"E, Alt. 27m), 12 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40051 (Culture FC-2799), Hukkonomori, Shikotsu lake, chitose-shi, Hokkaido (42º45'35.1"N, 141º26'23.1"E, Alt. 243m), 14 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40074, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'23.8"N, 138º20'53.2"E, Alt. 1311m), 28 Sep. 2011, Col. Y.-J. Zhao.

Notes – The present species was reported as a new record for Japan by Hosoya et al. (1993) on *F. mandshurica* under the name "*Lambertella albida* (Gillet) Korf", and spermatia was produced. In Europe and North America, "*L. albida*" is commonly occurring on petioles of common ash (*Fraxinus excelsior*), and it was reported as the casual agent of an emerging infectious disease on common ash called ash dieback (Kowalski and Holdenrieder 2009). Further research revealed that "*L. albida*" was a

mix-conception of two species which can be easily distinguished by molecular methods, and the closely relationship with *Hymenoscyphus* was suggested (Queloz et al. 2011; Zhao et al. 2012). The phylogenetic analysis showed that Japanese "*L. albida*" is conspecific with *H. pseudoalbidus* (Zhao et al. 2012).

8. *Hymenoscyphus scutula* (Pers.) W. Phillips [as '*scutulus*'] **Figs. 5.22–5.24**

Man. Brit. Discomyc. (London): 136 (1887)

Peziza scutula Pers., Mycol. eur. (Erlanga) 1: 284 (1822)

Helotium scutula (Pers.) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 110 (1871)

Phialea scutula (Pers.) Gillet, Champignons de France, Discom. (4): 108 (1881) [1879]

Hymenoscyphus scutula var. *fucatus* W. Phillips, Man. Brit. Discomyc. (London): 137 (1887)

Apothecia stipitate, occurring on decaying herb stems; disc flat, cupulate or concave, 0.4–1.8 mm in diameter in dried specimen; hymenium ochraceous-yellow to pale brown (150 PC = C0 M35 Y70 K0) when dry; receptacle smooth, yellow to ochraceous when dry; stipe yellow to ochre, concolorous with the receptacle or slightly darker, 0.6–1.4 mm long when dry, flocculose. **Ectal excipulum** two layered: outer layer textura prismatica, 12.5–50 μm thick, composed of smooth, hyaline, brick-shaped cells, 3–5×6–18 μm in the margin, thin-walled or slightly thick-walled in flank; inner layer composed of thin walled, hyaline, smooth, separate hypha of 2.5–3 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, separate hypha of 1.5-3 μm wide. **Asci** 110–135×8–10 μm, cylindric-clavate, 8-spored, arising from simple septa;

apex conical, 3–4 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 17–26×3.5–5 μm (23.2 ± 2.4×4.1 ± 0.3 μm on average ± SD, n= 20), biseriate, scutuloid, often curved, non-septate or 1-septate, some with cilia on one or both ends, 1–2 large guttules, rarely with muliti guttules or lacking guttules. **Paraphyses** filiform, apex obtuse, septate, hyaline, sometimes with granular deposit in the upper, enlarged at the apex up to 3–4 μm wide.

Cultural characteristics – Colonies on PDA grows fast, nearly covering the whole plate after 4 weeks incubation, circular, cottony, grayish to pale brown, curled; Aerial mycelium gray, partly developed; Margins entire; Reverse curled, gray to reddish brown, with orange pigmentation.

Specimens examined – On decaying herb stems: TNS-F-17507 (Culture FC-1080), Chino City, Nagano Pref. (35º59'0.1"N, 138º18'9.3"E), 2004, Col. T. Hosoya.

Notes – *Hymenoscyphus scutula* was firstly reported in Japan occurring on the stem of *Artemisia* (Otani 1966). *Hymenoscyphus scutula* is worldwidely distributed, especially endemic in tropical or subtropical regions, mainly occurring on herbaceous stems (particularly Asteraceae) (Lizoň 1992). It is characterized as having the long-stipitate apothecia and the scutuloid ascospores.

9. *Hymenoscyphus varicosporioides* Tubaki **Figs. 5.25–5.27**

Trans. Br. mycol. Soc. 49: 345 (1966)

Apothecia stipitate, occurring on rotting twigs; disc flat to cupulate, 0.3–1 mm in diameter in dried specimen; hymenium whitish when fresh, becoming yellowish to orange-yellow (136C= R255 G188 B61) when dry; receptacle smooth, concolorous with hymenium; stipe whitish when fresh, becoming beige or whitish yellow when dry, 0.2–0.5 mm long when dry, smooth. **Ectal excipulum** two layered: outer ectal excipulum 15–28 μm thick, textura angularis, prismatic or glubosa, composed of irregular, variable cells, smooth, hyaline, thin-walled, 15–25×10–15 μm; inner layer 12.5–50 μm thick, thin-walled, hyaline, smooth, separate hyphae of 1.5–2.5 μm wide. **Medullary excipulum** textura intricata, subhyaline, smooth, thin-walled, separate hyphae of 1.5–2.5 μm wide. **Asci** 70–95×7–9 μm, cylindric-clavate, 8-spored, arising from simple septate; apex round or flat, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 9–17.5×4–5 μm (13 ± 2 × 4.6 ± 0.5 μm on average ± SD, n= 20), uniseriate or irregularly biseriate, clavate-ellipsoid or ellipsoid-fusoid, some slightly curved, 0–multi guttules, non-septate or 1-septate. **Paraphyses** filiform, apex obtuse, septate, hyaline, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows normal, reach about 55 cm after 4 weeks incubation, circular, cottony, zonate, gray to dark gray; Aerial mycelium gray, little developed; Margins entire; Reverse zonate, pale brown in the central, with brown to black pigmentation, margin pallid.

Specimens examined – On rotting twigs of unknow plant: TNS-F-16472 (Culture FC-2038), Yukiiri, Chiyoda-machi, Kasumigaura-shi, Ibaraki Pref., 5 May 2005, Col. R.

Sasagawa; TNS-F-17845 (Culture FC-1205), Tone-gun, Gunma Pref. (48º48'46.6"N, 139º2'5"E), 18 Sep. 2005, Col. T. Hosoya; TNS-F-31184 (Culture FC-1369), Towada-shi, Aomori Pref. (24º24'55.7"N, 140º24'55.7"E), 26 May 2006, Col. T. Hosoya.

Notes – *Hymenoscyphus varicosporioides* was firstly reported from Japan by Tubaki (1966), but he failed to given the detailed description on the structure of excipulum. *Hymenoscyphus imberbis* (Bull.) Dennis resembles *H. varicosporioide* in many aspects, but the former has the larger ascospores (8–11×3–4 μm, Dennis 1964).

10. *Hymenoscyphus* sp. 1 **Figs. 5.28–5.30**

Apothecia stipitate, occurring on decaying herb stems; disc cupulate to convex, 0.7–1.8 mm in diameter in dried specimen; hymenium greenish black when fresh, becoming black when dry; receptacle flocculose, dark yellowish green when fresh or dry; stipe concolorous with the receptacle, 0.6–2.6 mm long when dry, flocculose. **Ectal excipulum** textura prismatica, composed of slightly thick-walled, brick-shaped cells of 4–8×8–20 μm, smooth, pale brown to subhyaline. **Hairs** protruded from outermost layer of ectal excipulum, hyline, septate, clavate, abundance in stipe base. **Medullary excipulum** textura intricata, pale brown to subhyaline, smooth, separate hypha of 2.5–3.5 μm wide. **Asci** 83–98×8–9 μm, cylindric-clavate, 8-spored, arising from simple septa; apex conical, 3–4 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 19–24×3.5–4 μm (21.7 ± 1.1×4.0 ± 0.2 μm on average ± SD, n= 20), uniseriate or biseriate in asci, cylindric-fusoid, usually inequilateral,

slightly curved, some pointed on one end, non-septate, mostly with one big, irregular-shaped guttule. **Paraphyses** filiform, obtuse, septate, hyaline, exlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 20 mm after 4 weeks incubation, circular, flocculent, initially gray to pale brown, becoming dark in age; Aerial mycelium grayish, little developed; Margins undulate, pale brown; Reverse yellowish brown.

Specimens examined – On decaying herb stems: TNS-F-40079 (Culture FC-2818), University of Tsukuba, Tsukuba City, Ibaraki Pref., 13 Oct. 2011, Col. Y.-J. Zhao.

11. *Hymenoscyphus* sp. 2 **Figs. 5.31–5.33**

Apothecia stipitate, occurring on decaying herb stems; disc flat to cupulate, 0.5–1.0 mm in diameter in dried specimen; hymenium pale berge to yellowish when fresh, becoming beige to pale brown (719 PC = C0 M14 Y24 K0) when dry; receptacle flocculose, pale white to berge, paler than hymenium when fresh or dry; stipe concolorous with the receptacle or slightly darker, 0.3–1.1 mm long when dry, flocculose. **Ectal excipulum** two layered: outer layer textura prismatica, composed of slightly thick-walled, brick-shaped cells of 5–10×10–25 μm, smooth, hyaline; inner layer composed of thin walled, hyaline, smooth, separate hypha of 2.5–4 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, separate hypha of 2.5–3.5 μm wide. **Asci** 90–110×7–10 μm, cylindric-clavate, 8-spored, arising from simple septa;

apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 14–23×4–6 μm (19.2 ± 1.8×5.0 ± 0.6 μm on average ± SD, n= 20), uniseriate or biseriate in asci; hyaline spores fusoid, longate ellipsoid, some pointed on one end, non-septate, mostly with one big central guttule; pale brown spores fusoid, pointed on ends, 1-septate, 0-muliti guttules; spores becoming brown in germination, expanded to 7–9 μm in width, 1-septate, eguttulate, pointed on one end. **Paraphyses** filiform, obtuse, septate, hyaline, exlarged at the apex up to 3–4 μm wide.

Cultural characteristics – Colonies on PDA grows normal, reach about 30 mm after 4 weeks incubation, filamentous, flocculent, whitish; Aerial mycelium white, well developed; Margins filiform; Reverse pale yellow.

Specimens examined – On decaying herb stems: TNS-F-40193 (Culture FC-5110), Yuhuin-cho, Yuhu-shi, Oita Pref. (33º11'39.8"N, 131º19'1.4"E, Alt.684m), 6 Nov. 2012, Col. Y.-J. Zhao.

Notes – The ascospores of present fungus become brown in germination, and this character resembles that in *H. caudatus* which also shows the pigmentation prior to germination (Kimberough and Atkinson 1972). However, the present fungus has the longer asci than *H. caudatus* (85–95 μm), and phylogenetically these two species were separated.

12. "*Hymenoscyphus*" sp. 1 **Figs. 5.34–5.36**

Apothecia subsessile to stipitate, occurring on decaying leaves; disc discoid to convex, 0.5–1.3 mm in diameter in dried specimen; hymenium yellow (1225C = R255 G203 B79), margin irregular, slightly wrinkled; receptacle and stipe waxy, fragile, concolorous with hymenium, smooth. **Ectal excipulum** two layered: covering layer absent; outer ectal excipulum in margin textura prismatica to textura globulosa, composed of thin-walled, brick or globosa cells of 3–7×5–12 μm, smooth, subhyaline to yellowish; outer ectal excipulum in flank composed of thin-walled, globosa to ellipsoid cells of 8–12×10–22 μm, smooth, subhyaline to pale brown; inner layer composed of thin-walled, hyaline to pale brown, smooth, separate hypha of 2.5–3.5 μm wide, obscure in flank. **Medullary excipulum** textura intricata, subhyaline, smooth, thin-walled, separate hypha of 3–8 μm wide. **Asci** 95–113×7–8 μm, cylindric-clavate, 8-spored, croziers absent or obscure; apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 13–19×3–5 μm (16.4 ± 1.5×4.1 ± 0.4 μm on average ± SD, n= 20), uniseriate or biseriate above, fusoid or oblong-fusoid, slightly curved, non-septate, 0-multi guttules. **Paraphyses** filiform, septate, hyaline, slightly enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows very slowly, reach about 10 mm after 4 weeks incubation, circular, flocculent, whitish to pale brown; Aerial mycelium white to pale brown, little developed; Margins entire; Reverse pallid to pale brown, dark in the central.

Specimens examined – On decaying leaves of unknown plant: TNS-F-40156 (Culture FC-5090), Hanazono Shrine, Kitaibaraki-shi, Ibaraki Pref., 26 Sep. 2012, Col. Y.-J.

Zhao; On decaying leaves of *Fagus* sp.: TNS-F-40162, Bijinbayashi, Tokamachi-shi, Niigata Pref. (37º6'8.60"N, 138º37'17.10"E, Alt. 337m), 1 Oct. 2012, Col. Y.-J. Zhao.

13. "*Hymenoscyphus*" sp. 2 **Figs. 5.37–5.38**

Apothecia subsessile to stipitate, occurring on fallen acorns; disc discoid to convex, 0.7–2.7 mm in diameter in dried specimen; hymenium yellow when fresh, becoming orange yellow (137C = R255 G161 B0) when dry, margin irregular, slightly wrinkled; receptacle beige to pale brown when dry, smooth; stipe concolorous with hymenium, 0.2–0.6 mm long when dry, smooth. **Ectal excipulum** two layered: covering layer absent; outer ectal excipulum in margin textura prismatica, composed of slightly thick-walled, brick-shaped cells of 3–5×6–10 μm, smooth, subhyaline to pale brown; outer ectal excipulum in flank and stipe composed of slightly thick-walled, globosa to ellipsoid cells of 4–10×6–17 μm, smooth, subhyaline to pale brown; inner layer composed of thick-walled, subhyaline, smooth, separate hypha of 1–2 μm wide, obscure in flank and stipe. **Medullary excipulum** textura intricata, hyaline to subhyaline, smooth, thick-walled, separate hypha of 2–4 μm wide. **Asci** long-stipitate, 95–112×6.5–7.5μm, cylindric-clavate, 8-spored, arising from croziers; apex conical, 1–2 μm thick; pore not stained by Melzer's reagent with or without 3% KOH pretreatment. **Ascospores** 10.5–13.5×3–4.5 μm (11.7 ± 0.8×3.7 ± 0.4 μm on average ± SD, n= 20), uniseriate or biseriate above, ellipsoid, fusoid to oblong-fusoid, non-septate, some with 2 large, globosa guttules and multiple small guttules. **Paraphyses** filiform, septate, hyaline, slightly enlarged at the apex up to 2–2.5 μm wide.

Specimens examined – On fallen acorns of *Quercus* sp.: TNS-F-40168, Ohara, Nakayama, Koriyama-shi, Gunma Pref. (36º35'33.8"N, 138º57'3.90"E, Alt. 777m), 2 Oct. 2012, Col. Y.-J. Zhao.

14. "*Hymenoscyphus*" sp. 3 **Figs. 5.39–5.41**

Apothecia stipitate, occurring on fallen fruits; disc discoid or convex, 0.5–2.0 mm in diameter in dried specimen; hymenium whitish when fresh, becoming pallid to pale beige (155PC= C0 M12 Y32 K0) when dry; receptacle smooth, whitish when fresh, becoming beige to brown when dry; stipe concolorous with receptacle, 5–14 mm long when dry, smooth. **Ectal excipulum** two layered: outer ectal excipulum textura prismatic, 12.5–37.5 μm thick, individual cells narrow, 2.5–5 μm wide, pale brown to brown, smooth, thin walled; inner layer thin walled, subhyaline to hyaline, smooth, separate hyphae of 2–3 μm wide. **Medullary excipulum** textura intricata to porrecta, subhyaline, smooth, slightly thicken-walled, separate hypha of ca. 2.5 μm wide. **Asci** 150–160×8–12 μm, clavate-cylindric, 8-spored, arising from simple septate; apex conical to truncate, ca. 3–4 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 24–31×6–8 μm (27.7 ± 2.3×6.8 ± 0.6 μm on average ± SD, n= 20), uniseriate, elongate fusoid, non-septate, frequently containing a large eccentric oil globule, some with 2 guttules or several small guttules. **Paraphyses** filiform, septate, hyaline, smooth, expanded at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 40 mm in

diameter after 4 weeks incubation, circular, flocculent, white to gray; Aerial mycelium white to gray, partly developed; Margins entire, white; Reverse zonate, pale yellow; Stroma not observed on PDA.

Specimens examined – On fruits of *Cercidiphyllum japonicum* Sieb. & Zucc: TNS-F-40173 (Cuture FC-5096), Mt. Buko-san, Chichibu-shi, Saitama Pref. (35º56'31.5"N, 139º6'57.5"E, Alt.502m), 13 Oct. 2012, Col. Y.-J. Zhao.

15. "*Hymenoscyphus*" sp. 4 **Figs. 5.42–5.44**

Apothecia stipitate, occurring on fallen acorns; disc flat to cupulate, 0.5–0.8 mm in diameter in dried specimen; hymenium waxy, pale white when fresh, becoming pale yellow (1205PC = C0 M5 Y35 K0) when dry, some with irregular margin; receptacle and stipe concolorous with hymenium, waxy, smooth, stipe 0.2–0.4 mm long when dry. **Ectal excipulum** two layered: covering layer absent; outer ectal excipulum in margin textura prismatica, composed of thin-walled, brick-shaped cells of 2.5–5×5–13 μm, smooth, subhyaline; outer ectal excipulum in flank and stipe composed of slightly thick-walled, globosa to ellipsoid cells, 4.5–6 μm in diameter, smooth, subhyaline, outermost layer of pale brown; inner layer composed of thin-walled, hyaline to subhyaline, smooth, separate hypha of ca. 2.5 μm wide, obscure in flank. **Medullary excipulum** textura intricata, hyaline, smooth, thin-walled, separate hypha of 2.5–5 μm wide. **Asci** 135–147×9–11 μm, cylindric-clavate, gradually narrowed downwards to a short stipe, 8-spored, croziers absent; apex truncate, 3–3.5 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 24–31.5×3.5–6 μm (27.7

± 2.0×4.8 ± 0.6 μm on average ± SD, n= 20), uniseriate or irregular biseriate, hyaline, narrowly ellipso-fusoid, ends taper end but usually rounded, straight or more frequently somewhat convex, 0-multi guttules, some with one-septate in the middle. **Paraphyses** filiform, septate, hyaline, straight, slightly enlarged towards apex to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows very slowly, reach about 15 mm after 4 weeks incubation, circular, flocculent, whitish to gray; Aerial mycelium white to gray, partly developed; Margins entire; Reverse dark gray, white in the margin.

Specimens examined – On fallen acorns of *Fagus* sp.: TNS-F-40179 (Culture FC-5101), Mt. Takao, Oita-shi, Oita Pref. (33º12'42.10"N, 131º39'22.20"E, Alt. 71m), 4 Nov. 2012, Col. Y.-J. Zhao.

16. "*Hymenoscyphus*" sp. 5 **Figs. 5.45–5.47**

Apothecia stipitate, occurring on decaying branches; disc cuplate or convex, 0.4–1.0 mm in diameter in dried specimen; hymenium dirty gray (warm gray 1PC= C2 M3 Y4 K5); receptacle wax, brown; stipe dark brown to black, 0.1–0.4 mm long when dry, tomentose, becoming dark towards the base. **Ectal excipulum** two layered: outer ectal excipulum textura globulosa, or less commonly textura angularis, 20–37.5 μm thick, individual cells pale brown, smooth, slightly thicken walled; inner layer obviously in margin, difficult to distinguish from hyphae of medullary excipulum in franks, thin walled, pale brown to subhyaline, smooth, separate hyphae of 1.5–2.5 μm wide. **Medullary excipulum** textura intricata, subhyaline to pale brown, smooth, slightly

thicken-walled, separate hypha of ca. 2.5 μm wide. **Asci** 130–170×8–9 μm, clavate-cylindric, 8-spored, arising from simple septate; apex rounded to truncate, ca. 4–5 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 10–20×4.5–6 μm (13.1 ± 2.5×5.3 ± 0.5 μm on average ± SD, n= 20), uniseriate to irregular biseriate in asci, ellipsoid to elongate fusoid, non-septate, frequently containing a large eccentric oil globule. **Paraphyses** filiform, septate, subhyaline, smooth expanded at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 15 mm in diameter after 4 weeks incubation, circular, flocculent, initially white to pale brown, becoming darker in age; Aerial mycelium white to gray, partly developed; Margins entire; Reverse white to pale yellow.

Specimens examined – On wood of unknown plant: TNS-F-40186 (Cuture FC-5104), Kokonoe-machi, Kuzu-gun, Oita Pref. (33º10'3.30"N, 131º14'45.3"E, Alt.915m), 6 Nov. 2012, Col. Y.-J. Zhao.

Notes – The structure of ectal excipulum in present fungus is very similar to *Moellerodiscus lentus* (Berk. & Broome) Dumont (Dumont 1976a), but this fungus had no stroma and completely different in other morphological characters. The present fungus is characterized by its large asci and ascospores, and yellowish brown, globose cells in ectal excipulum.

17. *Lambertella advenula* (W. Phillips) Hosoya & Y. Otani, **Mycoscience 38: 303 (1997)** (Orignial identification)

***Luteidiscella advenula* (W. Phillips) Y.-J. Zhao & T. Hosoya. comb. nov.**

Figs. 5.48–5.50

Description has already given in Chapter 3–4–4**.**

18. *Lambertella corni-maris* Höhn. **Figs. 5.51–5.53**

Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 375 [47 repr.] (1918)

Stroma substratal, visible as the blackened, shrinking areas on the substrate. **Apothecia** stipitate, occurring on fallen fruits; disc flat, discoid, or concave, 0.3–3 mm in diameter in dried specimen; hymenium beige to pale brown (1375PC = C0 M45 Y95 K0) when fresh, becoming reddish brown to dark brown (4695PC= C29 M79 Y71 K73) when dry; receptacle lighter than hymenium, beige, pruinose; stipe concolorous with receptacle, 0.4–1 mm long when dry, pruinose, darkish towards the base. **Ectal excipulum** two layered: outer layer textura prismatica, composed of thin-walled or slightly thick-walled, brick-shaped cells of 15–40 × 5–14 μm in middle flanks and 6–25 × 4–10 μm at the margin, with slightly granulate or smooth surface, becoming pale brown towards the margin; inner layer obscure but present, composed of thin walled, subhyaline to pale brown, granulate or smooth, separately ca. 5 μm wide hyphae. **Hairs** arising from the outermost layers of the ectal excipulum, cylindrical, septate, mostly hyaline, occasionally expanded up to 3–7 μm at the apex. **Medullary excipulum** textura intricata, hyaline to subhyaline, smooth, separate hypha of 3–5μm wide. **Asci** 80–110×5.5–7 μm, cylindric to clavate, 8-spored, croziers absent or obscure; apex

rounded to truncate, 2–3μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–10.5×3.5–4.5 μm (8.1 ± 0.9×4.1 ± 0.2 μm on average ± SD, n= 25), uniseriate, broadly ellipsoid, non-septate; at first hyaline, then developing pale brown, finally becoming yellow-brown to golden-brown within the ascus; hyaline spores smooth, eguttulate or with 1–2 polar guttules; brownish spores with granulate and thickened surface, eguttulate or with 1–2 polar guttules; Germinated spores on PDA becoming paler colored to hyaline, expanded, globosa, 12–18 μm in diameter. **Paraphyses** filiform, septate, hyaline, expanded at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, initially pallid, becoming darker in age; Aerial mycelium grayish to pale brown, little developed; Margins entire, pale brown; Stroma easily formed, visible as the blackened area covering the entire exposed surface of agar; Reverse with blackened areas emerged in the agar, margin pale brown; Rind composed of a single layer of cells, well defined epidermoid structure in face view.

Specimens examined – On decayed leaves of *Mallotus japonicus* (L.f.) Müll.Arg.: TNS-F-30402 (Culture FC-2389), Sanshiro-ike, Tokyo University, Hongo, Bunkyo-ku, Tokyo, 7 Dec. 2009, Col. T. Hosoya. On fallen fruits of *Torreya nucifera* (L.) Sieb. et Zucc.: TNS-F-40083 (Culture FC-2821), Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref. (36º6'9.50"N, 140º6'44.0"E, Ala. 44m), 14 Oct. 2011, Col. T. Hosoya.

Notes – *Lambertella corni-maris* mainly occurs on the fruits of *Carya*, *Cornus* and *Pyrus* (Dumont 1971). This is the first time to find *L. corni-maris* occurring on leaves of

M. japonicus and fruits of *T. nucifera.*

19. *Lambertella pyrolae* Y.-J. Zhao & T. Hosoya **Figs. 5.54–5.56**

Phytotaxa 136: 55 (2013)

Stroma substratal, visible on surface of substrate as clear, irregular, black lines delimiting blackened zones. **Apothecia** stipitate, occurring on decaying leaves and petioles; disc flat when fresh, becoming discoid to cupulate when dry, 0.5–1.6 mm in diameter in dried specimen; hymenium dark gray to beige (warm gray 3C = R199 G194 B186) when fresh, becoming dark yellow to brown (4745PC = C4 M20 Y22 K12) when dry; receptacle hairy, slightly paler than hymenium when fresh, becoming light brown when dry; stipe concolorous with the receptacle, 0.5–2 mm long when dry, with hairy surface. **Ectal excipulum** two layered: outer layer textura prismatica, composed of thin-walled, brick-shaped cells 15–40 × 6–13 μm in the middle flanks and 6–15 × 5–10 μm at the margin, with granulate or smooth surface, sometimes becoming pale brown towards the margin; inner layer composed of thin-walled, subhyaline to pale brown, granulate or smooth, hypha of ca. 5 μm wide. **Hairs** arising from the outermost layers of the ectal excipulum, cylindrical, septate, 17–60 μm long, mostly hyaline, occasionally expanded up to 4–8 μm at the apex. **Medullary excipulum** textura intricata, composed of hyaline, smooth, loosely interwoven hyphae 3–5 μm wide. **Asci** 81–145 × 6–9.5 μm (109.7±18.5 × 7.2±0.9 μm on average ± SD, n = 20), clavate, 8-spored, arising from simple septa; apex rounded, some slightly truncate, 2–4 μm thick; pore never stained by Melzer's reagent with or without 3% KOH pretreatment; both pigmented and colorless ascospores can be seen in the same ascus, most asci collapsing when all the ascospores

have become pigmented. **Ascospores** 14–22 × 3–4.5 μm (18 ± 2×3.5 ± 0.5 μm on average ± SD, n = 30), irregularly biseriate or biseriate above and uniseriate below, elongate-elliptic to fusoid, non-septate; at first hyaline then changing to pale brown, finally becoming yellow-brown to golden-brown within the ascus; hyaline spores smooth, 1–multi guttulate; pale brown spores 1–3 guttulate, remaining smooth or becoming somewhat granulate; brown spores eguttulate, with coarsely granulate surface, some with one side banded; germinated spores becoming paler colored to hyaline, expanded at the middle, 7–14 μm in width, aseptate, with ends pointed. **Paraphyses** straight, septate, hyaline, simple or branched near the base, expanded at the apex up to 2–5 μm wide.

Cultural characteristics – Growth on PDA slow, attaining a diameter of 50 mm in 14 d at 20℃, surface floccose, whitish to pale brown (4755PC = C3 M14 Y16 K7), becoming darker with age. Aerial mycelium white, becoming brown with age, not well developed, forming short mycelial strands. Rind becoming distinct in prolonged incubation up to 1 month. The rind delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to a various extent, epidermoid in face view.

Speciemens examined – On decayed leaves and petioles of *Pyrola incarnata* Fisch. ex DC.: TNS-F-25246, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref., July 2006, Col. T. Shirouzu; TNS-F-40033 (Culture FC-2726), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt. 1340m), 2 July 2011, Col. Y.-J. Zhao;

TNS-F-40132 (Culture FC-2996), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt. 1340m), 23 June 2011. Col. Y.-J. Zhao.

Notes – The distinctive characters of *L. pyrolae* are the combination of elongate-elliptic to fusoid ascospores, J– MLZ at ascal apex, presence of hairs, and ascospores pigmented before discharge.

Four species are similar to *L. pyrolae* in characters of asci and fusoid to subfusoid ascospores. They are: *Lambertella copticola* Korf & V.P. Tewari, *Lambertella rhamnicola* (L.R. Batra) Dumont, *Lambertella tetrica* (Quél.) Dumont and *Lambertella thindii* Dumont. However, *L. thindii* possesses attenuated paraphyses and acuminate hairs (Dumont 1976b), *L. rhamnicola* possesses brown paraphyses and absence of hairs (Batra 1968; Dumont 1971), and these two species can also be easily distinguished from *L. pyrolae* which having the usual type of paraphyses without coloration and cylindrical hairs. *Lambertella tetrica* differs from *L. pyrolae* notably in its conspicuously short stipe (0.1–0.3 mm long), broader asci (10–12 μm broad), and arising from easily visible croziers (Dumont 1971); *L. copticola* differs from *L. pyrolae* in ascospores becoming pigmented only after discharge and J+ ascal apex in MLZ (Dumont 1971; Tewari 1963).

20. *Lambertella yunnanensis* (S.H. Ou) W.Y. Zhuang & Yan H. Zhang Taxon 51: 769 (2002)

***Hymenoscyphus yunnanense* (S.H. Ou) Y. J. Zhao & T. Hosoya, comb. nov.**

Figs. 5.57–5.58

Description given in Chapter 3−4−5.

21. *Lambertella* sp. 1

***Brunneimargo camelliae* Y.-J. Zhao & T. Hosoya sp. nov.** **Figs. 5.59–5.60**

Description given in Chapter 3−4−3.

22. *Lambertella* sp. 2

***Crassitunica tsubakii* Y.-J. Zhao & T. Hosoya sp. nov.** **Figs. 5.61–5.63**

Description given in Chapter 3−4−2.

23. *Lanzia longipes* (Cooke & Peck) Dumont & Korf **Figs. 5.64–5.66**

Mycotaxon 7: 185 (1978)

Peziza longipes Cooke & Peck, Bull. Buffalo Soc. nat. Sci. 1: 295 (1875)

Phialea longipes (Cooke & Peck) Sacc., Syll. fung. (Abellini) 8: 267 (1889)

Hymenoscyphus longipes (Cooke & Peck) Kuntze, Revis. gen. pl. (Leipzig) 3: 485 (1898)

Rutstroemia longipes (Cooke & Peck) W.L. White, Lloydia 4: 203 (1941)

Stroma substratal, visible as blackened zones on petioles of host. **Apothecia** long-stipitate, occurring on decaying petioles; disc flat to discoid when fresh, becoming concave when dry, 1.5–4 mm in diameter in dried specimen; hymenium cream white (warm gray 1 PC= C2 M3 Y4 k5) when fresh, becoming buff (720 PC= C0 M20 Y32 K2) to pale brown when dry; receptacle concolorous or slightly paler than hymenium, furfuraceous; stipe subcylindric, concolorous with the receptacle, becoming much

darker toward base, up to 30 mm long when dry. **Ectal excipulum** three layered: Covering layer thin-walled, hyaline to pale brown, cells of irregular shaped, 2–4 μm wide; outer ectal excipulum textura prismatica, subhyaline to pale brown, composed of slightly thick-walled, brick-shaped cells 8–25×5–10 μm, becoming slightly globose in the margin; inner layer hyaline, separate hyphae of 4–8 μm wide. **Hairs** arising from outer covering layer, septate, hyaline to pale brown, smooth, usually with cylindrical or swollen apices. **Medullary excipulum** textura intricata, loosely interwoven, subhyaline to pale brown, smooth, separate hypha of 5–10 μm wide. **Asci** 70–95×8–11 μm, clavate, 8-spored, arising from crozier but obscure; apex slightly truncate, 4–5 μm thick; pore stained blue by Melzer's reagent. **Ascospores** 10–11×3.5–5 μm (10.6 ± 0.5×4.2± 0.4 μm on average ± SD, n= 23), uniseriate, ellipsoid to obovoid-reniform, usually inaequilateral, with one side much broader, smooth, some with 1–2 guttules; Some discharged spores of pale-brown, usually with one septum; When germinated in PDA, becoming 1-septate near to the narrow side, the cell in broad side with thickened and dark walls, much browner than the cell in narrow side; When germinated in 1% Agar, the whole spore becoming brown, thicken-walled and 1–2 septate, spermatia were extruded from the small tubules on the ascospore. **Paraphyses** filiform, septate, hyaline, slightly enlarged at the apex up to 2 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, whitish to grayish; Aerial mycelium white, cottony, partly developed; Margins entire; Reverse pale brown, with the blackened areas diffusing from center; Rind delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, epidermoid in face view.

Specimens examined – On unknown petioles: Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., Japan (140º6'44.0"N, 36º6'9.5"E, Alt. 44m), Col. Y.-J. Zhao: TNS-F-40082 (14 Oct. 2011), TNS-F-40097 (Culture FC-2832, 7 Nov. 2011), TNS-F-40177 (Culture FC-5098, 12 Oct. 2012); TNS-F-40148, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (138º20'51.2"N, 36º41'30"E, Alt. 1349 m), 15 Sep. 2012, Col. Y.-J. Zhao; TNS-F-40160, Hanazono Shrine, Kitaibaraki-shi, Ibaraki Pref., 26 Sep. 2012, Col. Y.-J. Zhao; TNS-F-40167, Takayama-mura, Gunma Pref. (138º57'36.5"N, 36º35'19.3"E, Alt. 242 m), 2 Oct. 2012, Col. Y.-J. Zhao.

Notes – *Lanzia longipes* is a new record in Japan. It is easily distinguished by its long stipe of apothecia and remarkably large asci and ascospores.

Lanzia longipes was firstly described from New York under the name *Peziza longipes* in 1895 by Cooke and Peck who emphasized the length of the stipe. The substratum was simply indicated as “leaf petioles”. White (1941) noted that it has a simple structured apothecium differerated from others. Korf and Gruff (1978) proposed it as a new combination of *Lanzia* for its prismatic cells in ectal excipulum.

24. *Lanzia pruni-serotinae* (Whetzel & W.L. White) M.P. Sharma & R.M. Sharma

Int. J. Mycol. Lichenol. 2: 109 (1985) **Figs. 5.67–5.68**

Rutstroemia pruni-serotinae Whetzel & W.L. White, Lloydia 4: 221 (1941)

Stroma substratal, visible as blackened zones on leaf veins and petioles of host. **Apothecia** stipitate, occurring on decaying leaves and petioles; disc flat to cupulate, 0.5–2.5 mm in diameter when fresh; hymenium beige to brown (137 PC= C0 M38 Y95 K0) when fresh, becoming dark brown (1615 PC=C11 M74 Y100 K50) to black when dry; receptacle beige when fresh, becoming brown when dry, flocculose; stipe concolorous with receptacle, 1–11 mm long when fresh, flocculose, becoming dark brown towards the base, sometimes the in-continuous brown hyphae mass attached in the surface. **Ectal excipulum** three layered: covering layer thin-walled, hyaline to pale brown, slightly granulate, 2–5 layers of hyphae; outer ectal excipulum textura prismatica, hyaline to pale brown, composed of slightly thick-walled, brick-shaped cells of 2.5–8 μm wide; inner layer hyaline to pale brown, smooth, slightly thick-walled, separate hyphae of 5–10 μm wide. **Hairs** protruded from outermost layer of ectal excipulum, hyaline to pale brown, smooth to roughened, septate, some expanded at the apex. **Medullary excipulum** textura intricata, hyaline, smooth, tightly interwoven, merged into gelatinously materials, separate hypha of 3–6 μm wide. **Asci** 60–75×6–8 μm, clavate, 8-spored, arising from repeated croziers; apex rounded to truncate, 1.5–2 μm thick; pore very faintly stained by Melzer's reagent. **Ascospores** 6–9×3–4.5 μm (8.0 ± 1.0×3.4 ± 0.5 μm on average ± SD, n= 20), uniseriate or irregular biseriate at upper, elongate ellipsoid, non-septate, smooth, 0–2 guttules. **Paraphyses** filiform, septate, hyaline, simple or branched near the base, usually expanded at apex up to 2.5–3 μm wide.

Specimens examined – On leaves and petioles of *Prunus grayana* Maxim.: TNS-F-40119, Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref. (140º6'44.0"N,

36º6'9.5"E, Alt. 44m), 13 June 2012, Col. Y.-J. Zhao.

Notes – The morphological features well agreed with the previous description of *L. pruni-serotinae* (Sharma and Sharda 1985; White 1941, as *Rutstroemia pruni-serotinae*). It is often confused with *Rutstroemia renispora* (Ellis) W.L. White in apothecial structure and stromatic characters (White 1941), but *L. pruni-serotinae* has the shorter asci, smaller non-curved ascospores and occurrence on *Prunus*.

Lanzia pruni-serotinae is a new record of Japan.

25. *Lanzia* sp. 1 **Figs. 5.69–5.71**

Stroma substratal, appearing in leaf surface as blackened rind. **Apothecia** stipitate to subsessile, mainly occurring on decaying leaves; disc discoid to cupulate, 0.5–1.8 mm in diameter in dried specimen; hymenium pallid to pale beige (434 C = R207 G195 B195) when fresh, the color nearly unchanged in drying; receptacle rough, brownish to red brownish when fresh or dry; stipe concolorous with the receptacle, 0.2–0.8 mm long when dry, rough. **Ectal excipulum** three layered, 50–87.5 μm thick: covering layer yellowish brown, composed of 1-4 layers, granulate, 2.5–5 μm width hypha; outer layer textura prismatica, composed of thin-walled or slightly thickened, brick-shaped cells of 7.5–17.5×15–42.5 μm, smooth or slightly rough, hyaline, becoming yellowish brown towards the margin; inner layer composed of thin walled, subhyaline to pale brown, granulate, separate hypha of 5–7.5 μm wide. **Medullary excipulum** textura intricata, subhyaline, smooth, hypha of 5–7.5 μm wide. **Asci** 70–85×7–8 μm, cylindric-clavate, 8-spored, arising from simple septa; apex rounded or truncate, 2–3 μm thick; pore

stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–13×3–5 μm (9.9 ± 1.7×3.9 ± 0.6 μm on average ± SD, n= 20), uniseriate or biseriate in upper part, ellipsoid to clavate-ellipsoid, non-septate, mostly with one big guttule in the central. **Paraphyses** filiform, obtuse, septate, hyaline, slightly enlarged towards the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows very slowly, reach about 10 mm in diameter after 4 weeks incubation, irregular, floccose, initially grayish (Warm gray 2C = R213 G210 B202), becoming darker in age; Aerial mycelium grayish, little developed; Margins undulate; Reverse pale brown to black, forming blackened areas diffusing from center; Rind vertically not well developed, textura globulosa in face view.

Specimens examined – On decaying leaves of unknown plant: TNS-F-40038 (Cuture FC-2790), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt.1340m), 3 July 2011, Col. Y.-J. Zhao.

26. *Lanzia* sp.2 **Figs. 5.72–5.74**

Stroma substratal, the rind not obviously. **Apothecia** stipitate, occurring on fallen involucres and spines of fruits; disc flat to cupulate, 0.7–2.1 mm in diameter in dried specimen; hymenium beige, gray yellow (4665 PC = C4 M27 Y33 K11) or black when fresh, the color nearly unchanged in drying; receptacle floccose, gray brown to brown when fresh or dry; stipe concolorous with the receptacle, 0.7–2.3 mm long when dry,

floccose. **Ectal excipulum** three layered: covering layer yellowish light brown, composed of 2–3 layers, slightly granulate, 2.5–5 μm width hypha; outer layer textura prismatica, composed of thin-walled, brick-shaped cells of 7–10×10–40 μm, smooth, subhyaline to pale brown, becoming yellowish brown towards the margin; inner layer composed of thin walled, subhyaline, smooth, separate hypha of 6.5–9 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, hypha of 5–8 μm wide. **Asci** 60–75×5–6 μm, cylindric-clavate, 8-spored, arising from simple septa; apex rounded or truncate, 2–3 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 6–9×3–4 μm (7.6 ± 0.8×3.2 ± 0.4 μm on average ± SD, n= 20), uniseriate, ellipsoid, non-septate, mostly eguttule. **Paraphyses** filiform, obtuse, septate, hyaline, granulate, slightly enlarged towards the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, filamentous, flocculose, zinitially whitish, becoming brown in age, blackened areas forming zonatly; Aerial mycelium white to grayish, little developed; Margins filiform; Reverse pale brown to black, blackened areas zonatly diffusing from center; Rind not well developed in culture, but clear in vertical view, textura angularis to epidermoid in face view. **Spermatia and Spermatiophores** produced abundantly on PDA; Spermatia hyaline, globosa to subglobose, 2–3 μm in diameter, containing a single eccentric oil globule; Spermatiophores phialidic, hyaline, septate or asceptate, 6–9 μm long.

Specimens examined – On fallen fruits of *Quercus* sp.: TNS-F-40081 (Cuture FC-2820), University of Tsukuba, Ibaraki Pref., 13 Oct. 2011, Col. Y.-J. Zhao.

27. *Lanzia* sp. 3 **Figs. 5.75–5.77**

Stroma substratal, forming irregular, blackened areas, the rind not clear. **Apothecia** stipitate, occurring on fallen involucres and spines of fruits; disc flat to cupulate, 0.5–2.5 mm in diameter in dried specimen; hymenium beige, pale brown, brown (726 C = R229 G203 B177) or black when fresh, the color nearly unchanged in drying; receptacle floccose, beige, pale brown when fresh or dry, surface wrinkling when dry; stipe concolorous with the receptacle or brownish, becoming blackish towards base, 0.5–1.0 mm long when dry, floccose. **Ectal excipulum** three layered: covering layer 7.5–12.5 μm width, yellowish brown, composed of 1-3 layers, granulate hypha; outer layer textura prismatica, composed of thin-walled or slightly thicken-walled, brick-shaped cells of 7–15×10–30 μm, 5–10×8–15 μm in the margin, smooth or slightly granulate, yellowish brown to subhyaline, becoming yellowish brown towards the margin; inner layer composed of thin or slightly thicken walled, subhyaline to pale brown, granulate, separate hypha of ca. 5 μm wide. **Medullary excipulum** textura intricata, hyaline to subhyaline, smooth, hypha of 5–8 μm wide. **Asci** long stipitate, 76–93×5 μm, narrowly cylindric-clavate, 8-spored, arising from simple septa or croziers; apex rounded or truncate, 2–3 μm thick; pore faintly staining by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–9×3–4 μm (7.8 ± 0.7×3.4 ± 0.5 μm on average ± SD, n= 20), uniseriate, ellipsoid, non-septate, mostly eguttule. **Paraphyses** filiform, obtuse, septate, hyaline, granulate, slightly enlarged towards the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, filamentous, cottony, initially beige to pale brown, becoming dark brown in age; Aerial mycelium beige to pale brown, well developed; Margins entire; Reverse brown to black; Rind not well developed in culture, diffused, blackish, textura globulosa to angularis in face view. **Spermatia and Spermatiophores** produced abundantly on PDA; Spermatia hyaline to pale brown, globosa to subglobose, 2–3 μm in diameter, containing a single eccentric oil globule; Spermatiophores phialidic, hyaline to plale brown, septate or asceptate, 7.5–9 μm long.

Specimens examined – On decaying involucres and spines of *Castanea crenata* Siebold & Zucc.: TNS-F-40095 (Cuture FC-2830), University of Tsukuba, Ibaraki Pref., 20 Oct. 2011, Col. Y.-J. Zhao; TNS-F-40096 (Cuture FC-2831), Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., 27 Oct. 2011, Col. Y.-J. Zhao; TNS-F-40178, Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., 29 Oct. 2012, Col. Y.-J. Zhao; TNS-F-40192 (Cuture FC-5109), Yuhuin-cho, Yuhu-shi, Oita Pref. (33º11'39.8"N, 131º19'1.40"E, Alt.684m), 6 Nov. 2012, Col. Y.-J. Zhao.

Notes – There are two species known to occur on the involucres and spines of *C. crenata*: *Poculum elatinum* (Alb. & Schwein.) M.P. Sharma and *Poculum americanum* (E.J. Durand) M.P. Sharma & K.S. Thind. However, both *P. elatinum* and *P. americanum* have the gelatinous matrix in ectal excipulum (white, 1941), which completely differs from the present species.

The present species resembles *Lanzia prasinum* (Massee) Spooner var. *nigripes* Spooner in having the same shape and size of asci and ascospores, and all of them have

yellowish brown covering layer in ectal excipulum. However, *L. prasinum* var. *nigripes* have the conspicuous dark brown vertical streaks in receptacle and with the paler colored margin, and the ascal apex are not staining in Melzer's reagent (Spooner 1987). These characters differ from the present fungus.

The present species is characterized by the long stipitate asci, granulate and brownish covering layer in the ectal excipulum and the brownish margin. However, its taxonomic status needs further study.

28. *Lanzia* sp. 4 **Figs. 5.78–5.79**

Stroma substratal, visible on both surfaces of the host leaf as clearly, irregular, black lines delimiting blackened zones. **Apothecia** stipitate, cupulate, occurring on decaying leaves, 0.9–1.7 mm in diameter in fresh specimen; hymenium cream white to pale brown (155PC= C0 M12 Y32 K0) when fresh, becoming beige to dark brown in drying; receptacle pale brown to reddish brown, hairy; stipe concolorous with the receptacle, 0.5–1.5 mm long in fresh, hairy, with incontinuous brown hyphae mass attached in the surface, becoming dark brown at the base. **Ectal excipulum** of textura prismatica, composed of slightly thick-walled, brick-shaped cells of 5–20×5–15 μm, becoming brown at the margin; inner layer composed of 2–5 μm wide, paralleled hyphae, pale brown, smooth and slightly refractive; cover layer composed of roughened, pale brown, incontinuous hyphae, usually attached with the brown colored and roughened hyphae mass in the stipe. **Medullary excipulum** of textura intricata, composed of hyaline to pale brown, smooth, tightly interwoven, 1.5–2 μm thick hyphae. **Asci** 38–52×4–5 μm, clavate, 8-spored, arising from repeated croziers; apex 2.5–3 μm thick, pore very faintly

blue in Melzer's reagent. **Ascospores** 4–6.5×2–4μm (5.5 ± 0.6×3.0 ± 0.4 μm on average ± SD, n= 25) uniseriate, ellipsoid to round, non-septate, smooth, some with one big guttule in the central when young, becoming eguttule when mature; brownish, punctate spores were observed out of asci in specimens. No germination was observed in culture. **Paraphyses** filiform, septate, hyaline, simple or branched near the base, 2–2.5 μm wide of apex.

Speciemens examined – On decaying leaf of *Swida controversa* (Hemsl. ex Prain) Soják.: TNS-F-40120, Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., 13 June 2012, Col. Y.-J. Zhao; TNS-F-40136, Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., 5 July 2012, Col. Y.-J. Zhao.

29. *Lanzia* sp.5 **Figs. 5.80–5.81**

Stroma substratal, visible as blackened zones delimited by irregular, black lines. **Apothecia** stipitate, occurring on decaying leaves and petioles; disc flat to cupulate, 0.8–2.5 mm in diameter when fresh; hymenium pale brown (137 PC= C0 M38 Y95 K0) when fresh, becoming dark brown (1615 PC=C11 M74 Y100 K50) to black when dry; receptacle pale brown, some with slightly greenish cast, smooth; stipe concolorous with receptacle, 1.5–10 mm long when fresh, smooth to flocculose, becoming dark brown towards the base, sometimes with in-continuous brown hyphae mass attached in the surface. **Ectal excipulum** three layered: covering layer in-continuous, thin-walled, pale brown, smooth or slightly roughened, absent in margin; outer ectal excipulum textura prismatica, hyaline, composed of slightly thick-walled, brick-shaped cells of 10–20 μm

wide, becoming slightly globose and small towards margin; inner layer hyaline to subhyaline, smooth, slightly thick-walled, separate hyphae of 3–5 μm wide. **Hairs** absent. **Medullary excipulum** textura intricata, hyaline, smooth, loosely interwoven, merged into gelatinously materials, separate hypha of 5–10 μm wide. **Asci** 45–53×4.5–6 μm, clavate, 8-spored, arising from repeated croziers; apex rounded to truncate, 2–3 μm thick; pore very faintly stained by Melzer's reagent. **Ascospores** 8–9.5×2.5–3.5 μm (8.9 ± 0.5×2.9 ± 0.3 μm on average ± SD, n= 20), uniseriate or irregular biseriate, elongate ellipsoid to fusoid, non-septate, smooth, mostly with one big guttule in the central, rarely company with 1–2 small guttules. **Paraphyses** filiform, septate, hyaline, simple or branched near the base, slightly expanded at apex up to 1.5–2 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, initially beige to pale brown, becoming grayish in age; Aerial mycelium gray, cottony, little developed; Margins entire; Reverse pale brown, with the blackened areas diffusing from center; Rind becoming distinct after 8 weeks incubation, delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, epidermoid in face view.

Specimens examined – Yatsugatake, kawakami forests, University of Tsukuba (385m, 35º55'24.80''N, 138º29'47.10''E), 20 July 2012, Col. Y.-J. Zhao: TNS-F-40137, on decaying leaves of *Prunus* sp.; TNS-F-40139, on decaying leaves and petioles of *Pyrus pyrifolia*; TNS-F-40143, on decaying leaves of *Tilia japonica*; TNS-F-40144, on decaying leaves of *Prunus* sp.

30. *Lanzia* sp. 6 **Figs. 5.82–5.84**

Stroma substratal, visible as black rind on substrate but obscure. **Apothecia** stipitate, occurring on decaying herb stems; disc flat to concave, 0.8–3 mm in diameter in dried specimen; hymenium greenish yellow (C26 M21 Y100 k68); receptacle concolorous with hymenium, flocculose; stipe concolorous with receptacle, 0.6–8 mm long when dry, flocculose, darkish towards the base. **Ectal excipulum** merged into gelatinous structure, two layered: covering layer absent; outer ectal excipulum textura prismatica, composed of thin-walled, brick-shaped cells of 4–10×5–20 μm, smooth, subhyaline, becoming pale brown towards margin; inner layer composed of thin-walled, subhyaline, smooth, separate hypha of 2.5–4 μm wide. **Medullary excipulum** textura intricata, merged with gelatinous structure, subhyaline, smooth, thin-walled, separate hypha of 2.0–3.5 □m wide. **Asci** 55–70×4.5–7 μm, cylindric-clavate, mostly 4-spored or 3-spored with one spore abortion, rarely 5-spored, arising from croziers; apex conical, 3–5 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–14×4–5 μm (10.8 ± 1.6×4.9 ± 0.4 μm on average ± SD, n= 20), uniseriate, ellipsoid, mostly hyaline, partly greenish yellow, some becoming greenish yellow in asci; hyaline spores non-septate, mostly with 1–2 globosa guttules; greenish yellow spores 0–1 septate, with 0–2 irregular-shaped guttules. **Paraphyses** filiform, obtuse, septate, hyaline, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, reach about 80 mm after 4 weeks incubation, rhizoid, flocculent, pale brown to brown; Aerial mycelium gray, little developed; Margins lobate; Reverse pale brown to brown, lobate; Rind not well

developed in culture, diffused, brownish.

Specimens examined – On decaying herb stems: TNS-F-40055 (Culture FC-2802), Kokenodomon Gulley, Shikotsu Toya National Park, Chitose-shi, Hokkaido, (42º42'45.6"N, 141º19'11.80"E, Alt. 448m), 14 Sep. 2011, Col. Y.-J. Zhao.

Note – The remarkable characters of present fungus are the greenish yellow apothecia, 4-spored asci, presence of greenish yellow ascospores and the simple excipular structure.

31. *Lanzia* sp. 7 **Figs. 5.85–5.86**

Stroma substratal, visible as clearly, irregular, black lines delimiting blackened zones. **Apothecia** stipitate, occurring on decaying leaves; disc flat to cupulate, 0.3–1.4 mm in diameter in dried specimen; hymenium pale brown to brown (1365 PC= C0 M33 Y75 K0) when fresh, becoming dark brown (1615 PC=C11 M74 Y100 K50) to black when dry; receptacle concolorous with hymenium, flocculose; stipe concolorous with receptacle, 0.5–2.3 mm long when dry, flocculose, becoming darker in the base. **Ectal excipulum** three layered: covering layer composed of 1–3 layers hyphae, thin-walled, smooth or slightly roughened, hyaline to pale brown, absent in margin; outer layer textura prismatica, slightly thick-walled, hyaline, composed of 1.8–6.3 μm wide, brick-shaped cells; inner layer thin-walled, hyaline to pale brown, separate hyphae of 3–5 μm wide, becoming pale brown towards margin. **Hairs** arising from the outermost layers of the ectal excipulum, hyaline to pale brown, smooth to roughened, septate,

expanded at the apex. **Medullary excipulum** textura intricata, composed of slightly thick-walled, hyaline to pale brown, smooth to roughened, loosely interwoven hyphae of 2.5–7 μm wide. **Asci** 54–68×5–7 μm, clavate, 8-spored, arising from croziers; apex rounded to truncate, 2–3 μm thick, pore faintly stained blue in Melzer's reagent. **Ascospores** 5–7.5×2.5–4 μm (6.2 ± 0.7×3.1 ± 0.4 on average ± SD, n= 20), uniseriate or irregular biseriate, ellipsoid, non-septate, smooth, 0–2 guttules. **Paraphyses** filiform, septate, hyaline, simple or branched near the base, slightly expanded at apex of 2–3 μm wide.

Speciemens examined – On decaying leaves of *Betula* sp.: TNS-F-40036, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (1340m, 36º31'32.10"N, 138º20'52.00"E), 3 July 2011, Col. Y.-J. Zhao.

32. *Moellerodiscus pinicola* Y. Otani Bull. natn. Sci. Mus., Tokyo, B 5: 51 (1979)

(Original identification)

***Rutstroemia pinicola* (Y. Otani) Y.-J. Zhao & Hosoya, comb. nov.**

Figs. 5.87–5.89

Description given in Chapter 3−4−6.

33. *Moellerodiscus* sp. 1 **Figs. 5.90–5.92**

Stroma not observed on the substrate. **Apothecia** stipitate, occurring on decaying petioles; disc flat, discoid, or cupulate, 0.5–1.5 mm in diameter in dried specimen;

hymenium dark brown (476 C = R76 G51 B39) to black when fresh, becoming darker when dry; receptacle tomentose, yellow-brown; stipe concolorous with receptacle, 0.3–1.3 mm long when dry, tomentose, becoming dark towards the base. **Ectal excipulum** textura globulosa, composed of prismatic cells in margin, pale brown in the margin and hyaline in flank, dia. 8–15μm, smooth, thin or slightly thicken walled, frequently attached with pale brown fragments of hyphae in flank and stipe. **Medullary excipulum** textura intricata, subhyaline to pale brown, smooth, separate hypha of 2–3μm wide. **Asci** 135–150×12–15 μm, narrow clavate to long cylindric, 8-spored, arising from croziers; apex rounded to truncate, 4–6 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 22–33×4–7 μm (26.9 ± 2.5×5.5 ± 0.8 μm on average ± SD, n= 20), uniseriate to biseriate in asci, allantoid; hyaline spores non-septate, eguttulate or with 1–2 big guttules, in some spores guttules becoming pale brown; pale brown to brown ascospore 0–3 septate, eguttulate or with several tiny guttules, smooth or rough. **Paraphyses** filiform, septate, subhyaline to pale brown, expanded at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows very slowly, reach about 15 mm in diameter after 4 weeks incubation, irregular, flocculent, initially g pallid, merged with pale brown to brown hyphae, becoming darker in age; Aerial mycelium pallid to grayish, short-haired, little developed; Margins undulate; Reverse dark brown, zonate, with the blackened areas; Rind becoming distinct after 12 weeks incubation, delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, textura globulosa to epidermoid in face view.

Specimens examined – On decaying petioles of unknown plant: TNS-F-40085 (Cuture FC-2823), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'32.10"N, 138º20'52.00"E, Alt.1340m), 17 Oct. 2011, Col. Y.-J. Zhao.

Notes – The present fungus is characterized by the large, allantoid ascospores and the pale brown paraphyses. Because the ectal excipulum is composed of globulose cells, it should be included into *Moellerodiscus* based on current definition.

34. *Moellerodiscus* sp. 2 **Figs. 5.93–5.95**

Stroma not observed on the substrate. **Apothecia** stipitate, occurring on decaying branches; disc discoid or concave, 0.7–4.1 mm in diameter in dried specimen; hymenium pale brown when fresh, becoming dark brown (4625PC= C29 M78 Y91 K78) when dry; receptacle tomentose, berge to pale brown; stipe dark brown to black, 11–30 mm long when dry, tomentose, becoming dark towards the base. **Ectal excipulum** three layered: covering layer 6.5–10.5 μm width, yellowish brown, composed of 1–3 layers, granulate hyphae, discontinuous; outer layer textura globulosa to angularis, 25–75 μm thick, composed of slightly thicken-walled, round or irregular cells, smooth, subhyaline to pale brown, becoming yellowish brown towards the margin; inner layer composed of thin walled, subhyaline to pale brown, granulate or smooth, separate hypha of 5–7 μm wide. **Medullary excipulum** textura intricata, subhyaline to pale brown, smooth, slightly thicken-walled, separate hypha of 5–8 μm wide. **Asci** 81–96×5–7 μm, clavate-cylindric, 8-spored, arising from simple septate;

apex rounded to truncate, ca. 3 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 8–13×3–4 μm (10.4 ± 1.2×3.3 ± 0.3 μm on average ± SD, n= 20), uniseriate to biseriate in asci, ellipsoid, non-septate, eguttulate or with 2 small polar guttules. **Paraphyses** filiform, septate, subhyaline, smooth or granulate, expanded at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, initially grayish to pale brown, becoming darker in age, forming the brownish hyphae striate zonatly, produced the black exudates near the margin; Aerial mycelium gray to pale brown, little developed; Margins even; Reverse gray to pale brown; Rind not well developed in culture, diffused, blackish; **Spermatia** hyaline, globosa, arising from small tubes at each end of ascospore when ascospores germinated on PDA or produced from phialidic spermatiophores in PDA, 2–3 μm in diameter, some containing a single eccentric oil globule.

Specimens examined – On branches unknown plant: TNS-F-40188 (Cuture FC-5105), Kokonoe-machi, Kuzu-gun, Oita Pref. (33º10'3.30"N, 131º14'45.3"E, Alt.915m), 6 Nov. 2012, Col. Y.-J. Zhao.

35. *Phaeohelotium epiphyllum* (Pers.) Hengstm. **Figs. 5.96–5.98**

Mycotaxon 107: 272 (2009)

Peziza epiphylla Pers., Tent. disp. meth. fung. (Lipsiae): 72 (1797)

Helotium epiphyllum (Pers.) Fr., Summa veg. Scand., Section Post. (Stockholm): 356 (1849)

Calycina epiphylla (Pers.) Kuntze, Revis. gen. pl. (Leipzig) 3: 448 (1898)

Hymenoscyphus epiphyllus (Pers.) Rehm ex Kauffman, Pap. Mich. Acad. Sci. 9: 177 (1929) [1928]

Apothecia stipitate, occurring on decaying leaves; disc discoid to concave, 0.8–1.6 mm in diameter in dried specimen; hymenium whitish yellow to pale yellow (1365C = R255 G182 B82); receptacle and stipe waxy, concolorous with hymenium or slightly darker, smooth. **Ectal excipulum** merged into gelatinous structure, three layered: covering layer pale brown or yellowish brown, granulate, sometimes extending toeward the margin, composed of 1–2 layers of hyphae; outer ectal excipulum textura prismatica, composed of thin-walled, brick-shaped cells of 4–10×8–20 μm, smooth or slightly granulate, hyaline; inner layer composed of slightly thick-walled, hyaline, smooth, separate hypha of 2.5–4 μm wide. **Medullary excipulum** textura intricata, hyaline, smooth, thick-walled, separate hypha of 2–3.5 μm wide. **Asci** 80–95×6–8 μm, cylindric-clavate, 8-spored, arising from croziers; apex conical, 2–3 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 12–19×3–4 μm (15.2 ± 1.6×3.4 ± 0.4 μm on average ± SD, n= 20), uniseriate or biseriate above, fusoid or oblong-fusoid, curved, some pointed on one end, non-septate, mostly with two big guttules. **Paraphyses** filiform, obtuse, septate, hyaline, slightly granulate above, enlarged at the apex up to 2–3 μm wide.

Cultural characteristics – Colonies on PDA grows slowly, reach about 20 mm after 4

weeks incubation, circular, flocculent, whitish to gray; Aerial mycelium white, little developed; Margins entire; Reverse pallid to pale brown, dark in the central.

Specimens examined – On decaying leaves of *Populus maximowiczii* A.Henry: TNS-F-40042 (Culture FC-2792), Nishikionuma Park, Tomakomai-shi, Hokkaido, (42º36'39.6"N, 141º27'10.0"E, Alt. 27m), 12 Sep. 2011, Col. Y.-J. Zhao.

Notes – The present fungus is identifified as "*H.epiphyllus*" by ITS-5.8S region (Fig. 3.1). *Hymenoscyphus epiphyllus* was included into genus *Phaeohelotium* because of the gelatinized textura prismatica in ectal excipulum (Hengstmengel 2009).

36. *Poculum* sp. **Figs. 5.99–5.101**

Stroma substratal, visible on the substrate as blackened areas. **Apothecia** stipitate, occurring on decaying branches; disc discoid to flat, 1.2–6 mm in diameter in dried specimen; hymenium dark brown when fresh, becoming black when dry; receptacle smooth, pale brown to dark brown when fresh, becoming dark brown to black when dry; stipe concolorous with receptacle, 0.7–10 mm long when dry, smooth, becoming dark towards the base. **Ectal excipulum** three layered: covering layer poorly defined but present, 2.5–6 μm wide, hyaline to pale brown, composed of 1–3 layers, granulate hyphae, discontinuous, obviously in the base of stipe; outer ectal excipulum highly refractive, 30–65 μm thick, hyaline, composed of narrow hyphae 2–5 μm wide, imbedded in a gelatinous matrix, parallel to each other or slightly interwoven; inner layer subhyaline, composed of thin walled, smooth, separate hypha of 4–6 μm wide.

Medullary excipulum textura intricata, subhyaline to pale brown, smooth, slightly thicken-walled, separate hypha of 2–5 μm wide. **Asci** 100–125×10–12.5 μm, clavate-cylindric, 8-spored, arising from croziers; apex rounded to truncate, 3–5 μm thick; pore stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 13–16.5×3–5 μm (14.9 ± 0.9×4.0 ± 0.4 μm on average ± SD, n= 20), uniseriate or irregular biseriate in asci, curved, oblong-reniform, sometimes with 2–3 large guttules, 0–1 septate. **Paraphyses** filiform, septate, smooth, containing chestnut colored granular content in the upper two-thirds of their length, expanded at the apex up to 2.5–4 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, initially pale brown, becoming darker in age, forming the brownish hyphae striate zonatly, produced the black exudates near the margin; Aerial mycelium pale brown, cottony, well developed; Margins even; Reverse brown to black; Rind delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, textura globulosa to angularis in face view; **Spermatia** hyaline, globosa, extruding from small tubes at each end of ascospore when ascospores germinated or produced from phialidic spermatiophores in PDA culture, 2.5–4 μm in diameter, containing a small eccentric oil globule.

Specimens examined – On branches of unknown plant: TNS-F-40046, Nishikionuma Park, Tomakomai-shi, Hokkaido, (42º36'39.6"N, 141º27'10.0"E, Alt. 28m), 12 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40048 (Cuture FC-2796), Midorinotonneru, Tomakomai-shi, Hokkaido (42º43'21.5"N, 141º33'58.6"E, Alt. 94m), 13 Sep. 2011, Col.

Y.-J. Zhao.

Notes – The remarkable characters of the present fungus are the gelatinous ectal excipulum, curved and oblong-reniform ascospores, and the spermatia directly produced from ascospores.

Lanzia echinophila (Bull.) Korf resembles the present fungus in the shape, size of asci and ascospores, and both of them have the globose spermatia produced from ascospores. However, the present fungus has the gelatinous matrix in its ectal excipulum. This completely differs from *Lanzia echinophila* (Korf 1982; White 1941). *Poculum firmum* (Pers.) Dumont resembles the present fungus in having the same gelatinous structure in ectal excipulum and the globose spermatia produced from ascospores, but *P. firmum* differs from the present fungus in having the narrow ellipsoid ascospores and the long asci (125–150 μm) (Dumont 1976; White 1941).

37. *Poculum sydowianum* (Rehm) Dumont **Figs. 5.102–5.104**

Mycologia 68: 872 (1976)

Ombrophila sydowiana Rehm, in Sydow, Mycotheca marchia 7: no. 666 (1884)

Ciboria sydowiana Rehm, Hedwigia 24: 226 (1885)

Rutstroemia sydowiana (Rehm) W.L. White, Lloydia 4: 200 (1941)

Stroma substratal, visible on petioles as clear, irregular, black lines delimiting blackened zones. **Apothecia** stipitate, occurring on decaying petioles; disc flat to cupulate when fresh, becoming concave when dry, leathery, 0.6–1.7mm in diameter;

hymenium pale brownish yellow (1365 PC= C0 M33 Y75 k0) when fresh, becoming dark reddish brown to dark brown (175 PC= C18 M79 Y71 K56) when dry; receptacle slightly paler than hymenium when fresh, furfuraceous, with longitudinal striation of reddish brown hyphae running over the surface; stipe subcylindric, concolorous with the receptacle, 1–3.4 mm long when dry, becoming much darker toward base. **Ectal excipulum** three layered: Covering layer 5–10 μm thick, composed of thin walled, septate, pale brownish, 2–4 μm width hyphae; outer ectal excipulum 25–40 μm thick, colorless, gelatinous, highly refractive, composed of very thick-walled, parallel interwoven hyphae, distinct from the margin to the base of stipe; inner layer pale brownish, thin-walled, separate hyphae of 5– 8 μm wide. **Medullary excipulum** textura intricata, loosely interwoven, pale brown, separate hyphae of 3–5 μm wide. **Asci** 82.5–100×7.5–12.5 μm (90.8 ± 4.4×10.3± 1.1 μm on average ± SD, n= 25), clavate, 8-spored, arising from crozier; apex truncate, 4–5 μm thick; pore stained blue by Melzer's reagent. **Ascospores** 10–13.8×5–6.3 μm (12.5 ± 1.1×5.3± 0.5 μm on average ± SD, n= 25), uniseriate, broadly obovoid-reniform, straight to strongly curved, smooth, some with a large guttules in the center; When germinated in PDA, spores becoming 1–3 septate, pale brown to brown, cell walls becoming slightly thickened and darker. **Paraphyses** straight, septate, hyaline, slightly enlarged at the apex of 2–4 μm wide.

Cultural characteristics – Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, flocculent, whitish to grayish; Aerial mycelium white, cottony, fully developed; Margins entire; Reverse pale brown; Stroma formation not observed on culture; No stroma like structure were produced in culture. **Spermatia** hyaline, extruding singly or successively from small tubes at one end of ascospore when

ascospores germinated on PDA or in culture produced from phialidic spermatiophores, , broadly ellipsoid to subglobose, 2–3.2×1.8–3.3 μm.

Specimens examined – On petioles of *Quercus crispula*: TNS-F-40039, Nishikionuma Park, Tomakomai-shi, Hokkaido Pref., (42º36'39.60"N, 141º27'10.00"E, Alt. 27m), 12 Sep. 2011, Col. Y.-J. Zhao; TNS-F-42316 (Culture FC-2766), Green tunnel, Tomakomai-shi, Hokkaido Pref. (42º43'22.18"N, 141º33'59.89"E, Alt. 92m), 13 Sep. 2011, Col. T. Hosoya; TNS-F-40059, Tomakomai Experimental Forest, Hokkaido University, Tomakomai-shi, Hokkaido Pref. (42º40'21.70"N, 141º36'26.60"E, Alt. 22m), 15 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40071 (Culture FC-2813), Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'30.00"N, 138º20'51.20"E Alt. 1349m), 28 Sep. 2011, Col. Y.-J. Zhao; TNS-F-40165, Takayama-mura, Gunma Pref., Japan (36º35'19.3"N, 138º57'36.5"E, Alt. 242m),
2 Oct. 2012, Col. Y.-J. Zhao;
On petioles and branches of unknown plant: TNS-F-40172, Niigata Pref., Japan (37º0'56"N, 138º46'54.7"E, Alt. 846m), 3 Oct. 2012, Col. Y.-J. Zhao.
On petioles of *Quercus serrata*: TNS-F-40163 (FC-5091), Takayama-mura, Gunma Pref. (36º35'19.3"N, 138º57'36.5"E, Alt. 242m), 2 Oct. 2012, Col. Y.-J. Zhao.

Notes – The morphological features agreed with description of *P. sydowianum* although the asci are slightly larger, 115–130×10–12 μm (White 1941, as *Rutstroemia sydowiana*). *Poculum sydowianum* occurs on petioles of *Quercus* spp. and is characterized by the obovoid-reniform ascospores and reddish brown striation on the receptacle (White 1941).

Poculum myricae Spooner & Dennis resembles *P. sydowianum* in many respects, but differs in its small spores (10–11.5×5–5.5 μm), short asci (82–96 μm), no striation in receptacle, and occurs on branches of *Myrica gale* (Spooner and Dennis 1985). *Poculum petiolorum* (Roberge ex Desm.) Dumont & Korf also has the reniform spores and occurs on *Quercus* spp., but *P. petiolorum* is long stipitate reaching a height of 2 cm, much longer and thinner ascospores (14–17×4.5–5.5 μm), without reddish brown hyphae in receptacle.

The dark coloration of ascospore in germination was observed in Japanese material.

This is the first report of *P. sydowianum* from Japan.

38. *Rutstroemia bulgarioides* P. Karst. **Figs. 5.105–5.107**

Bidr. Känn. Finl. Nat. Folk 19: 165 (1871)

Peziza bulgarioides Rabenh., Fungi Europaei no. 1008 (1867)

Piceomphale bulgarioides (P. Karst.) Svrček, Česká Mykol. 11: 237, 240 (1957)

Chlorociboria bulgarioides (P. Karst.) C.S. Ramamurthi, Korf & L.R. Batra, Mycologia 49: 860 (1958) [1957]

Ciboria bulgarioides (P. Karst.) Baral, in Baral & Krieglsteiner, Beih. Z. Mykol. 6: 11 (1985)

Stroma obscure on substrate. **Apothecia** stipitate to subsessile, occurring on the cones of *Picea*; disc discoid or curved when fresh, becoming irregular shrinking when dry, 3–8 mm in diameter in dried specimen; hymenium black or olive green (444 PC= C38

M15 Y18 K43) when fresh, becoming totally black when dry; receptacle concolorous with hymenium, smooth; stipe short, concolorous with receptacle, 1–3 mm long when dry, smooth. **Ectal excipulum** two layered: covering layer absent; outer ectal excipulum composed of angularis to irregular cells, with globosa cells merged, thick-walled or with two layers of cell membranes, smooth, subhyaline, becoming brown towards margin, the outermost layer of pale brown to brown; inner layer obviously in the margin, obscure in the flank, dark brown, smooth or slightly granulate, separate hyphae of 2–4 μm width. **Medullary excipulum** textura intricata, subhyaline to pale brown, smooth, hypha of 2–4 μm wide, tightly interwoven. **Asci** 83–92×6–9 μm, clavate, 8-spored, crozier absent; apex slightly truncate, 3–3.5 μm thick; pore stained blue in Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–11×3–4 μm (8.9 ± 0.9×3.9 ± 0.3 μm on average ± SD, n=21), uniseriate or biseriate above, ellipsoid, smooth or warted, eguttulate; microspores globose to ellipsoid, with one eccentric guttule, 4–7 μm in diameter. **Paraphyses** filiform, septate, hyaline, slightly enlarged at the apex up to 3 μm wide.

Cultural characteristics – Colonies on PDA grows very slowly, reach about 17 cm after 4 weeks incubation, irregular, cottony, initially grayish (Warm gray 2C = R213 G210 B202), becoming pale brown in age, produced the black exudates in the surface; Aerial mycelium gray, poorly developed; Margins undulate; Reverse gray, with pale brown in the central, irregular, margin grayish, merged with rhizoid, intermittent, blacked lines delimiting irregular area; Rind diffused, dark brownish to black.

Specimens examined – On decaying fruits of *Picea jegoensis* (Sieb. et Zucc.) Carrière:

TNS-F-40005 (Culture FC-2715), Kamikawa-cho, Hokkaido, 23 April 2011, Col. S. Sato; TNS-F-40022, Oohora, Sugadaira, Ueda City, Nagano Pref. (36º29'59.4"N, 138º20'20.90"E, Alt. 1216m), 18 May 2011, Col. Y.-J. Zhao; TNS-F-40109, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (36º31'30.00"N, 138º20'51.20"E, Alt. 1349m), 7 May 2012, Col. Y.-J. Zhao; TNS-F-40110, Daido,Sugadaira Montane, University of Tsukuba, Ueda City, Nagano Pref. (36º30'17.80"N, 138º19'43.40"E, Alt. 39 m), 7 May 2012, Col. Y.-J. Zhao.

Notes – *Rutstroemia bulgarioides* P. Karst. occurs on the cones of *Picea* in late spring or early summer in temperate areas. It is distinguished by its greenish to black apothecia and the complicated ectal excipulum structure.

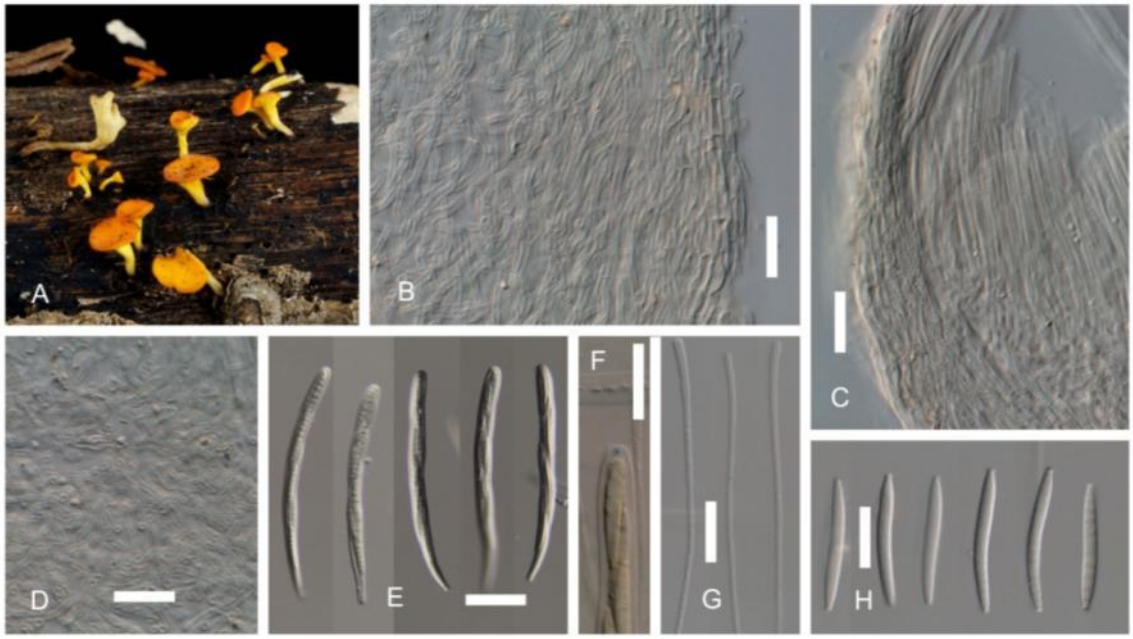

Fig. 5.1. *Dicephalospora rufocornea* (TNS-F-40024). **A:** Fresh apothecia on decayed branch. **B:** Close up of ectal excipulum. **C:** Close up of ectal excipulum at the margin. **D:** Structure in medullary excipulum. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Ascospores. *Bars* **B–D, F–H** 20 µm; **E** 40 µm.

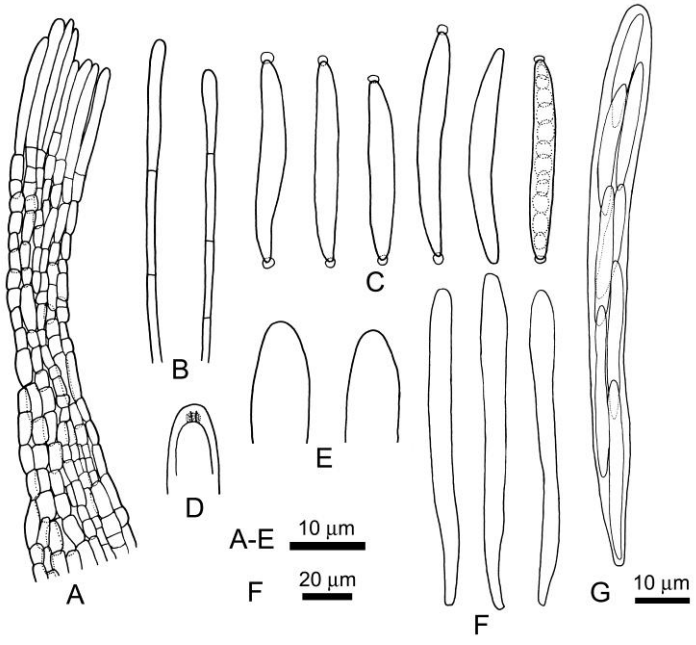

Fig. 5.2. Camera lucida illustration of *Dicephalospora rufocornea* (TNS-F-40024). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Paraphyses. **C:** Ascospores. **D:** Reaction of ascal apex to MLZ. **E:** Apex of asci. **F, G:** Asci. *Bars* **A–E** 10 µm; **F** 20 µm; **G** 10 µm.

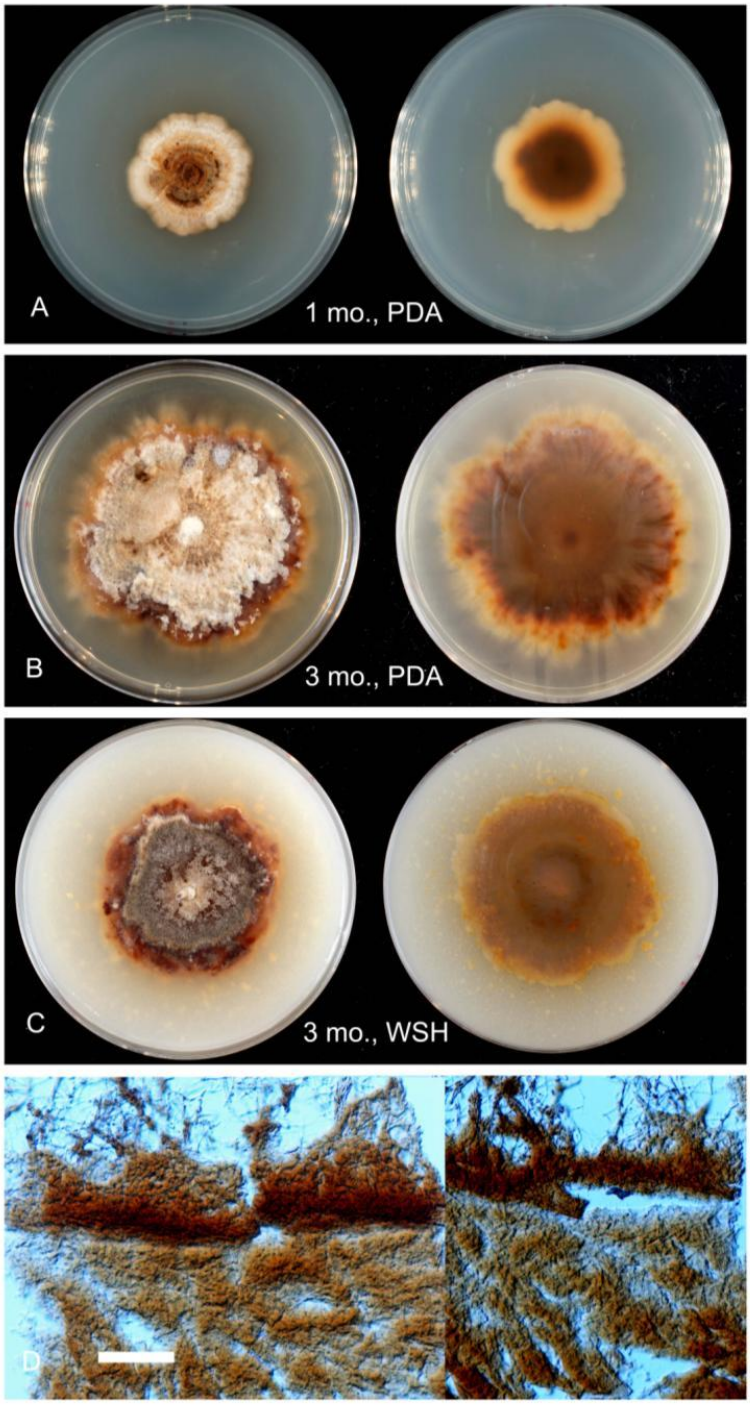

Fig. 5.3. Cultural characters of *Dicephalospora rufocornea* (FC-2730, Culture of TNS-F-40024). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.). **D:** Vertical view of blackened section produced in 3 month on PDA. *Bars* **D** 100 μm.

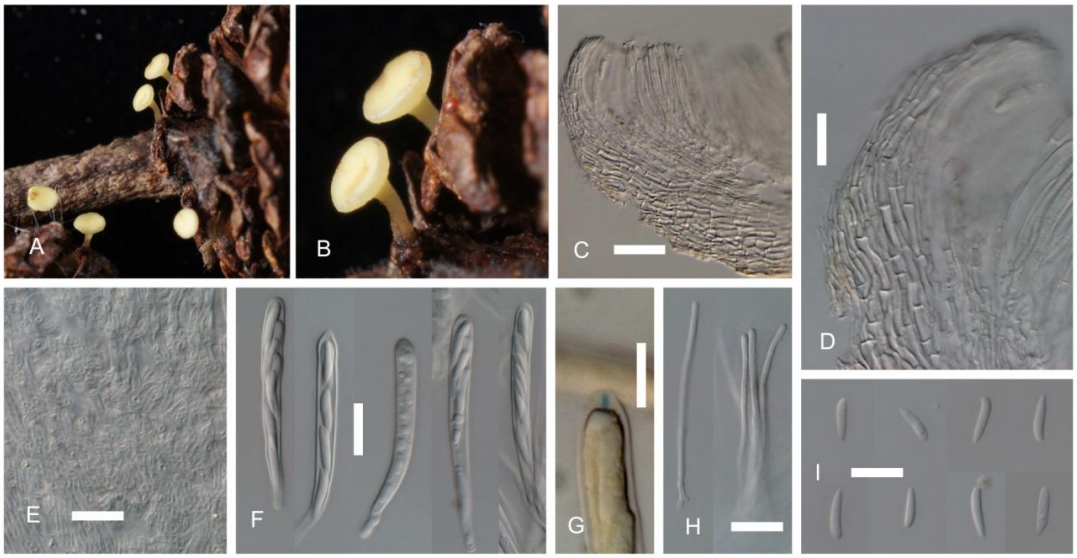

Fig.5.4. *Hymenoscyphus caudatus* (TNS-F-40056). **A:** Fresh apothecia on fallen fruits of *Alnus* sp. **B:** Close up of apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Structure in medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. *Bars* **C** 40 µm; **D–F, H–I** 20 µm; **G** 10 µm.

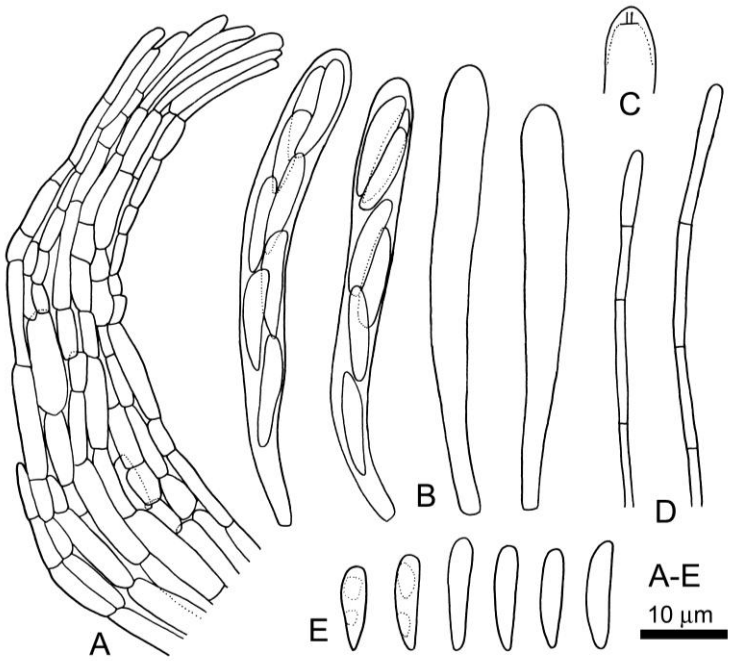

Fig. 5.5. Camera lucida illustration of *Hymenoscyphus caudatus* (TNS-F-40056). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

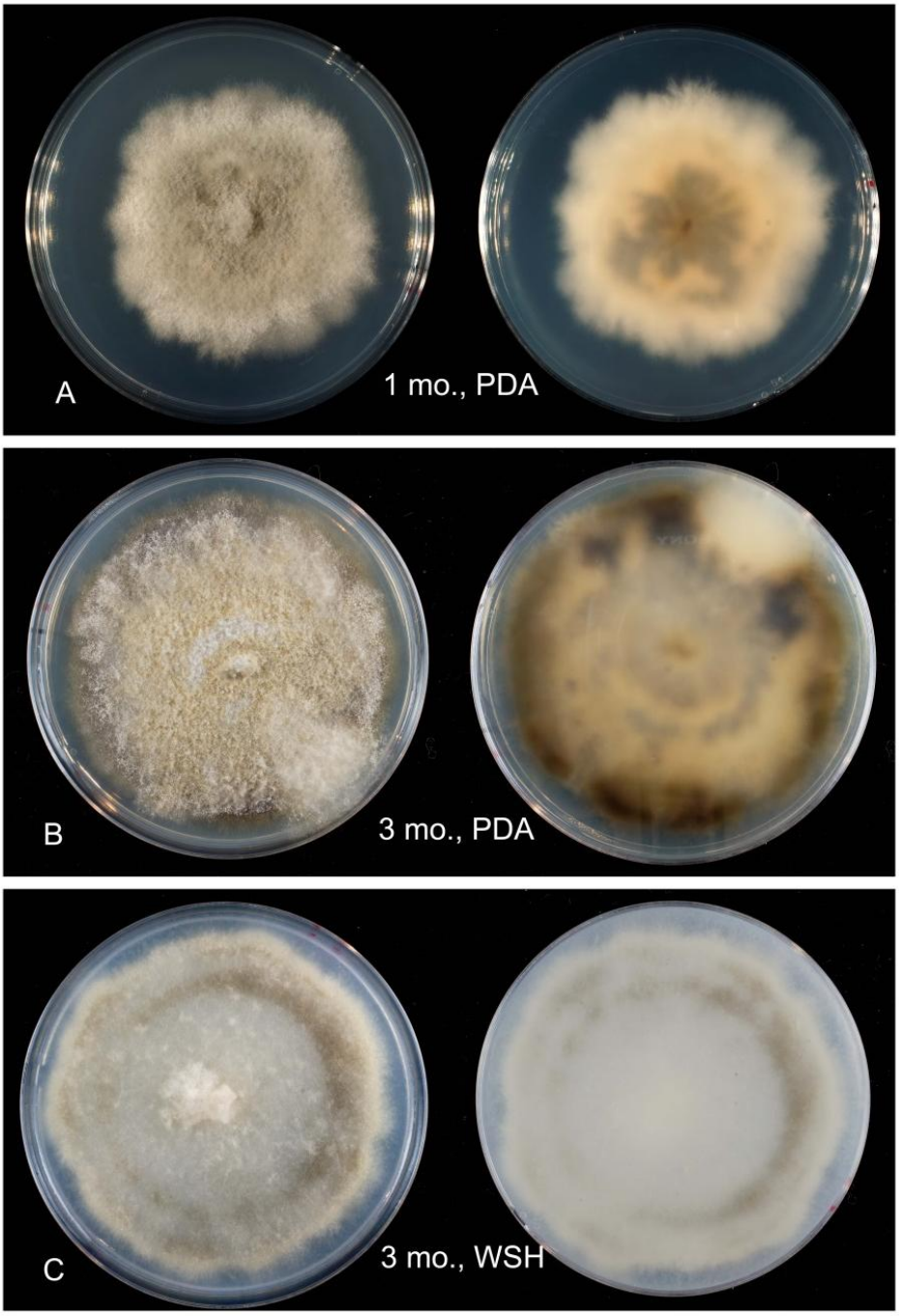

Fig. 5.6. Cultural characters of *Hymenoscyphus caudatus* (FC-2803, Culture of TNS-F-40056). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.).

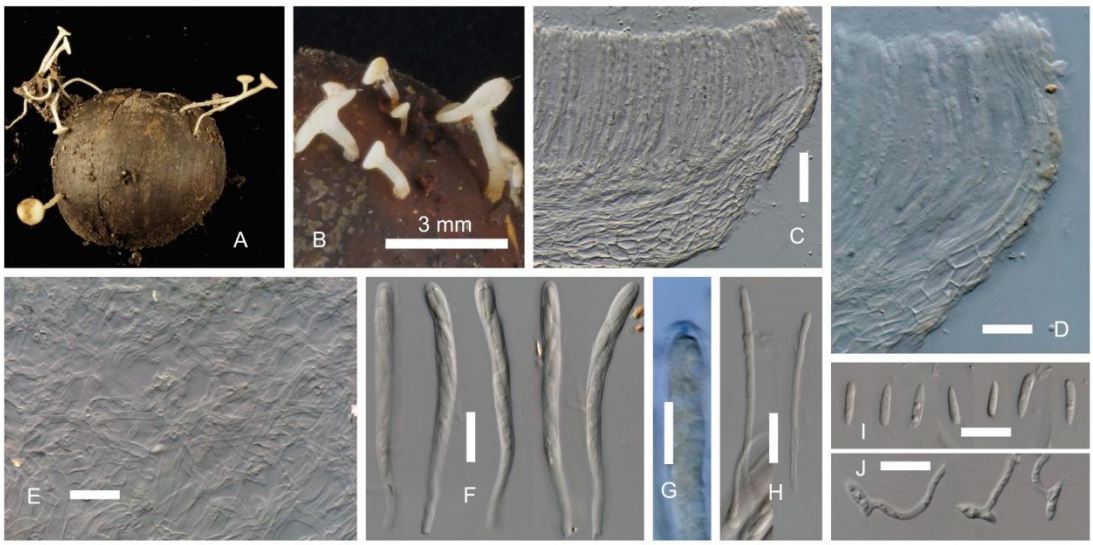

Fig. 5.7. *Hymenoscyphus fructigenus* (TNS-F-44644). **A:** Fresh apothecia on fallen acorns of *Quercus* sp. **B:** Close up of apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Structure in medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. **J:** Germinating ascospores. *Bars* **B** 3 mm; **C** 40 µm; **D–F, H–J** 20 µm; **G** 10 µm.

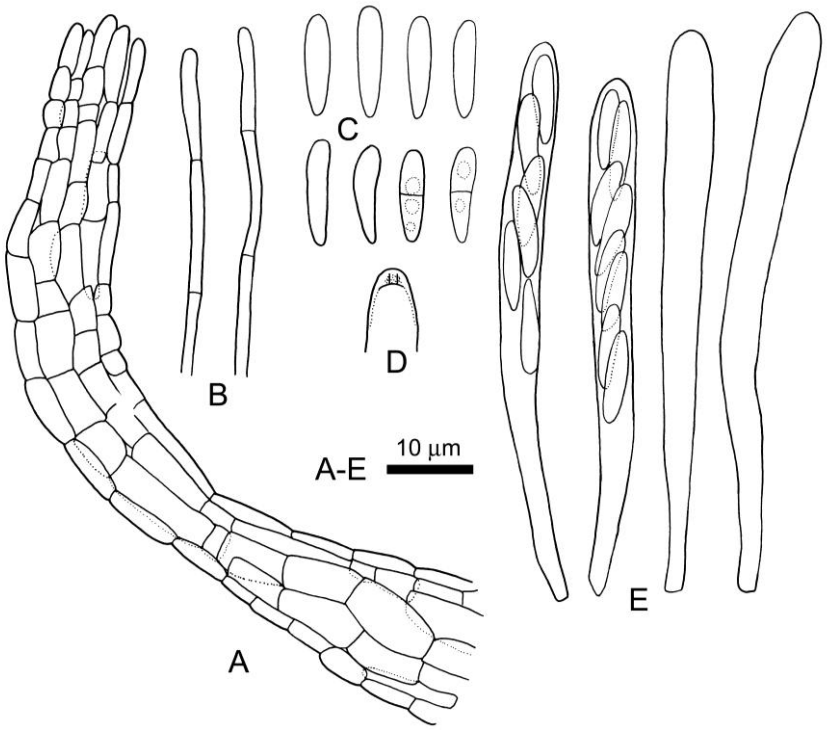

Fig. 5.8. Camera lucida illustration of *Hymenoscyphus fructigenus* (TNS-F-44644). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Paraphyses. **C:** Ascospores. **D:** Reaction of ascal apex to MLZ. **E:** Asci. *Bars* **A–E** 10 µm.

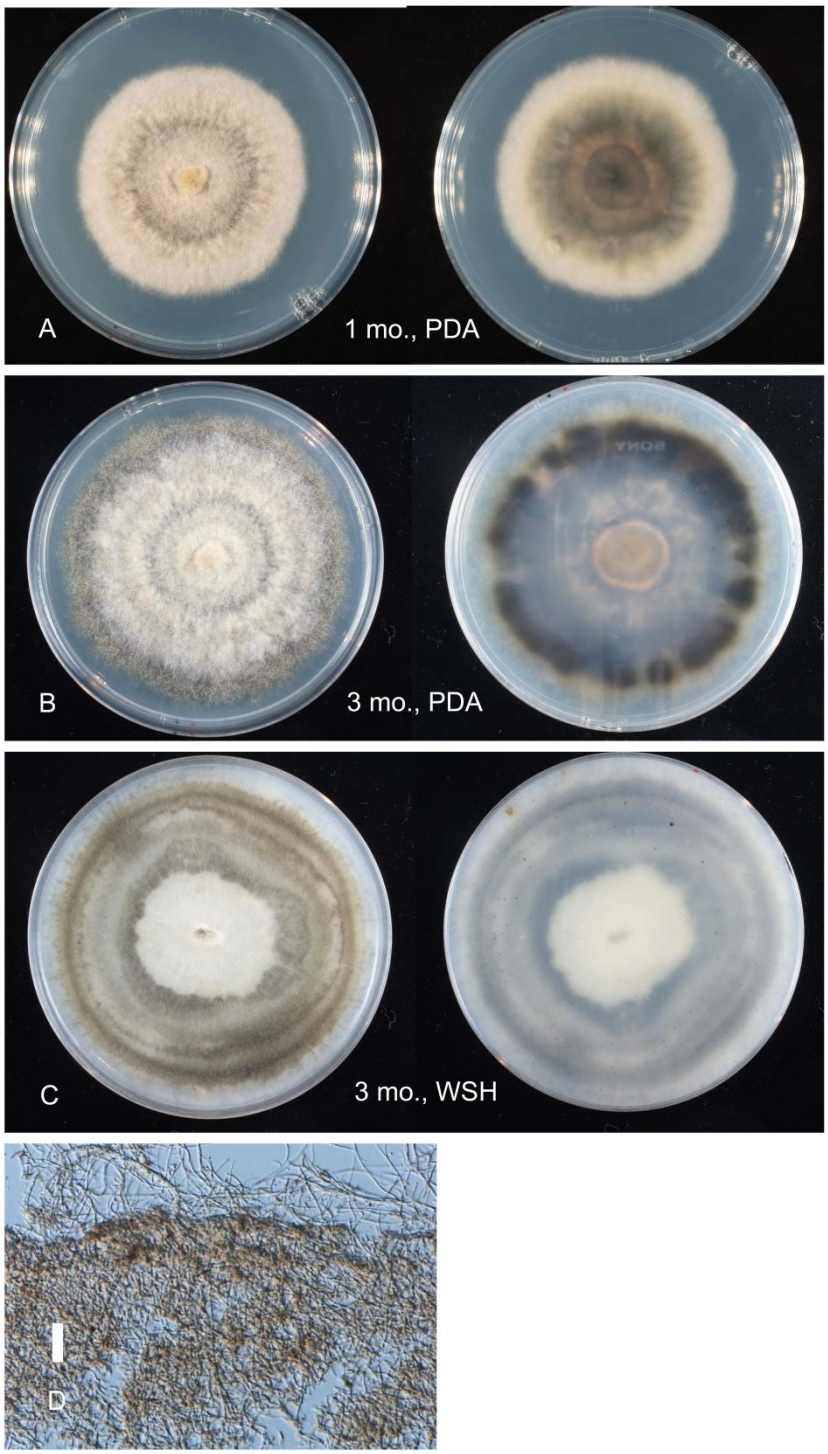

Fig. 5.9. Cultural characters of *Hymenoscyphus fructigenus* (FC-2855, Culture of TNS-F-44644). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.). **D:** Vertical view of blackened area produced in 3 month on PDA. *Bars* **D** 50μm.

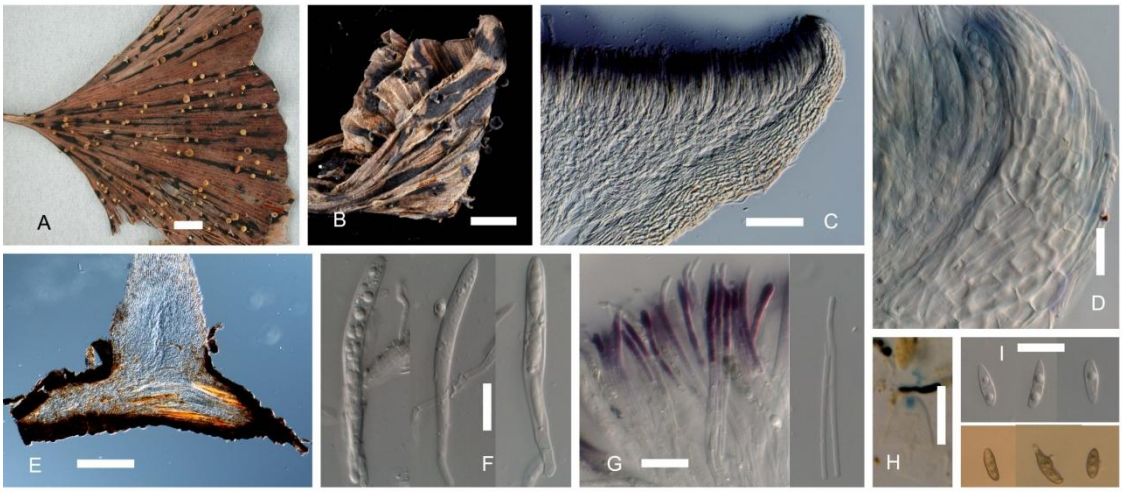

Fig. 5.10. *Hymenoscyphus ginkgonis* (TNS-F-11208). **A:** Fresh apothecia on fallen leaves of *Ginkgo biloba* **B:** Dried apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Vertical section at the base of the stipe showing the stroma. Note thin, black line (rind) surrounding the base of the stipe. **F:** Asci. **G:** Paraphyses. **H:** Reaction of ascal apex to MLZ. **I:** Hyaline ascospores and germinated ascospores. *Bars* **A** 5 mm; **B** 2 mm; **C** 100 µm; **D, F, G, I** 20 µm; **E** 200 µm; **H** 10 µm.

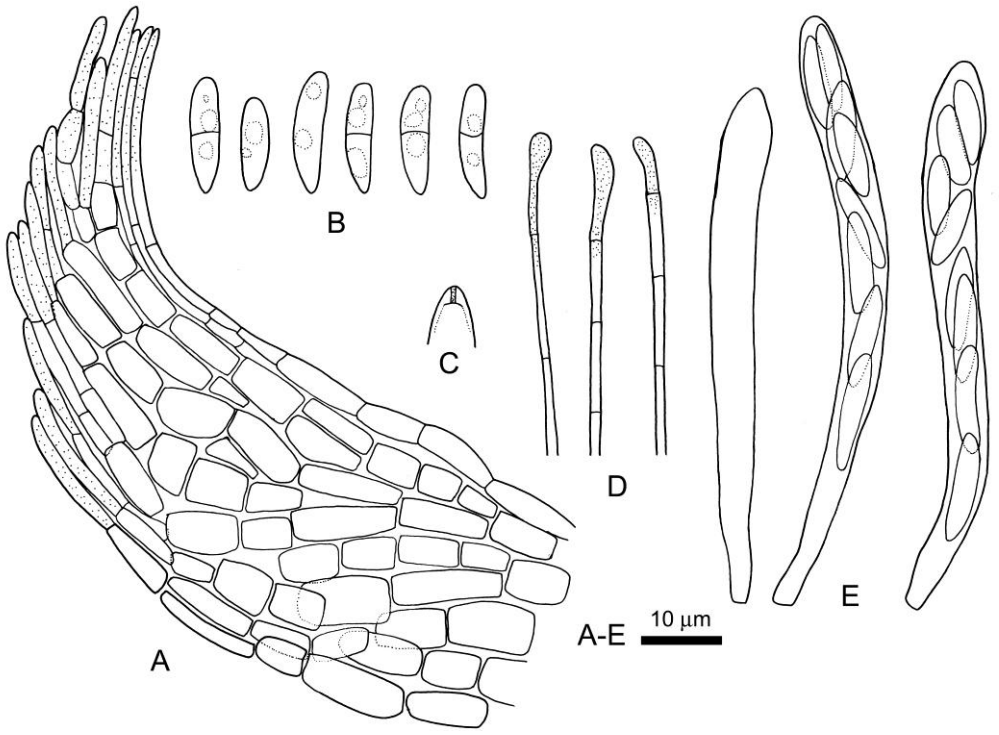

Fig. 5.11. Camera lucida illustration of *Hymenoscyphus ginkgonis* (TNS-F-11208). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Ascospores. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Asci. *Bars* **A–E** 10 µm.

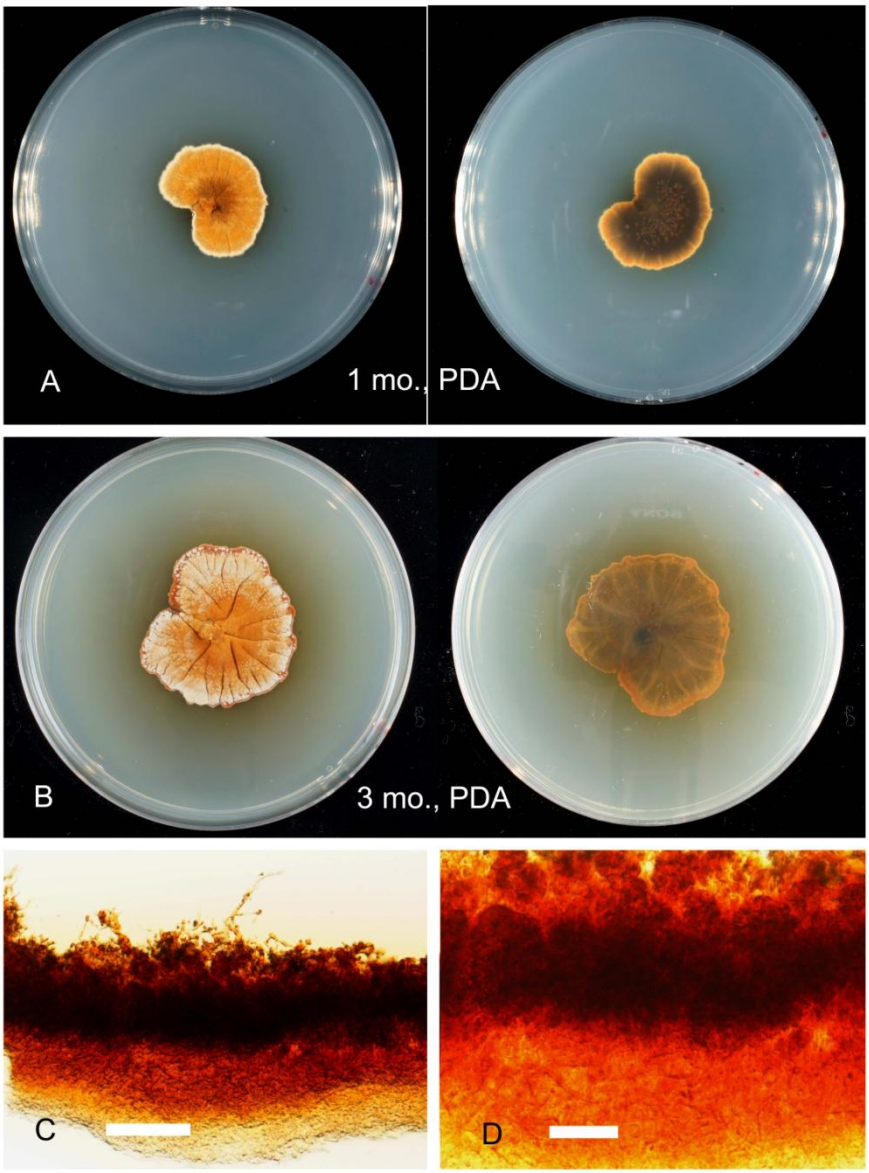

Fig. 5.12. Cultural characters of *Hymenoscyphus ginkgonis* (FC-1093, Culture of TNS-F-11208). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C, D:** Vertical view of blackened area produced in 3 month on PDA. *Bars* **C** 100μm**; D** 40μm.

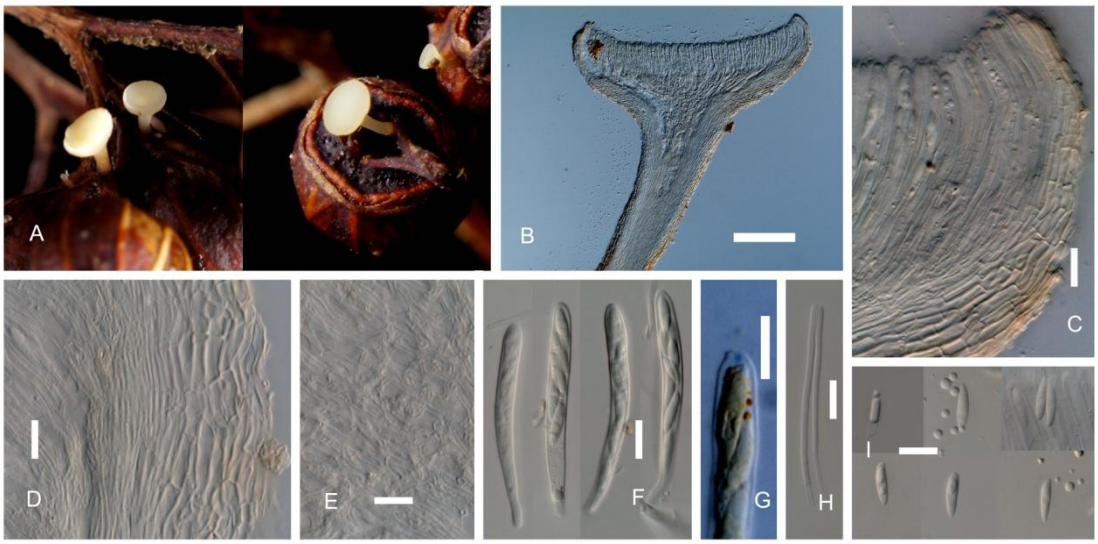

Fig. 5.13. *Hymenoscyphus menthae* (TNS-F-40052). **A:** Fresh apothecia on fallen fruits of *Hydrangea* sp. **B:** Vertical section of ectal excipulum. **C:** Close up of ectal excipulum at the margin. **D:** Close up of ectal excipulum in flank showing the layered structure. **E:** Structure in medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. *Bars* **B** 200 µm; **C–F, H–I** 20 µm; **G** 10 µm.

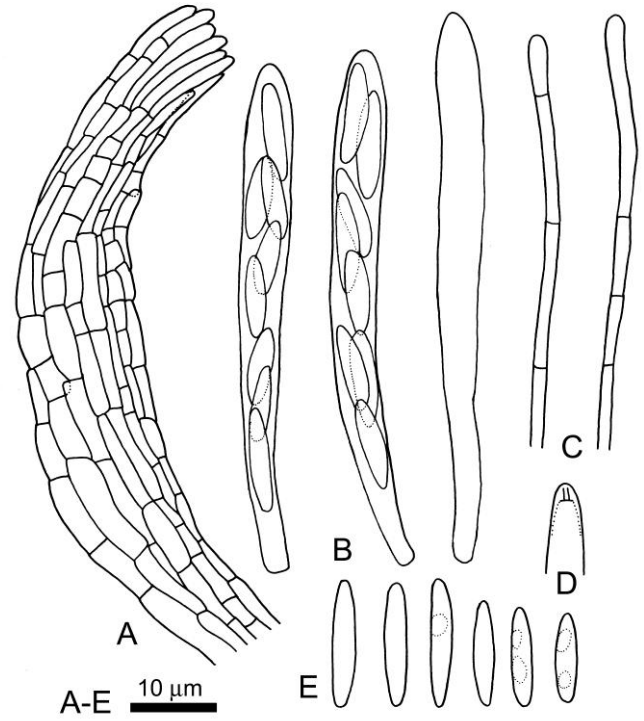

Fig. 5.14. Camera lucida illustration of *Hymenoscyphus menthae* (TNS-F-40052). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

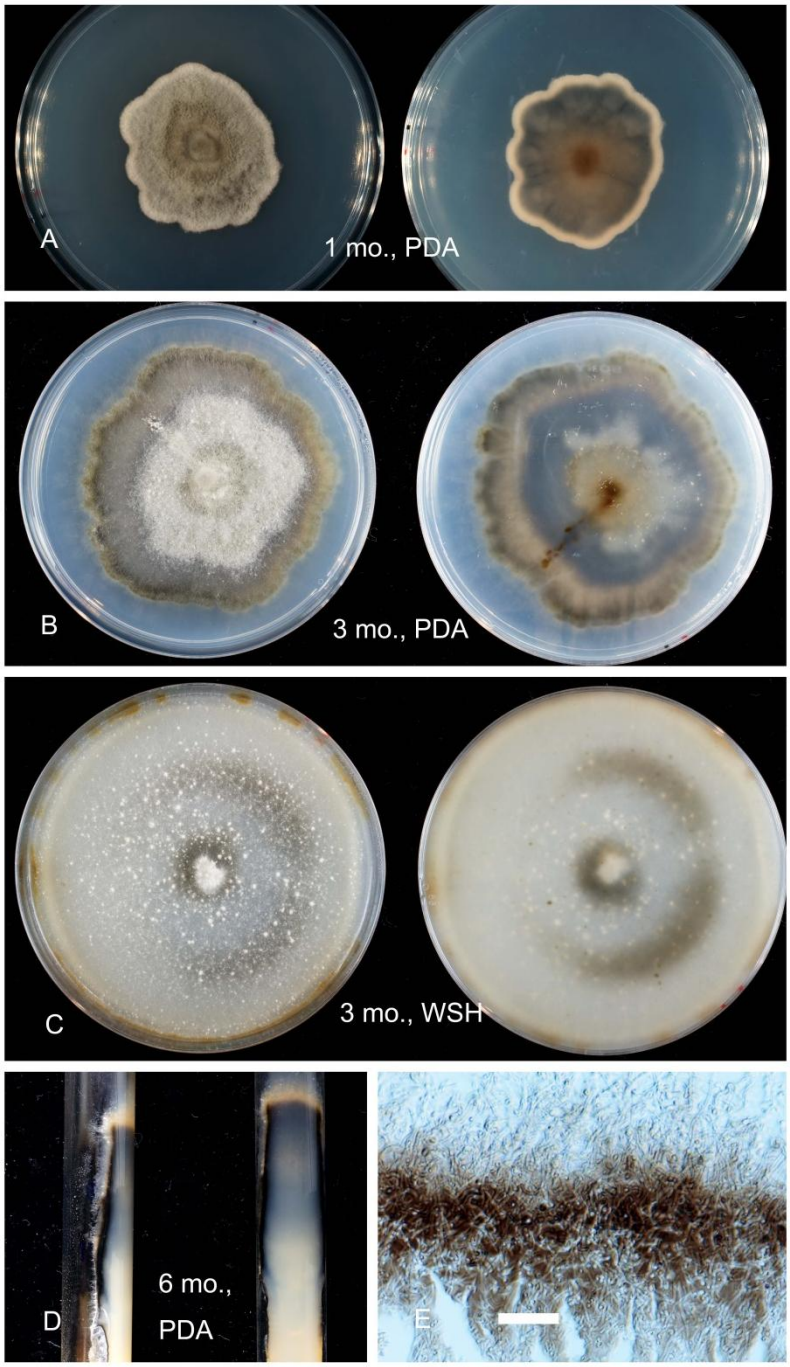

Fig. 5.15. Cultural characters of *Hymenoscyphus menthae* (FC-2800, Culture of TNS-F-40052). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.). **D:** Colony on PDA (20℃, 6 mo.), showing the blackened margin. **E:** Vertical view of blackened margin produced in 6 month on PDA. *Bars* **E** 40μm.

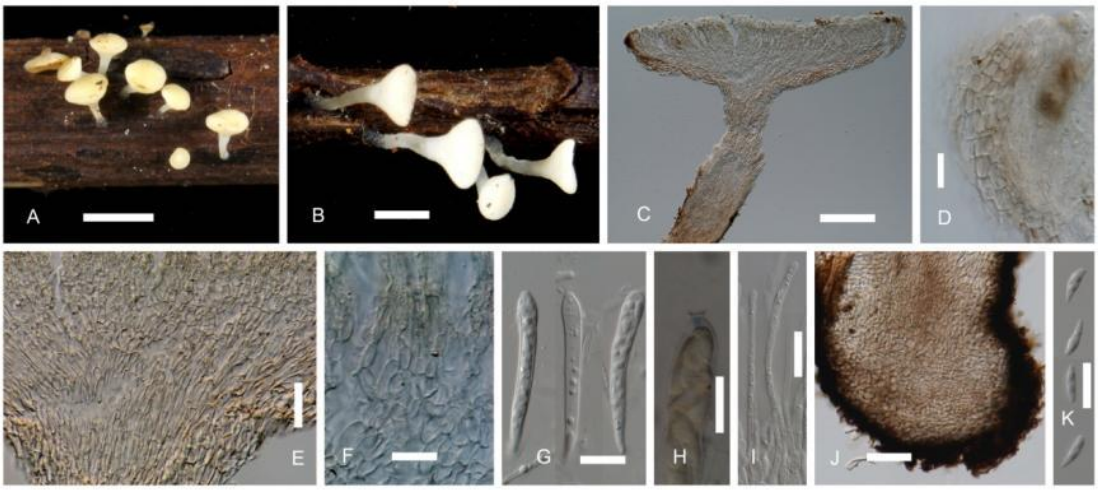

Fig. 5.16. *Hymenoscyphus microserotinus* (TNS-F-40067). **A: B:** Fresh apothecia on decayed petioles of *Aesculus turbinate* **C:**Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Structure in medullary excipulum. **F:** Close up of medullary excipulum. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Paraphyses. **J:** Rind at the base of apothecium. **K:** Ascospores. *Bars* **A** 2 mm**; B** 1 mm; **C** 200 µm; **D–G, I K** 20 µm; **H** 10µm; **J** 40 µm.

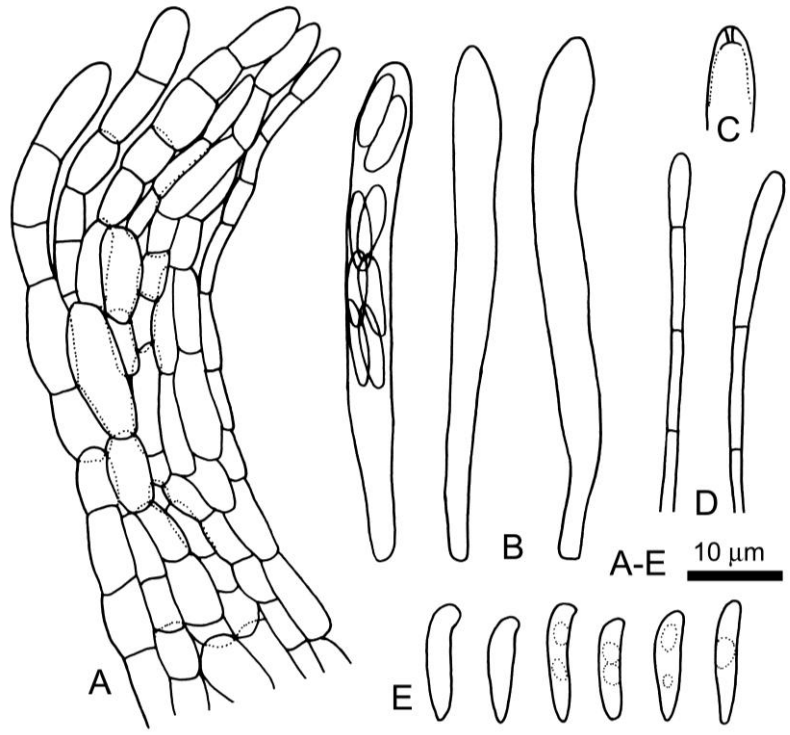

Fig. 5.17. Camera lucida illustration of *Hymenoscyphus microserotinus* (TNS-F-40067). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

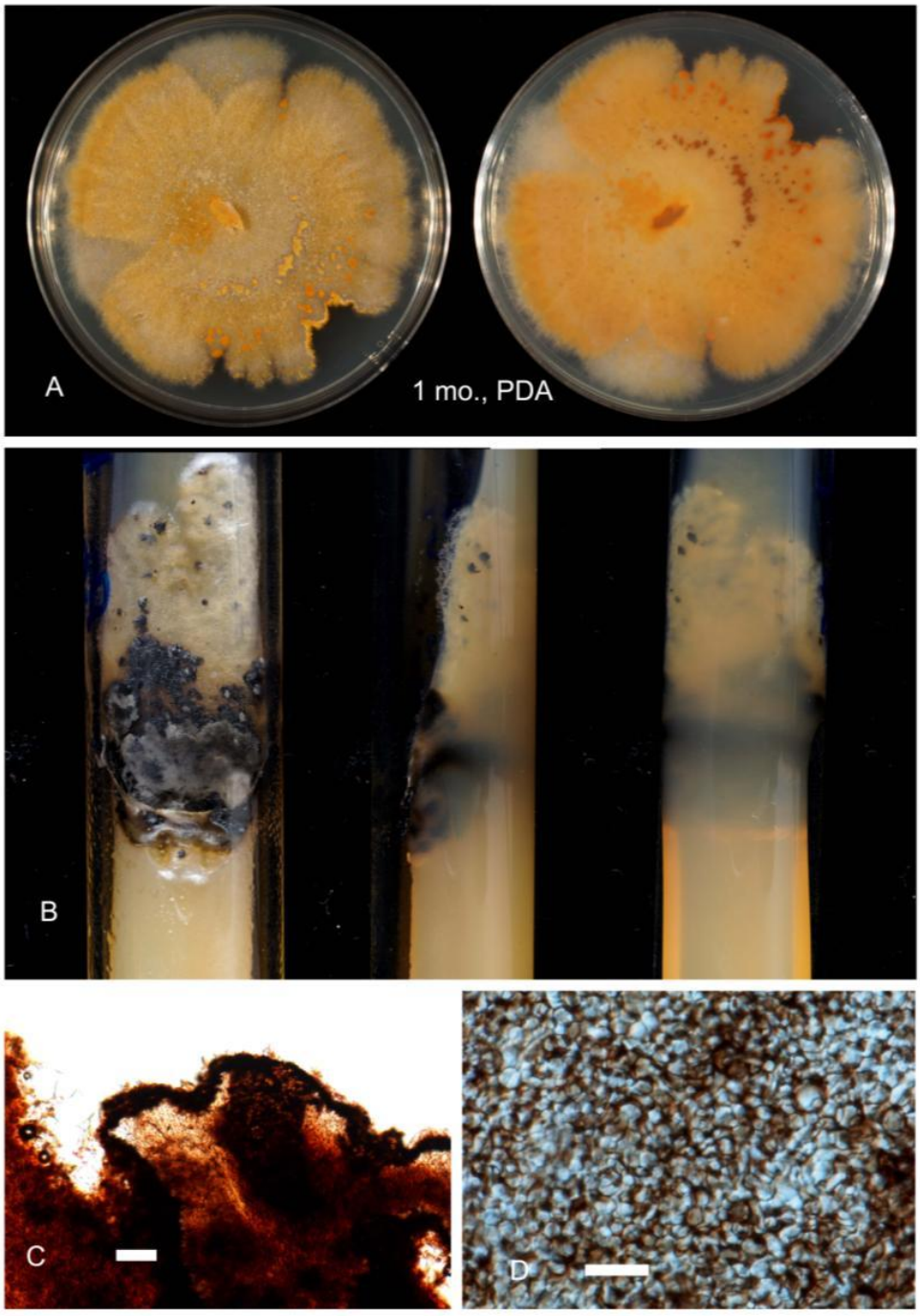

Fig.5.18. Cultural characters of *Hymenoscyphus microserotinus* (FC-2811, Culture of TNS-F-40067). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, > 5 mo.), showing the rind in culture. **C:** Vertical view of rind. D: Surface view of rind showing the textura globulosa cells. *Bars* **C** 50 μm; **D** 20μm.

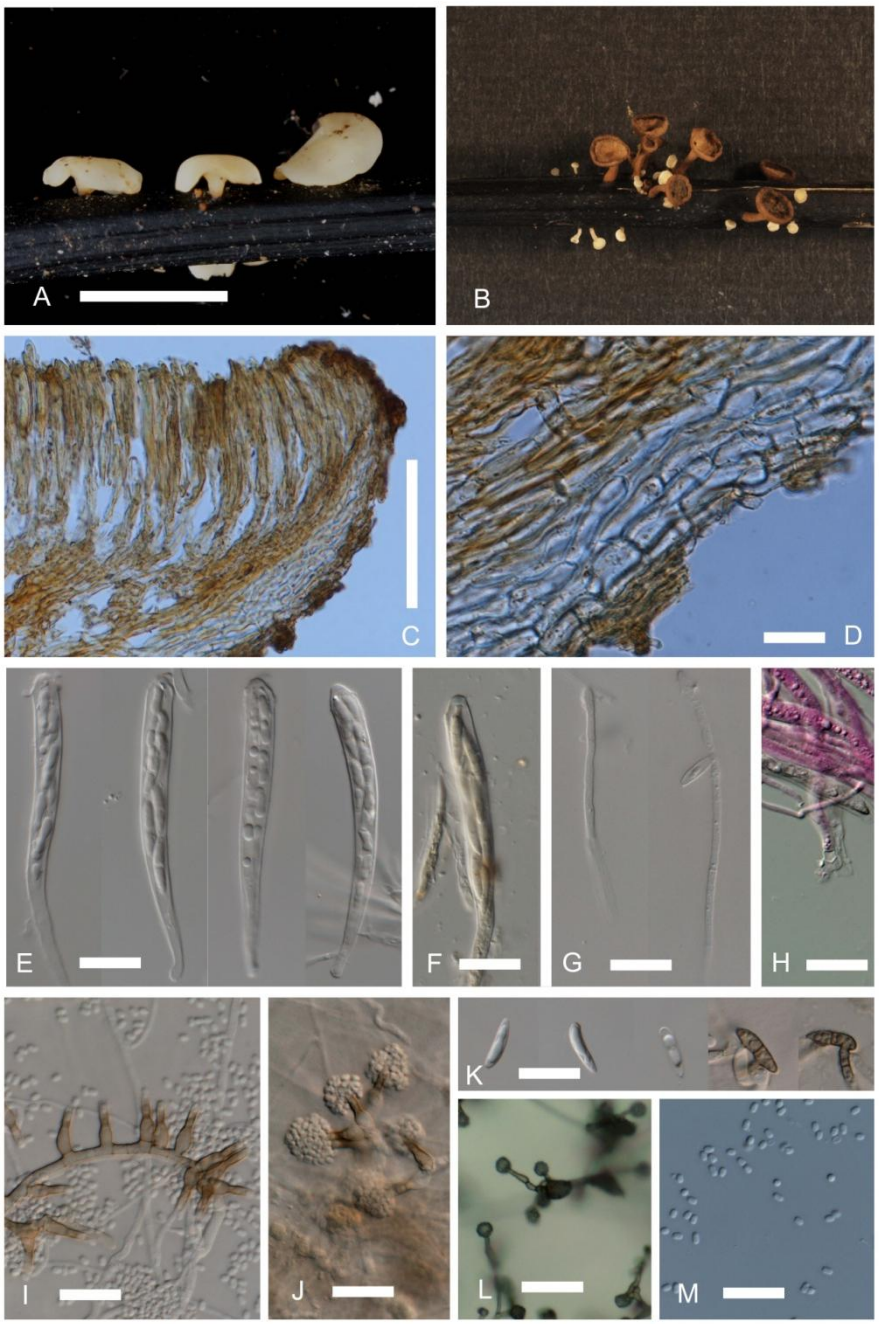

Fig. 5.19. *Hymenoscyphus pseudoalbidus* (TNS-F-40051). **A:** Fresh apothecia on fallen petioles of *Fraxinus mandshurica* **B:** Dried apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Crozier at the base of ascus. **I:** Spermatiophores. **J: L:** Spermatia and Spermatiophores. **K:** Hyaline ascospores and brown ascospores in germination. Spermatia and spermarphos. **M:** Spermatia. *Bars* **A** 5 mm; **C** 100 μm; **D–K, M** 20 μm; **L** 40 μm.

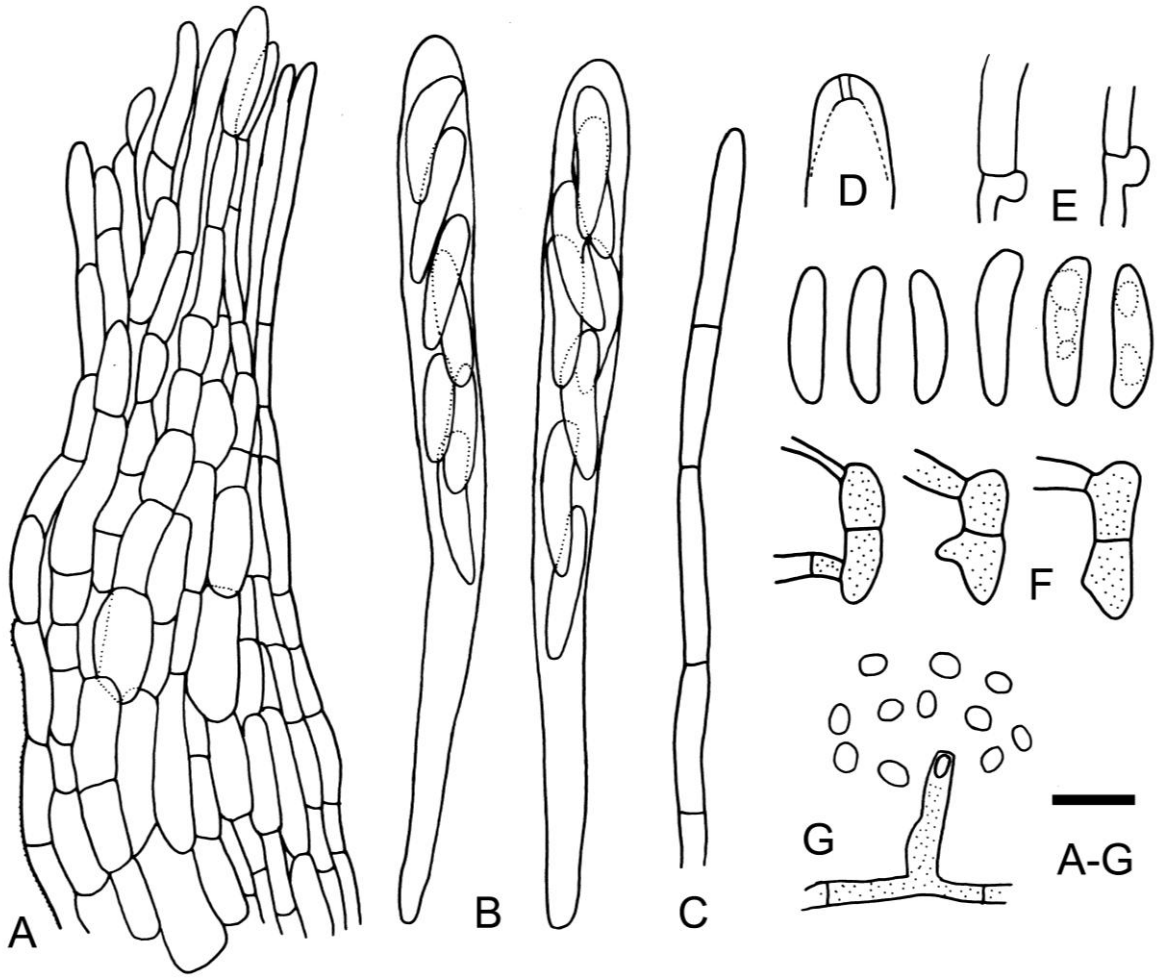

Fig. 5.20. Camera lucida illustration of *Hymenoscyphus pseudoalbidus* (TNS-F-40051). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Asci in the base of asci. **F:** Ascospores. **G:** Spermatia and spermatiophore. *Bars* **A–G** 10 μm.

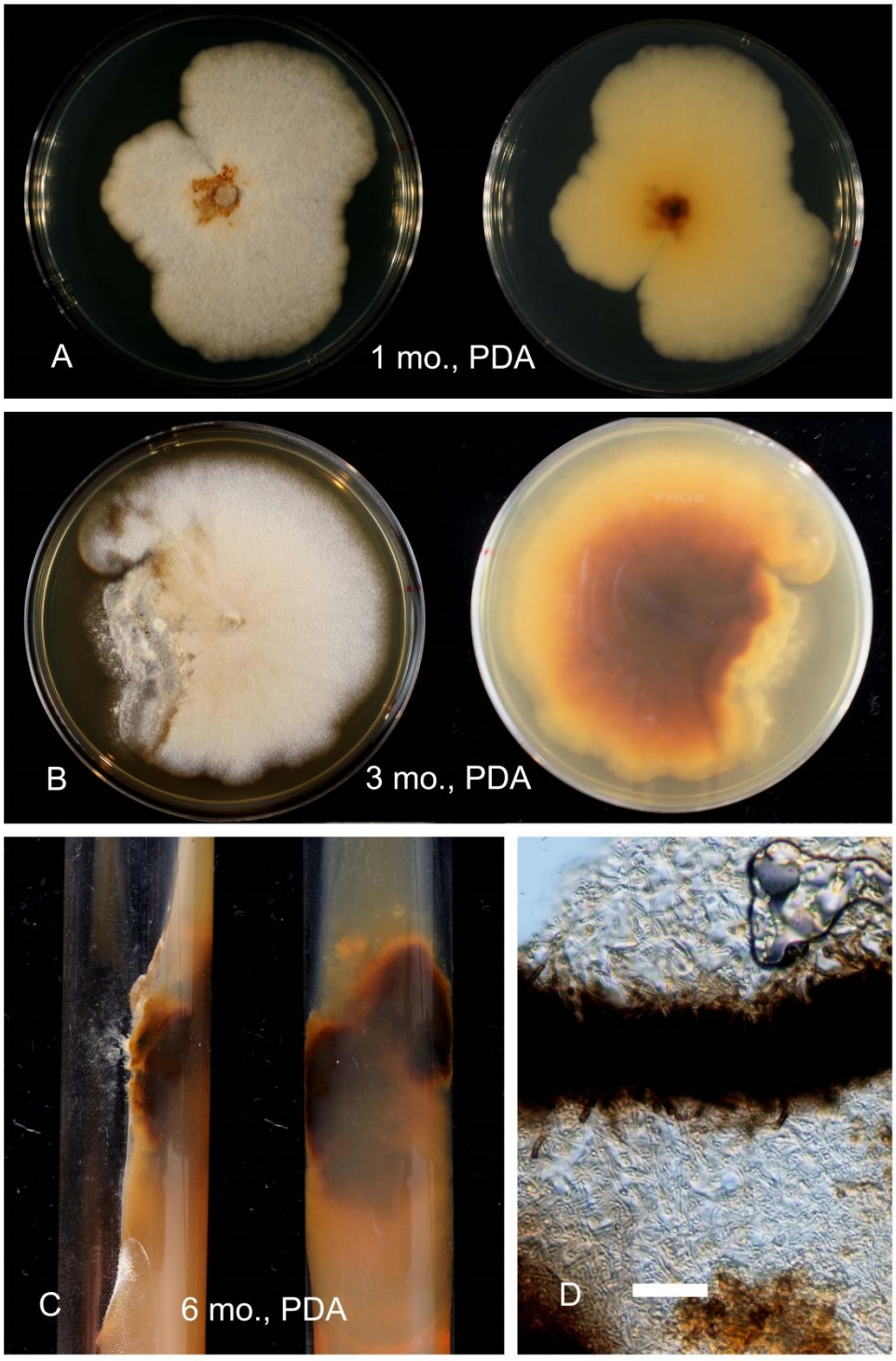

Fig. 5.21. Cultural characters of *Hymenoscyphus pseudoalbidus* (FC-2799, Culture of TNS-F-40051). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on PDA (20℃, 6 mo.), showing the blackened area. **D:** Vertical section of blackened area. *Bars* **D** 20μm.

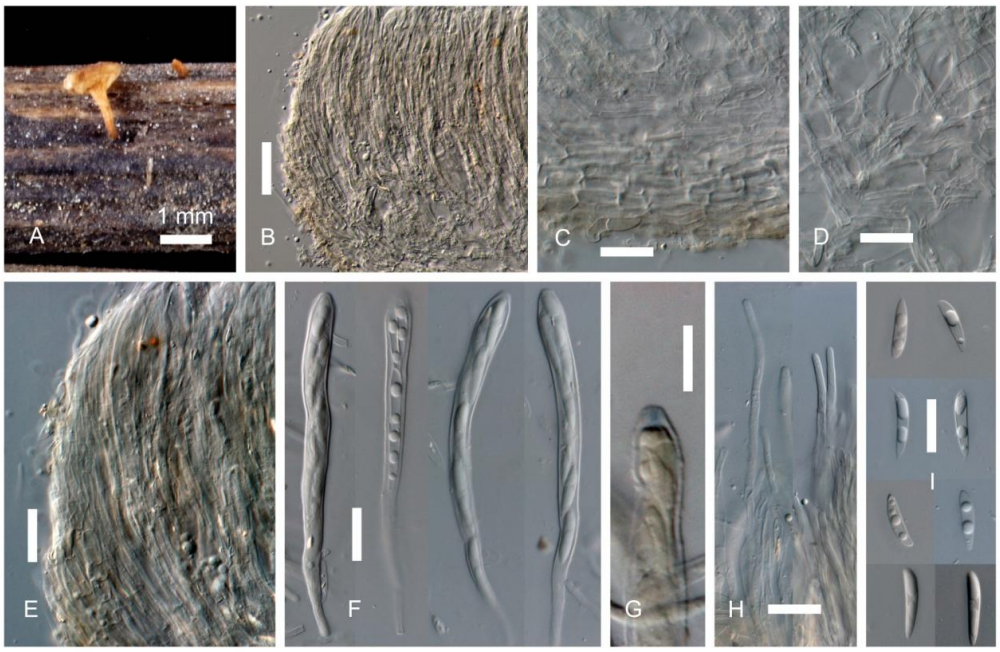

Fig. 5.22. *Hymenoscyphus scutula* (TNS-F-17507). **A:** Fresh apothecia on decaying herb stems**. B:** Vertical section of ectal excipulum. **C:** Close up of ectal excipulum in flank showing the layers. **D:** Structure in medullary excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores.

Bars **A** 1 mm; **B** 40 µm; **C–F, H–I** 20 µm; **G** 10 µm**.**

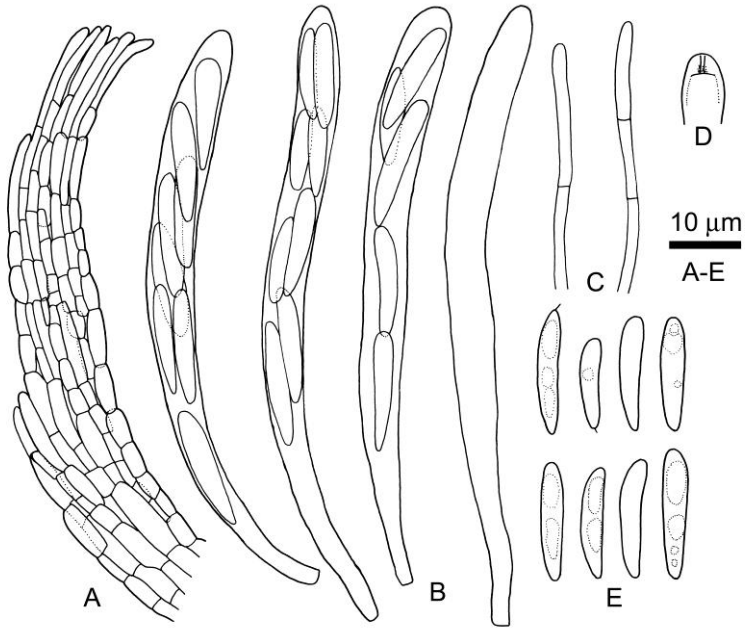

Fig. 5.23. Camera lucida illustration of *Hymenoscyphus scutula* (TNS-F-17507). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

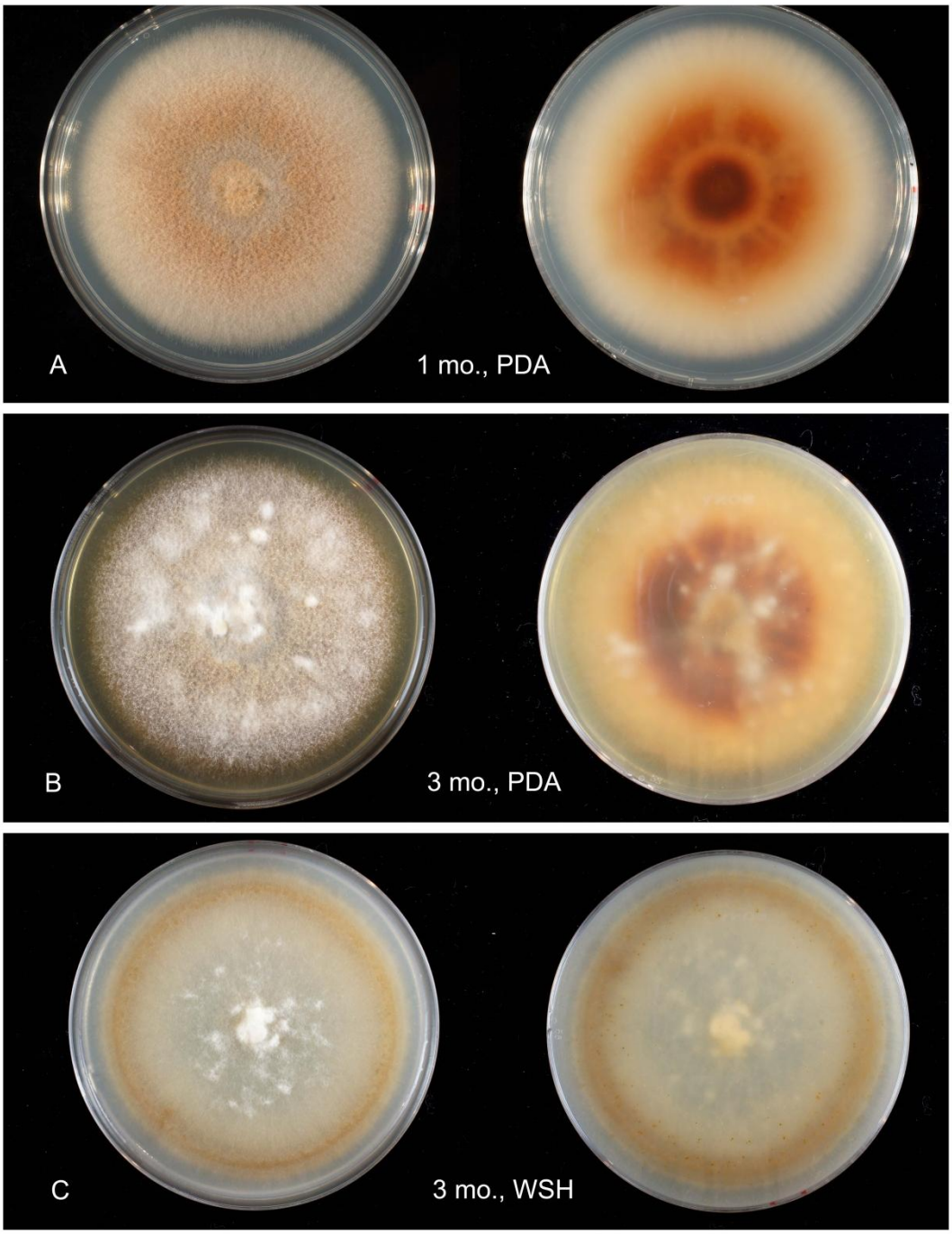

Fig. 5.24. Cultural characters of *Hymenoscyphus scutula* (FC-1080, Culture of TNS-F-17507). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.).

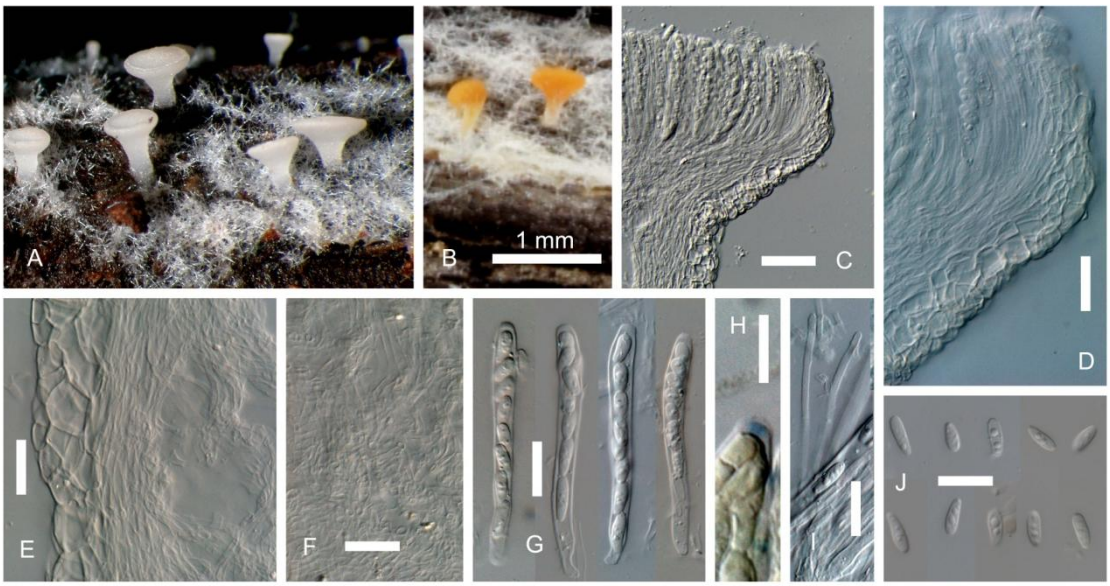

Fig. 5.25. *Hymenoscyphus varicosporioides* (TNS-F-16472). **A:** Fresh apothecia on rotting twigs**. B:** Dried apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** ectal excipulum in flank showing the layers. **F:** Structure in medullary excipulum. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Paraphyses. **J:** Ascospores. *Bars* **B** 1 mm; **C** 40 µm; **D–G, I J** 20 µm; **H** 10 µm.

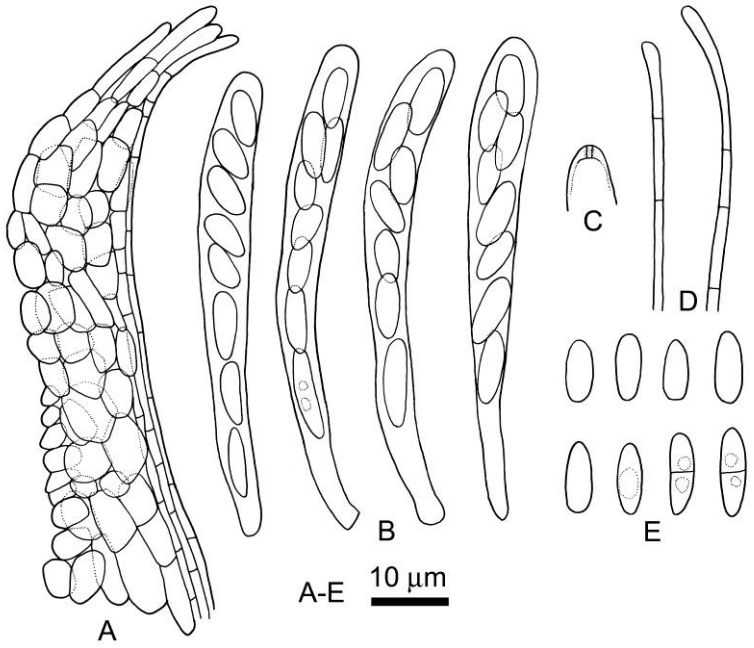

Fig. 5.26. Camera lucida illustration of *Hymenoscyphus varicosporioides* (TNS-F-16472). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

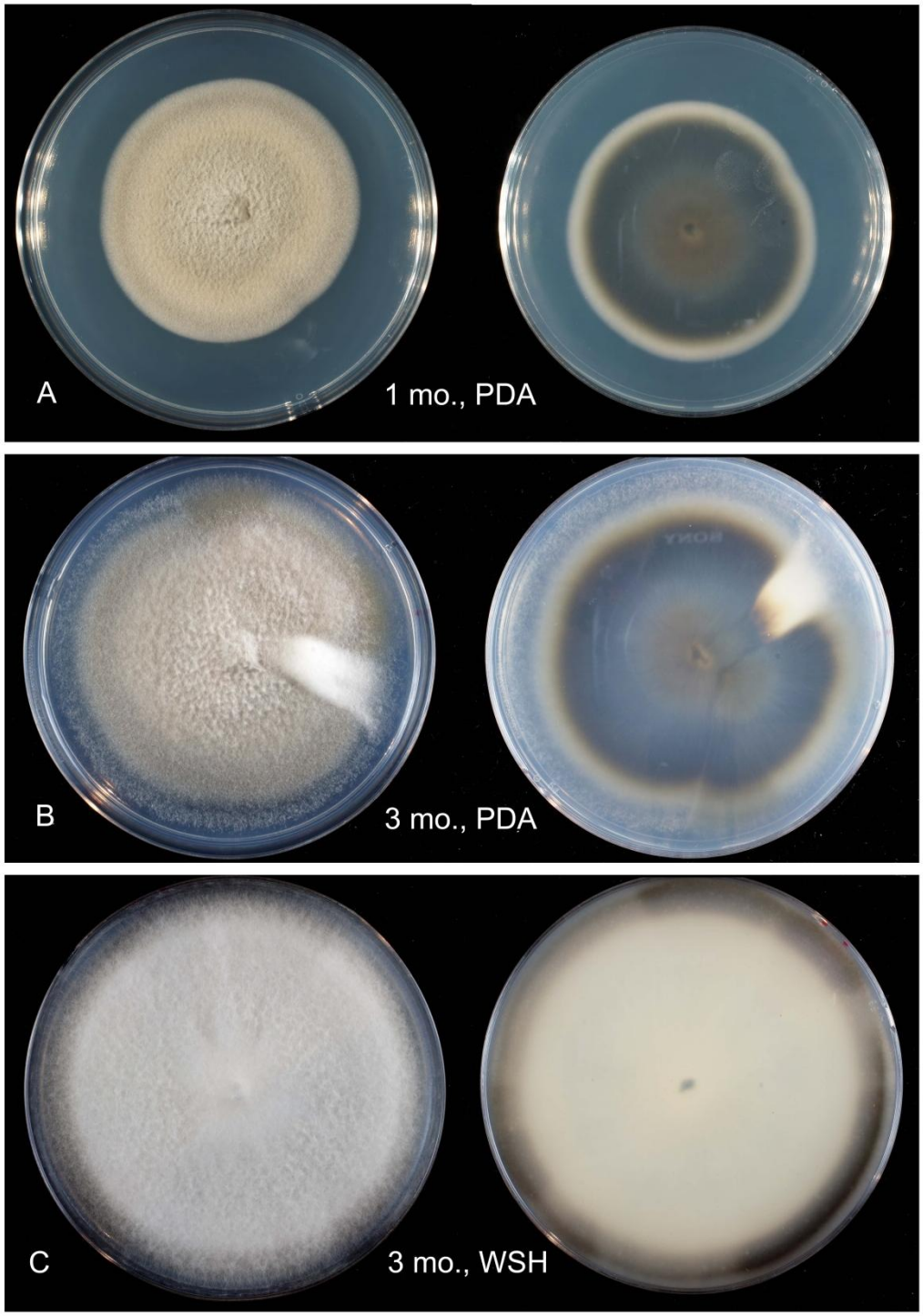

Fig. 5.27. Cultural characters of *Hymenoscyphus varicosporioides* (FC-2038, Culture of TNS-F-16472). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.).

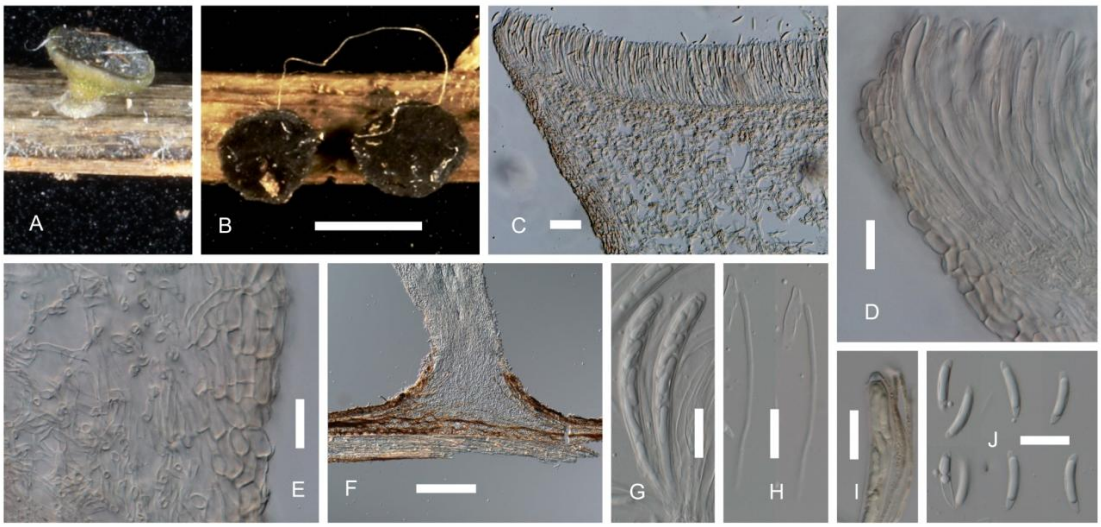

Fig. 5.28. *Hymenoscyphus* sp.1 (TNS-F-40079). **A:** Fresh apothecia on rotten twigs**. B:** Dried apothecia. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** ectal excipulum in flank showing the layers. **F:** Base of apothecium. **G:** Asci. **H:** Paraphyses. **I:** Reaction of ascal apex to MLZ. **J:** Ascospores. *Bars* **B** 1 mm; **C** 50 µm; **D–E, G–J** 20 µm; **F** 200 µm.

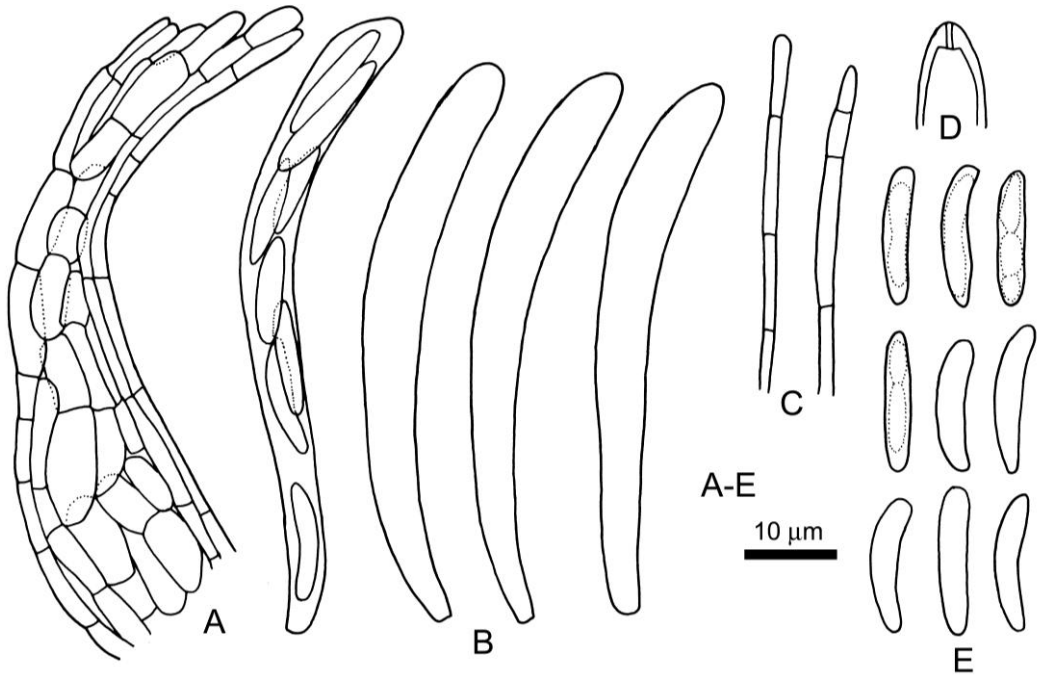

Fig. 5.29. Camera lucida illustration of *Hymenoscyphus* sp.1 (TNS-F-40079). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

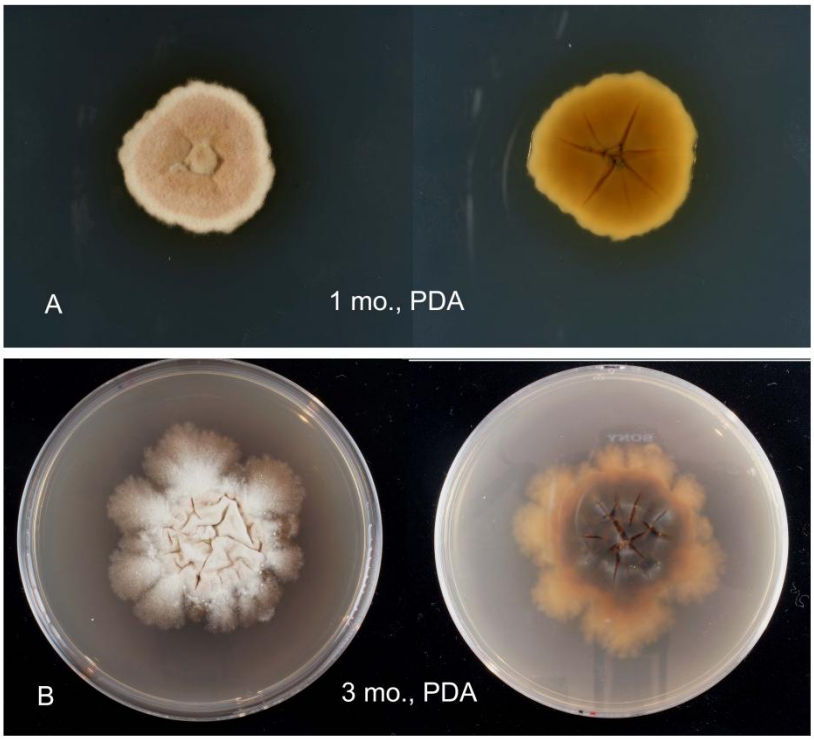

Fig. 5.30. Cultural characters of *Hymenoscyphus* sp.1 (FC-2818, Culture of TNS-F-40079). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.).

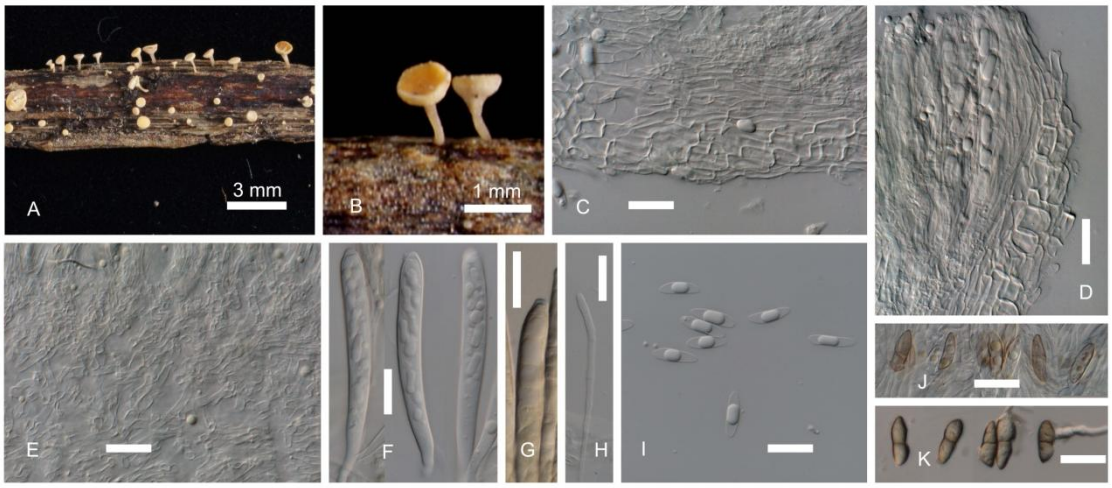

Fig. 5.31. *Hymenoscyphus* sp.2 (TNS-F-40193). **A:** Fresh apothecia on decaying herb stems**. B:** Close up of apothecia. **C:** Ectal excipulum in flank showing the layers. **D:** Close up of ectal excipulum at the margin. **E:** Structure in medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. **J:** Brown ascospores observed from dried specimen. **K:** Brown ascospores in germination.

Bars **A** 3 mm; B 1 mm; **C–K** 20 µm.

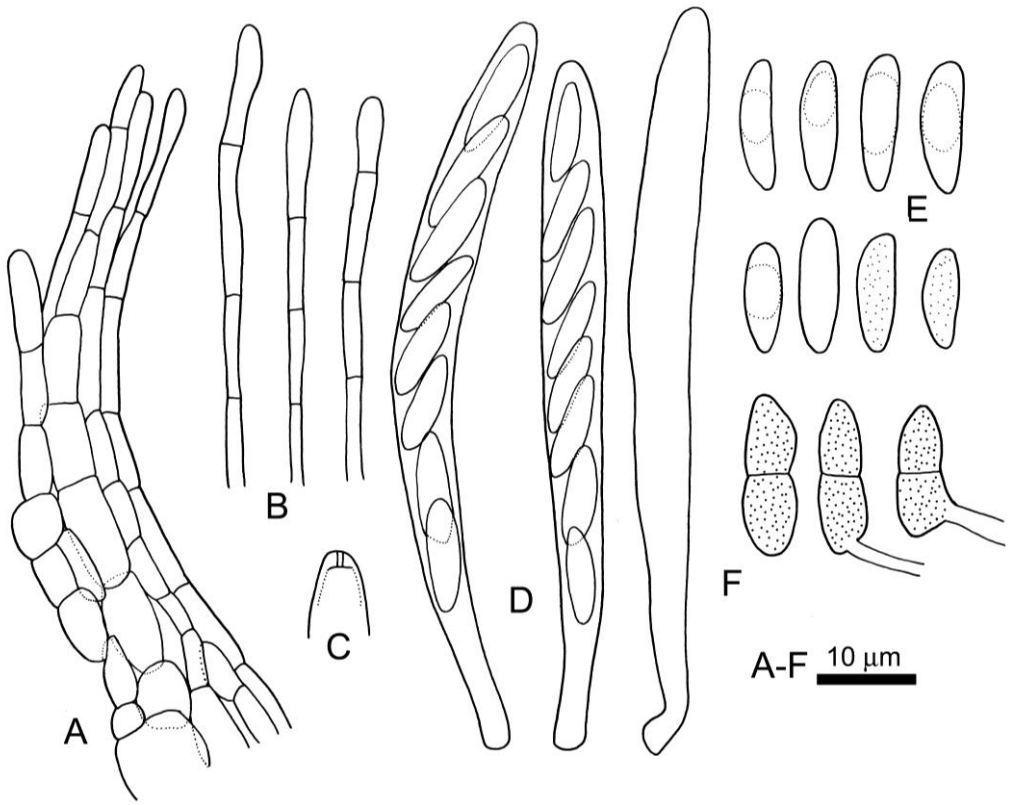

Fig. 5.32. Camera lucida illustration of *Hymenoscyphus* sp.2 (TNS-F-40193). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Paraphyses. **C:** Reaction of ascal apex to MLZ. **D:** Asci. **E:** Ascospores. **F:** Brown ascospores in germination. *Bars* **A–F** 10 µm.

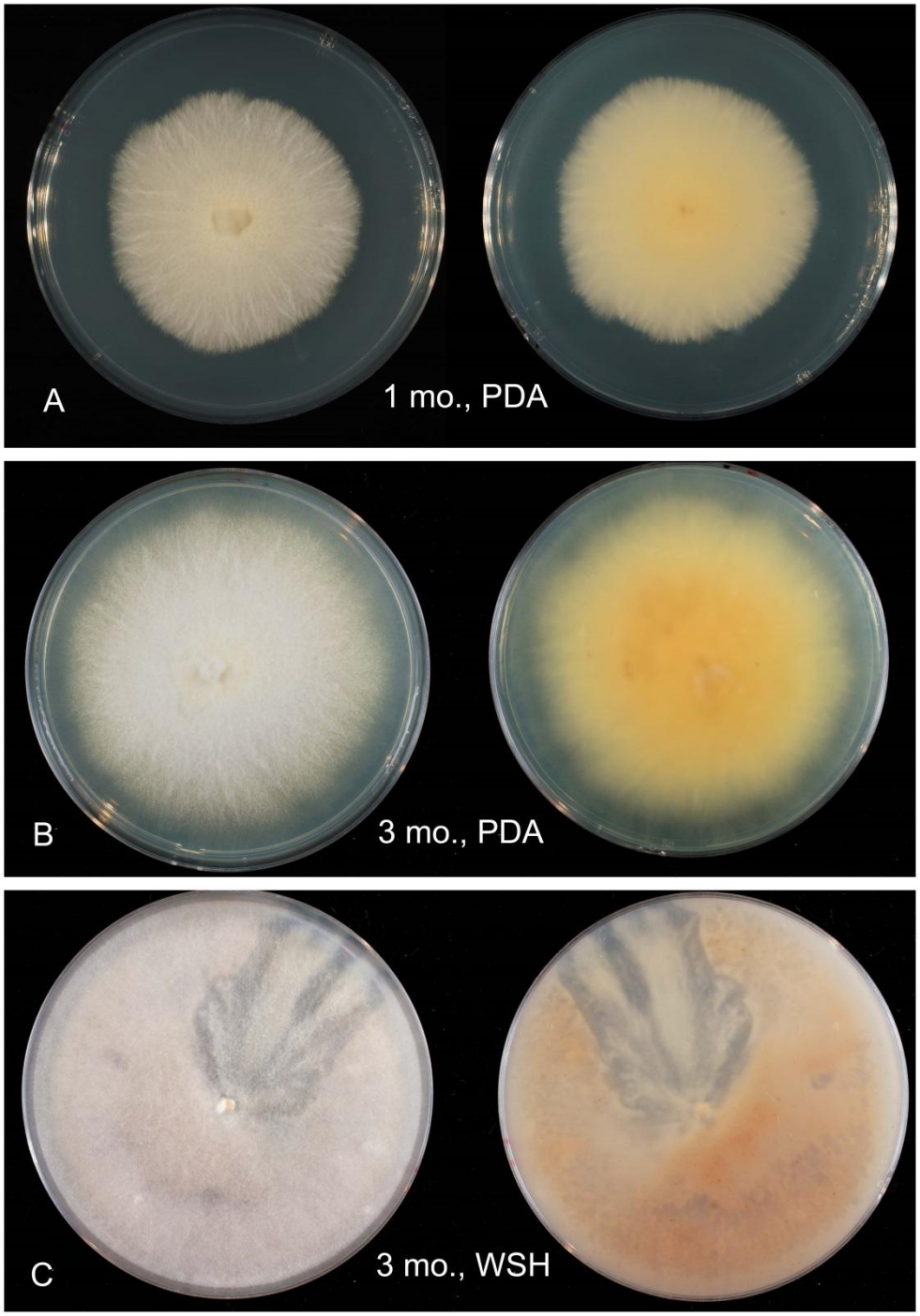

Fig. 5.33. Cultural characters of *Hymenoscyphus* sp.2 (FC-5110, Culture of TNS-F-40193). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). C: Colony on WSH (20℃, 3 mo.).

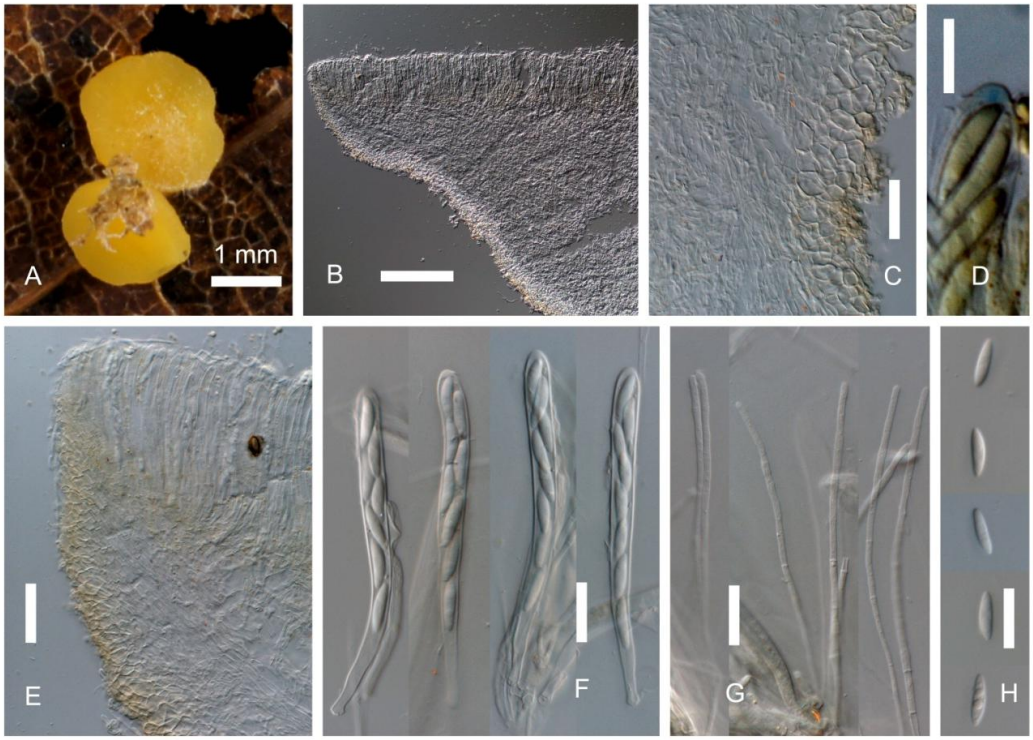

Fig. 5.34. "*Hymenoscyphus*" sp.1 (TNS-F-40156). **A:** Fresh apothecia on decaying leaves. **B:** Vertical section of ectal excipulum.**C:** Ectal excipulum in flank showing the layers. **D:** Reaction of ascal apex to MLZ. **E:** Close up of ectal excipulum at the margin. **F:** Asci. **G:** Paraphyses. **H:** Ascospores. *Bars* **A** 1 mm; **B** 200 µm; **C E** 40 µm; **D** 10 µm; **F–H** 20 µm.

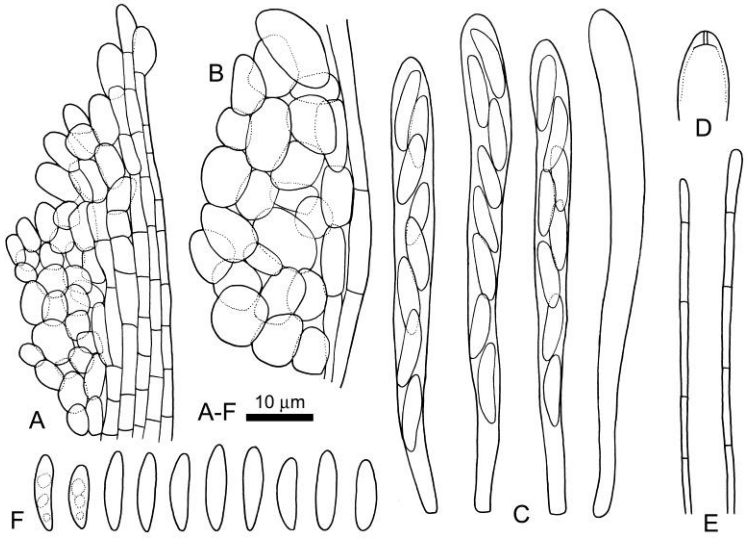

Fig. 5.35. Camera lucida illustration of "*Hymenoscyphus*" sp.1 (TNS-F-40156). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Close up of cells in ectal excipulum. **C:** Asci. **D:** Reaction of ascal apex to MLZ. **E:** Paraphyses. **F:** Ascospores. *Bars* **A–F** 10 µm.

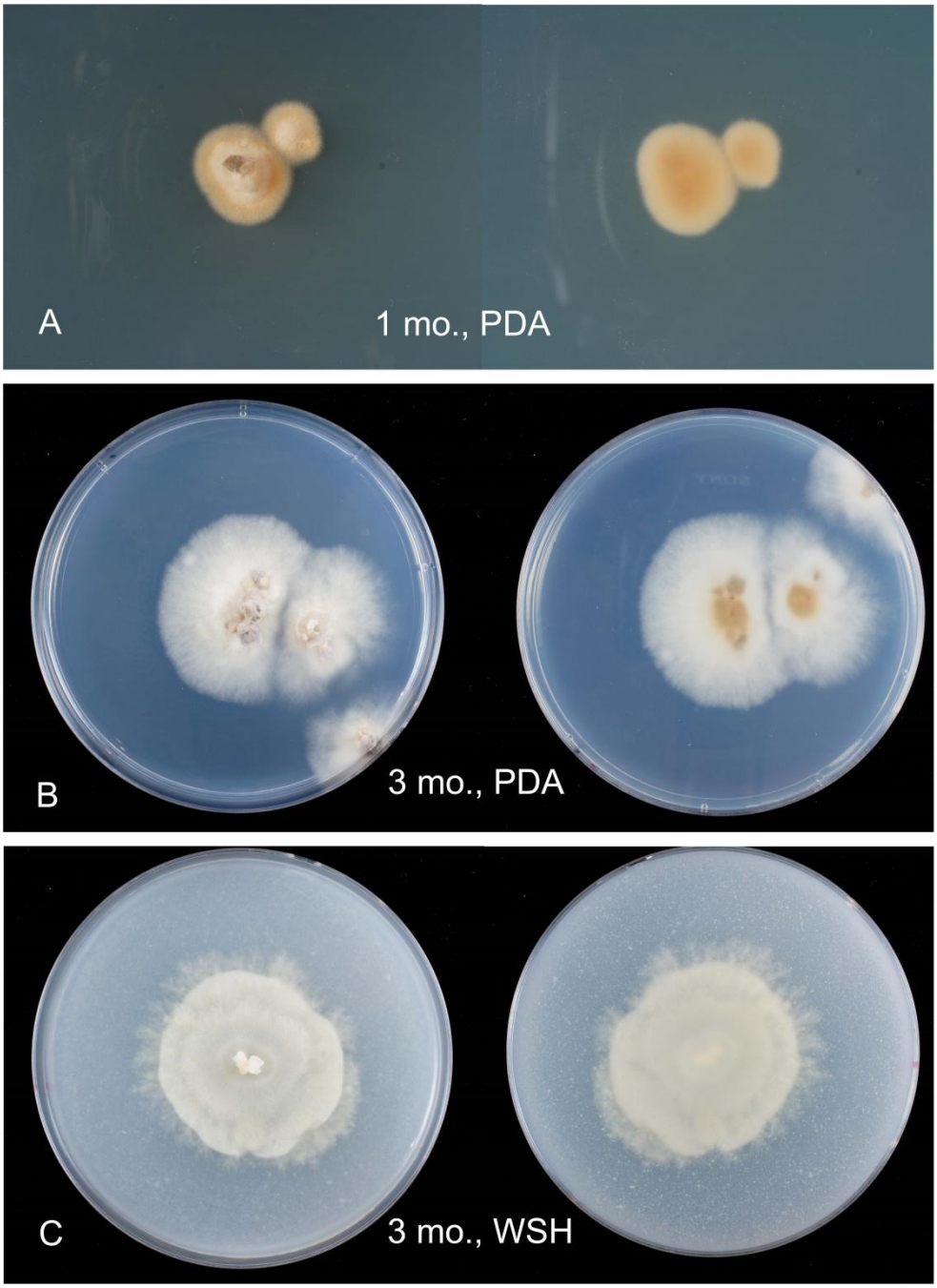

Fig. 5.36. Cultural characters of "*Hymenoscyphus*" sp.1 (FC-5090, Culture of TNS-F-40156). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). C: Colony on WSH (20℃, 3 mo.).

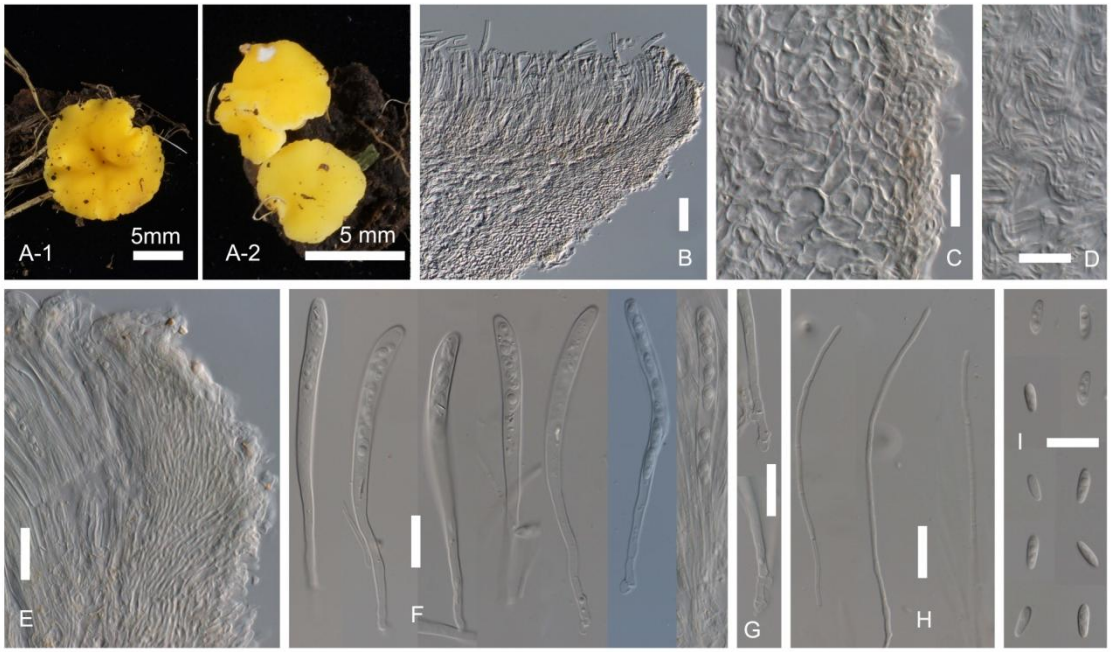

Fig. 5.37. "*Hymenoscyphus*" sp.2 (TNS-F-40168). **A-1, A-2:** Fresh apothecia on fallen acorns of *Quercus* sp. **B:** Vertical section of ectal excipulum. **C:** Ectal excipulum in flank showing the layers. **D:** Structure in medullary excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Asci. **G:** Croziers at the base of asci. **H:** Paraphyses. **I:** Ascospores. *Bars* **A-1, A-2** 5mm**; B:** 50 µm; **C–I** 20 µm.

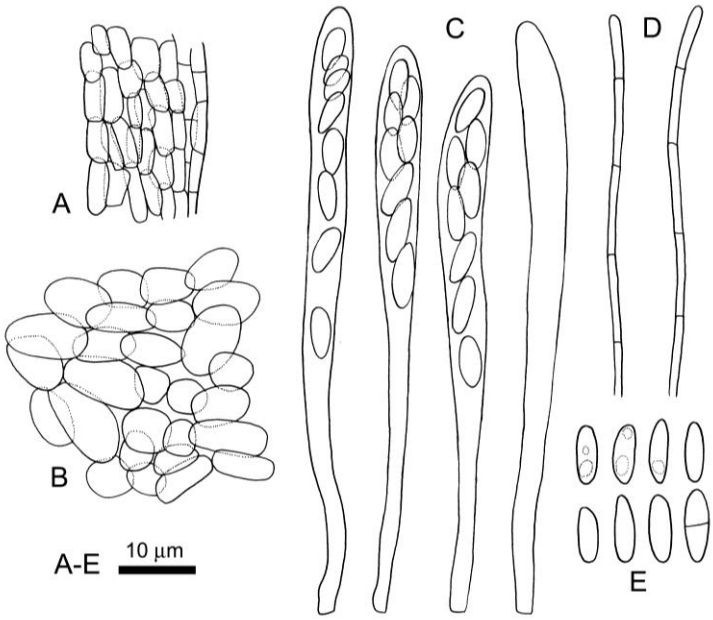

Fig. 5.38. Camera lucida illustration of "*Hymenoscyphus*" sp.2 (TNS-F-40168). **A:** Close up of cells in ectal excipulum at the margin. **B:** Close up of cells in ectal excipulum at the flank. **C:** Asci. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

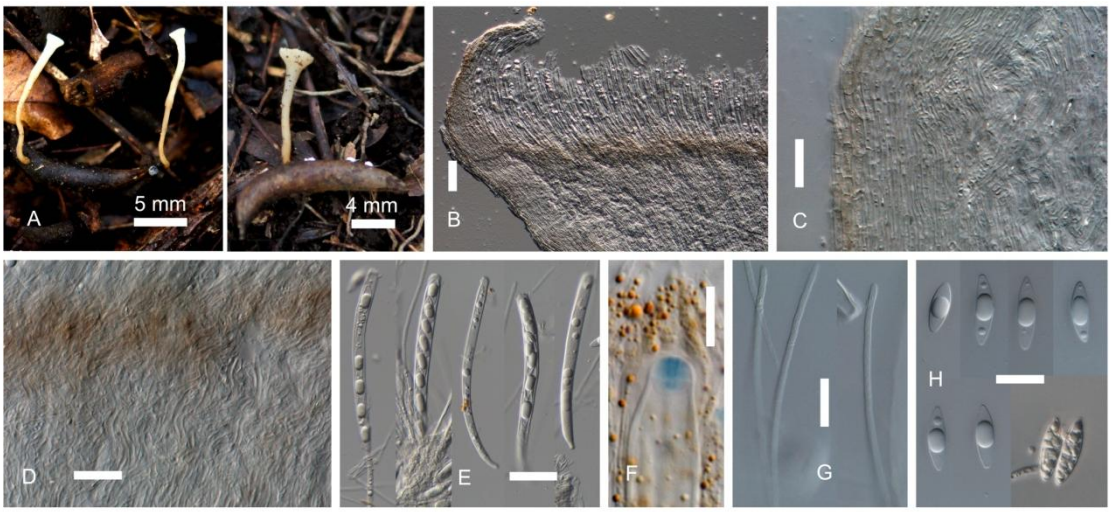

Fig. 5.39. "*Hymenoscyphus*" sp.3 (TNS-F-40173). **A:** Fresh apothecia on fruits of *Cercidiphyllum japonicum*. **B:** Vertical section of ectal excipulum. **C:** Ectal excipulum showing the layered structure. **D:** Structure in medullary excipulum. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Ascospores. *Bars* **B** 40 μm; **C–D, G–H** 20 μm; **E** 40 μm; **F** 10μm.

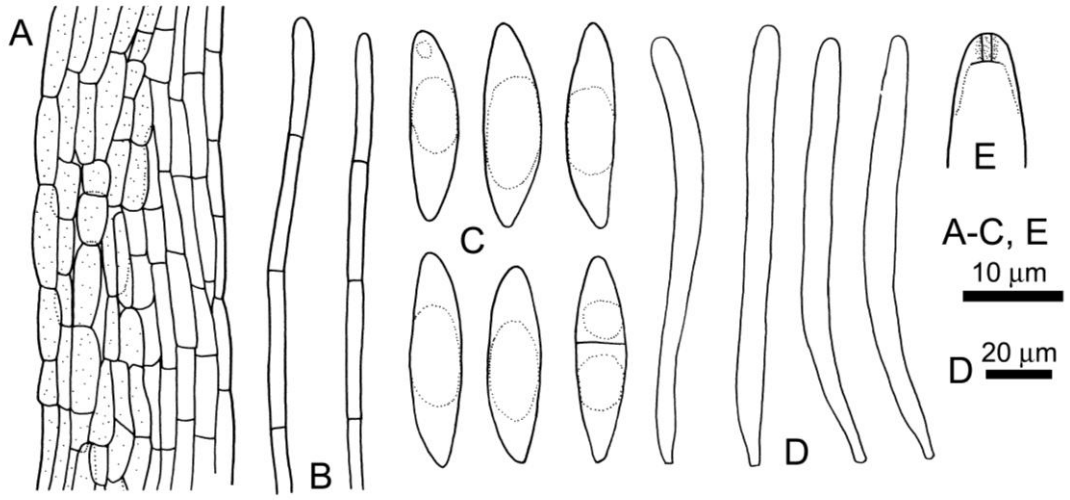

Fig. 5.40. Camera lucida illustration of "*Hymenoscyphus*" sp.3 (TNS-F-40173). **A:** Close up of cells in ectal excipulum in flank. **B:** Paraphyses. **C:** Ascospores. **D:** Asci. **E:** Reaction of ascal apex to MLZ. *Bars* **A–C, E** 10 μm; **D** 20 μm.

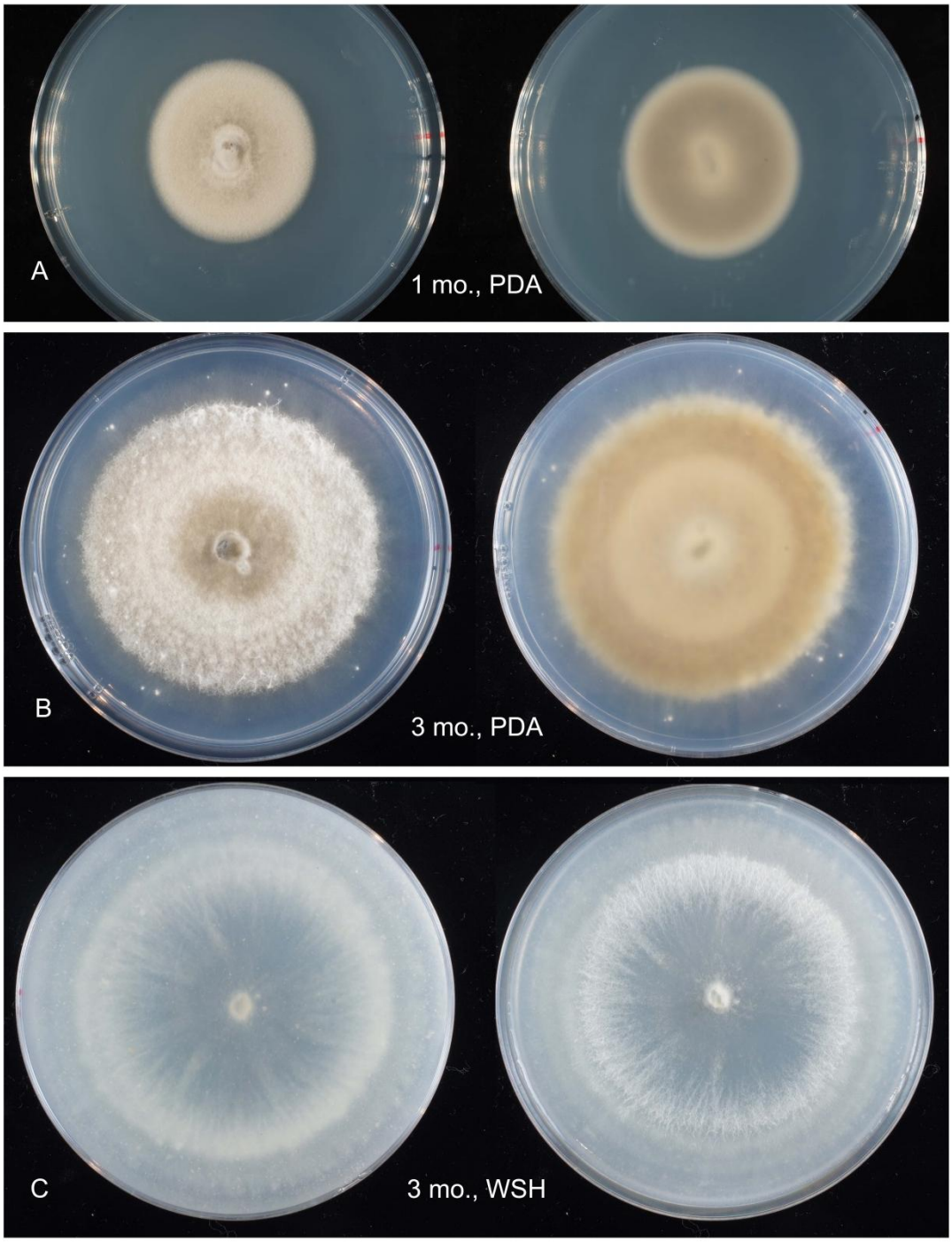

Fig. 5.41. Cultural characters of "*Hymenoscyphus*" sp.3 (FC-5096, Culture of TNS-F-40173). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). C: Colony on WSH (20℃, 3 mo.).

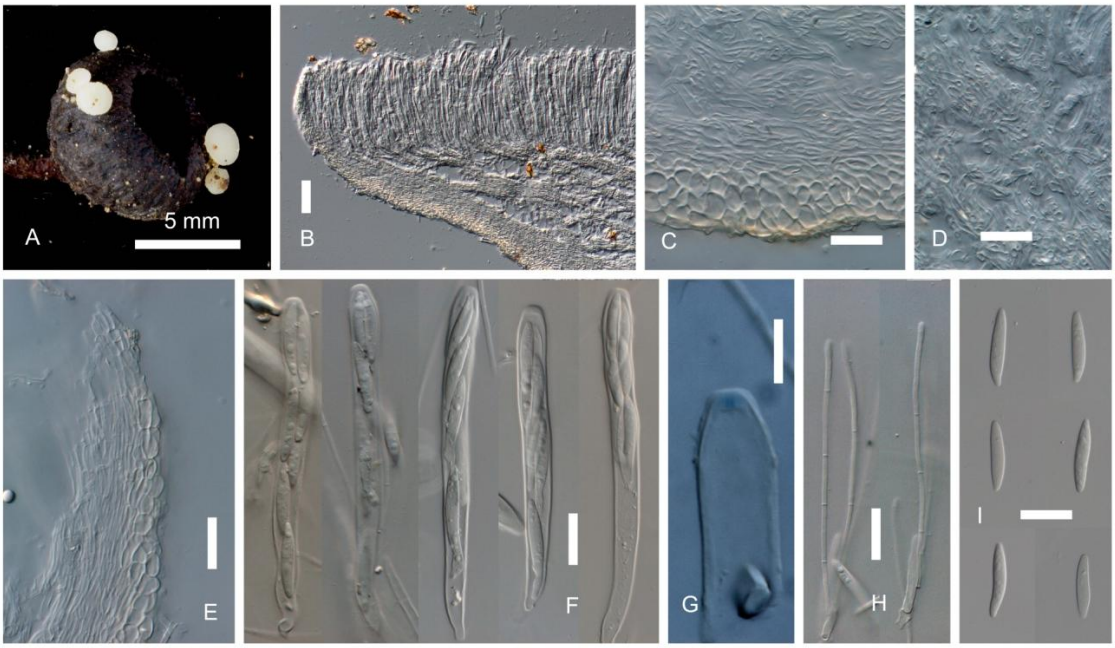

Fig. 5.42. "*Hymenoscyphus*" sp.4 (TNS-F-40179). **A:** Fresh apothecia on fallen acorns of *Fagus* sp. **B:** Vertical section of ectal excipulum. **C:** Ectal excipulum showing the layered structure. **D:** Structure in medullary excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I**: Ascospores. *Bars* **A** 5 mm; **B** 10µm; **C–F, H–I** 20 µm; **G** 10 µm.

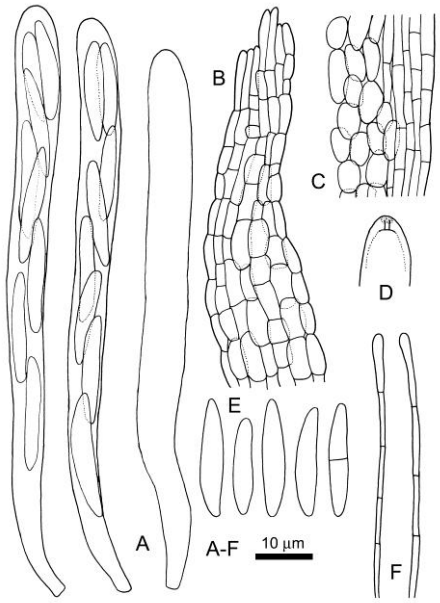

Fig. 5.43. Camera lucida illustration of "*Hymenoscyphus*" sp.4 (TNS-F-40179). **A:** Asci. **B:** Vertical section of an apothecium through the margin showing the ectal excipulum. **C:** Close up of cells in ectal excipulum in flank. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. **F:** Paraphyses. *Bars* **A–F**10 µm.

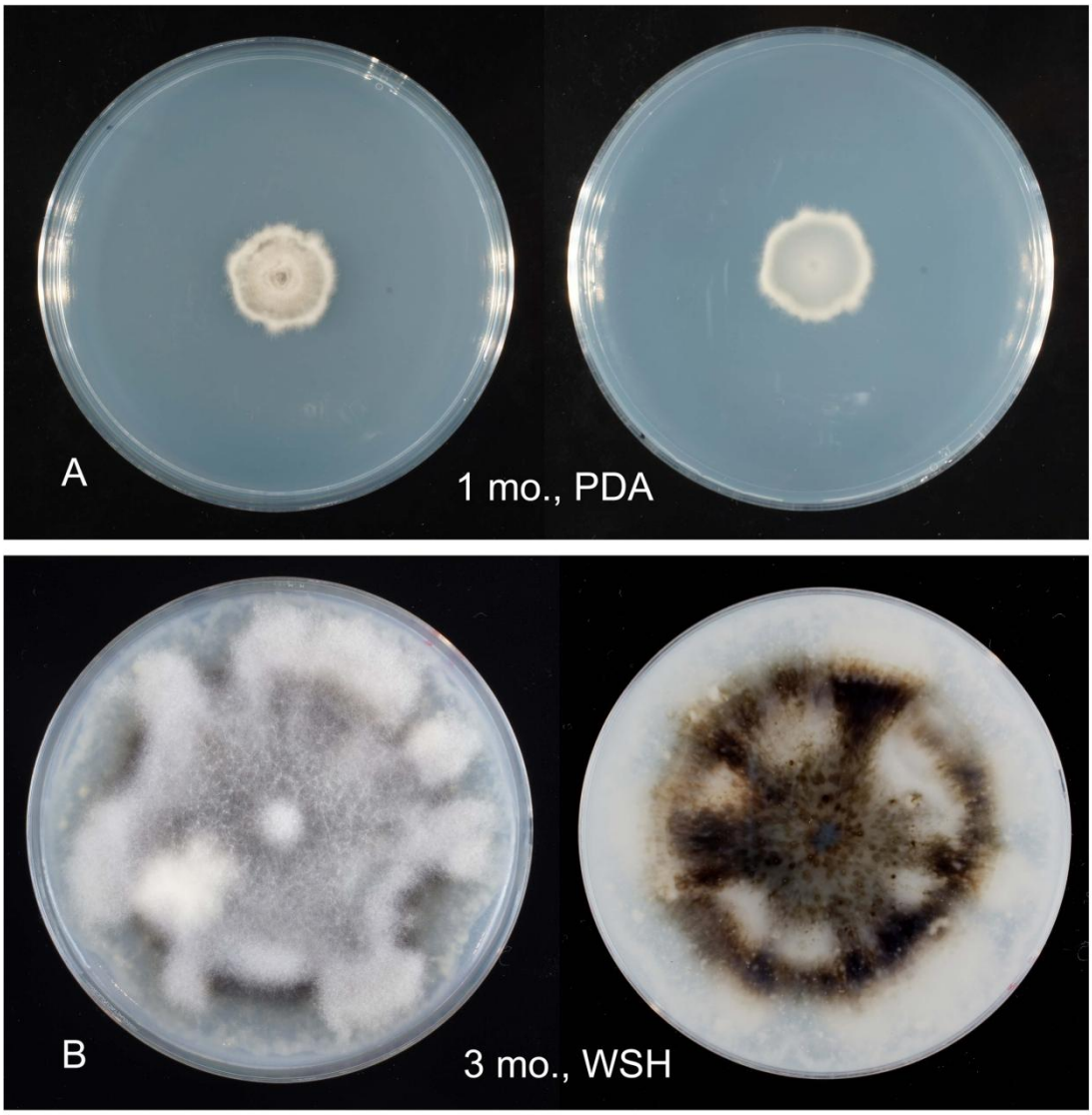

Fig. 5.44. Cultural characters of "*Hymenoscyphus*" sp.4 (FC-5101, Culture of TNS-F-40179). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on WSH (20℃, 3 mo.).

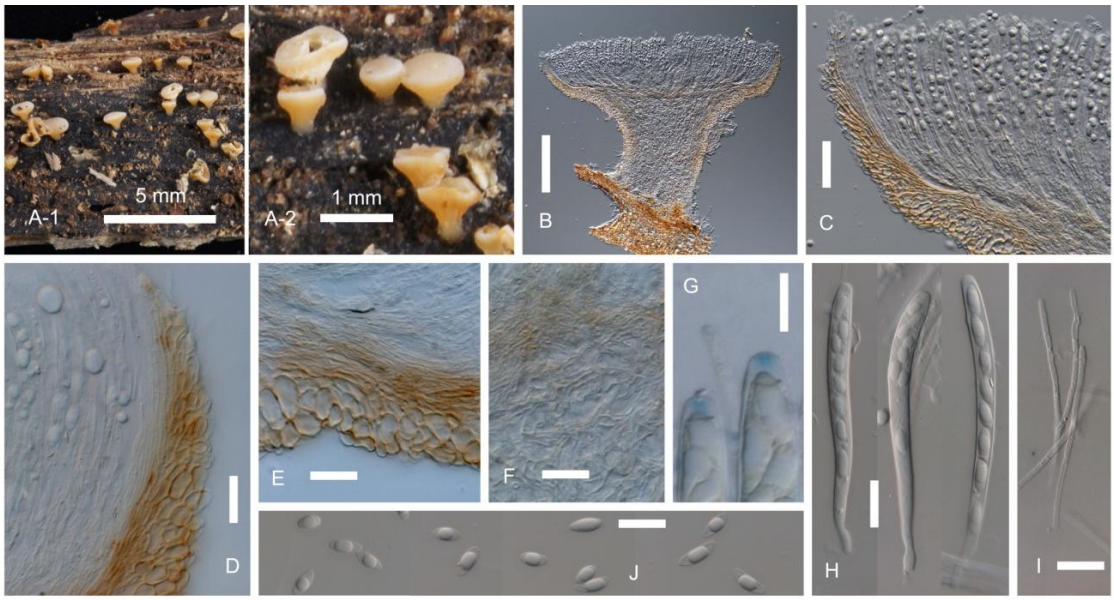

Fig. 5.45. "*Hymenoscyphus*" sp.5 (TNS-F-40186). **A-1:** Fresh apothecia on decaying wood. **A-2:** Close up of fresh apothecia. **B:** Vertical section of an apothecium. **C:** Ectal excipulum. **D:** Close up of ectal excipulum. **E:** Ectal excipulum showing the layered structure. **F:** Structure in medullary excipulum. **G:** Reaction of ascal apex to MLZ. **H:** Asci. **I**: Paraphyses. **J:** Ascospores.
Bars **A-1** 5 mm; **A-2** 1 mm; **B** 200 µm; **C** 40 µm; **D–F, H–J** 20 µm.

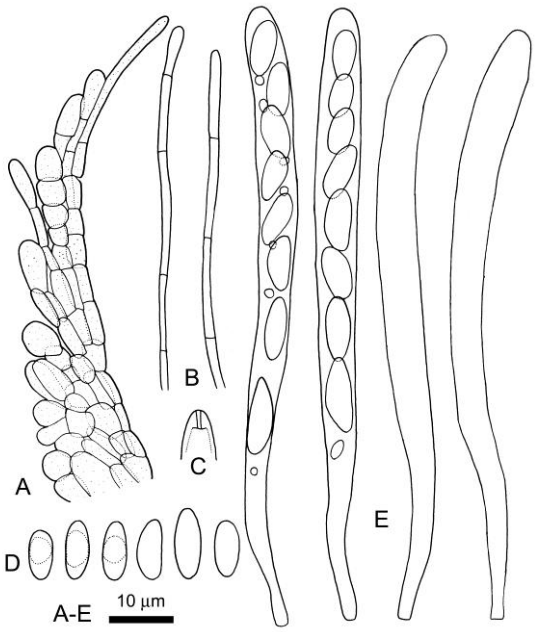

Fig. 5.46. Camera lucida illustration of "*Hymenoscyphus*" sp.5 (TNS-F-40186). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Paraphyses. **C:** Reaction of ascal apex to MLZ. **D:** Ascospores. **E:** Asci. *Bars* **A–E** 10 µm.

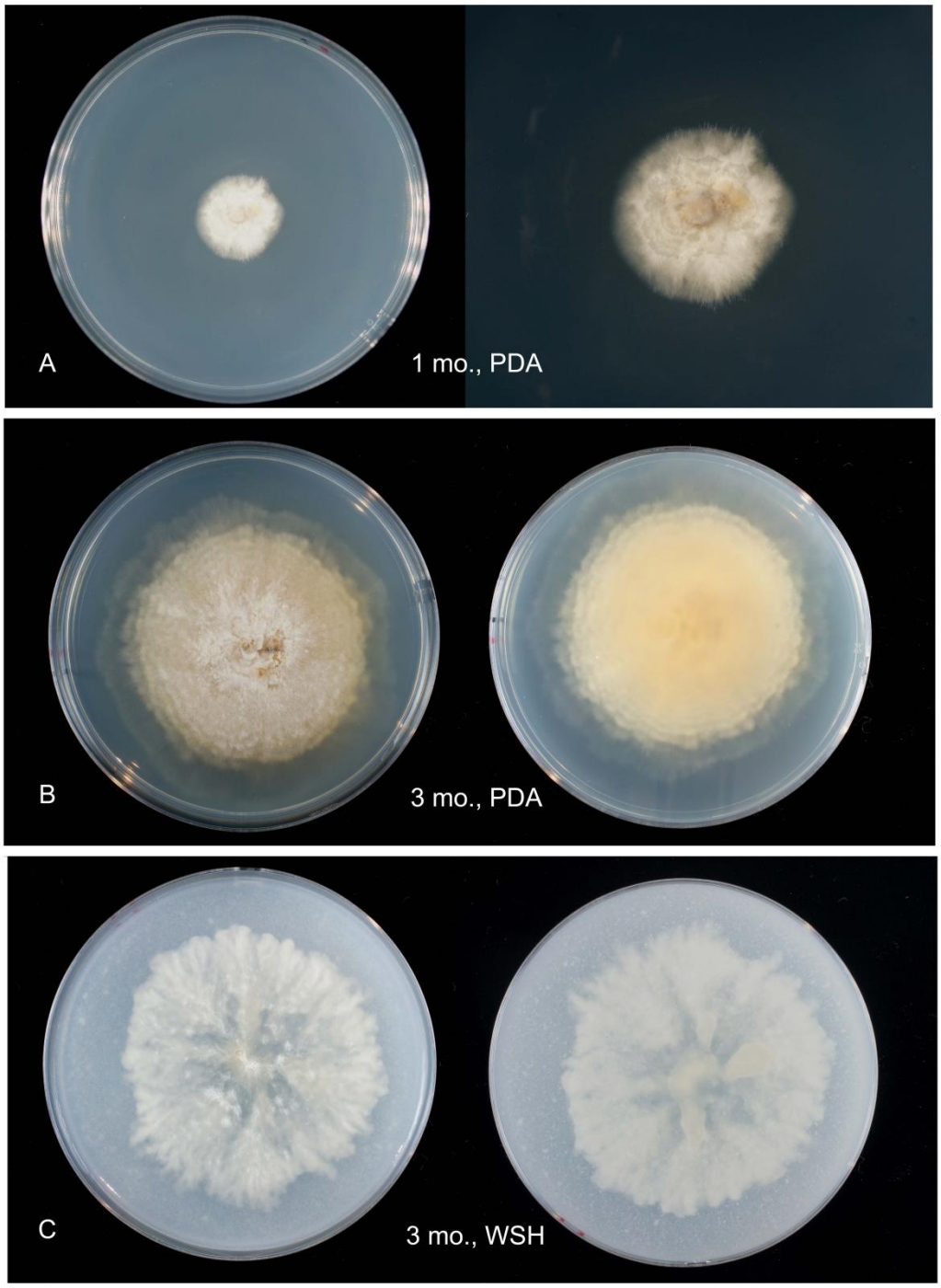

Fig. 5.47. Cultural characters of "*Hymenoscyphus*" sp.5 (FC-5104, Culture of TNS-F-40186). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). C: Colony on WSH (20℃, 3 mo.).

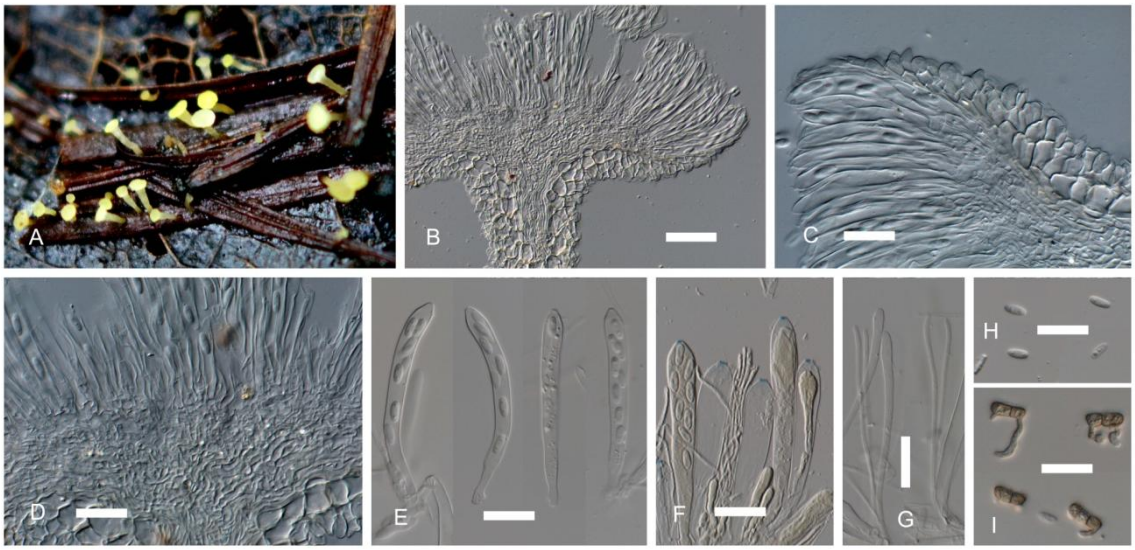

Fig. 5.48. *Luteidiscella advenulus* (TNS-F-40019). **A:** Fresh apothecia on decaying *Larix* sp. needles. **B:** Vertical section of an apothecium. **C:** Close up of ectal excipulum at the margin. **D:** Close up of medullary excipulum. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Hyaline ascospores. **I:** Germinating ascospores.
Bars **B** 40 µm; **C–I** 20 µm.

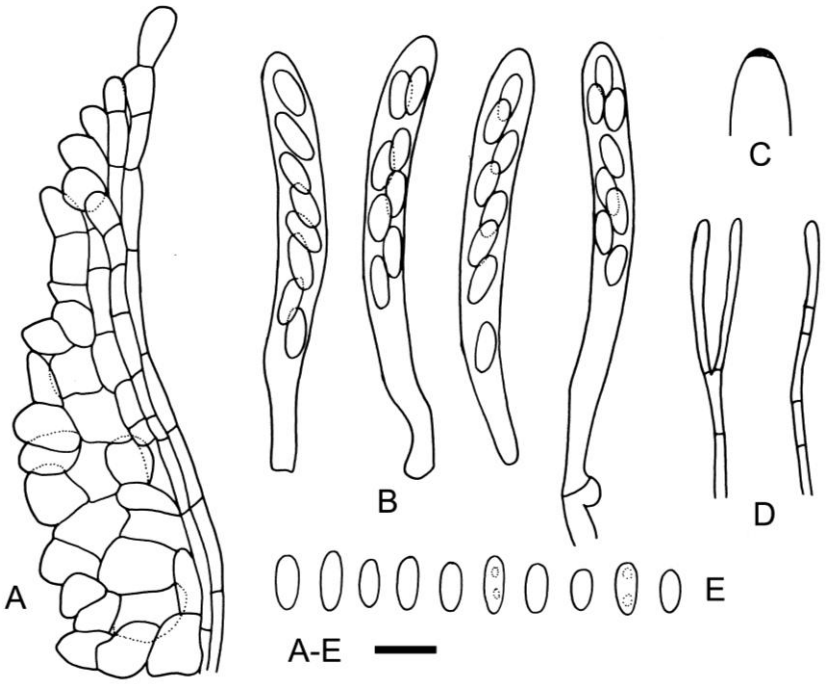

Fig. 5.49. Camera lucida illustration of *Luteidiscella advenulus* (TNS-F-40019). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

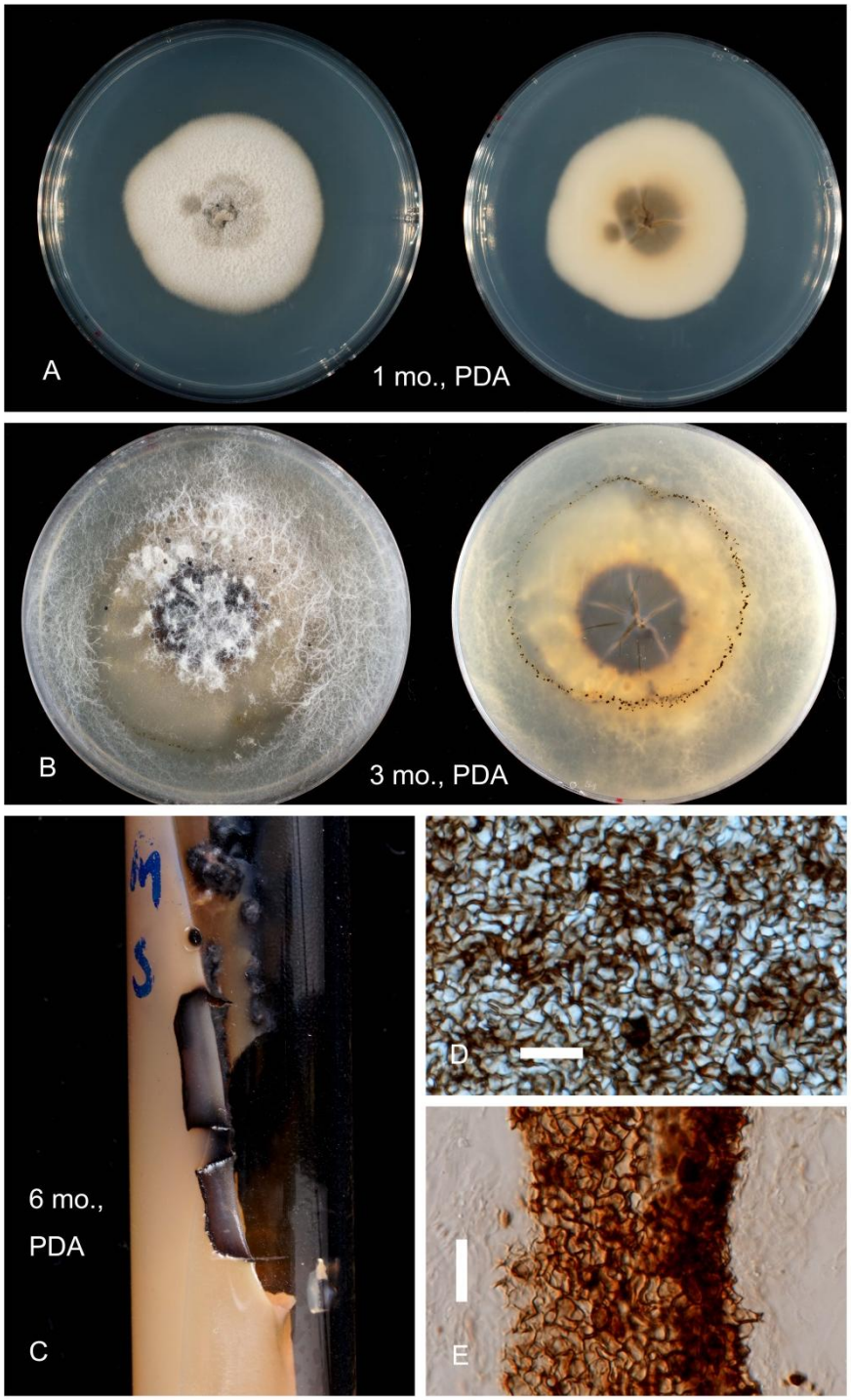

Fig. 5.50. Cultural characters of *Luteidiscella advenulus* (FC-2722, Culture of TNS-F-40019). **A:** Colony on PDA (20℃, 1 mo). **B:** Colony on PDA (20℃, 3 mo). **C:** Colony on PDA (20℃, 6 mo), showing the rind. **D:** Surface view of rind showing the textura globulosa to angularis cells. **E:** Vertical view of rind. *Bars* **D–E** 20μm.

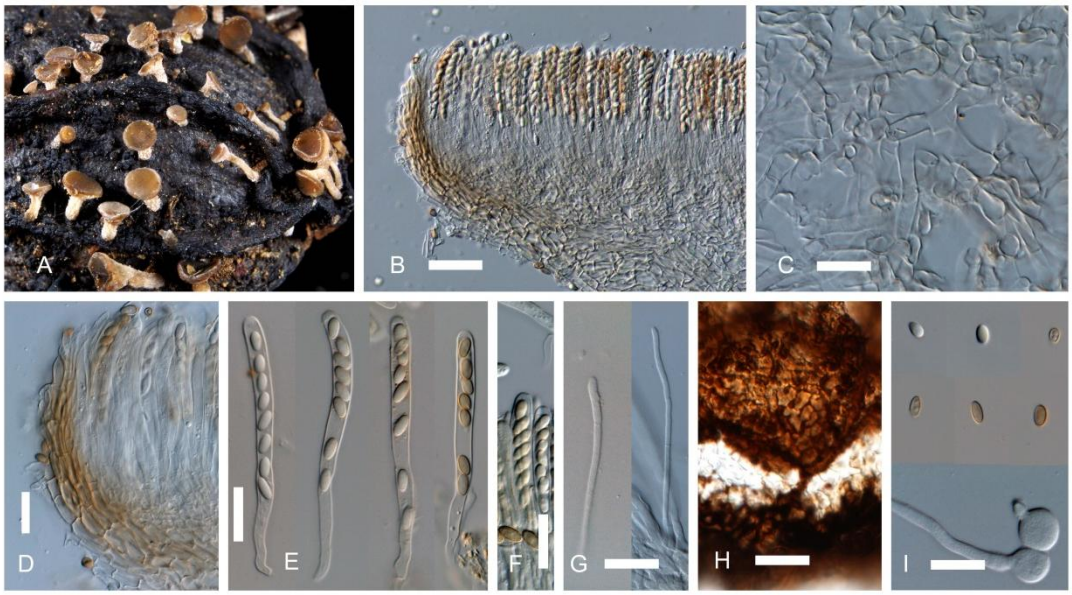

Fig. 5.51. *Lambertella corni-maris* (TNS-F-40083). **A:** Fresh apothecia on fallen fruits of *Torreya nucifera*. **B:** Vertical section of an apothecium. **C:** Close up of medullary excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Surface structure in blackened area on host substrate. **I:** Hyaline ascospores, brown ascospores and germinating ascospores. *Bars* **B** 40 µm; **C–I** 20 µm.

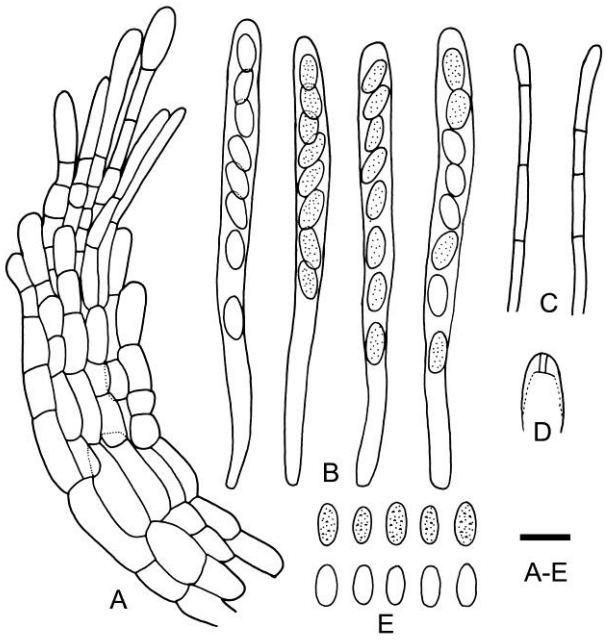

Fig. 5.52. Camera lucida illustration of *Lambertella corni-maris* (TNS-F-40083). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

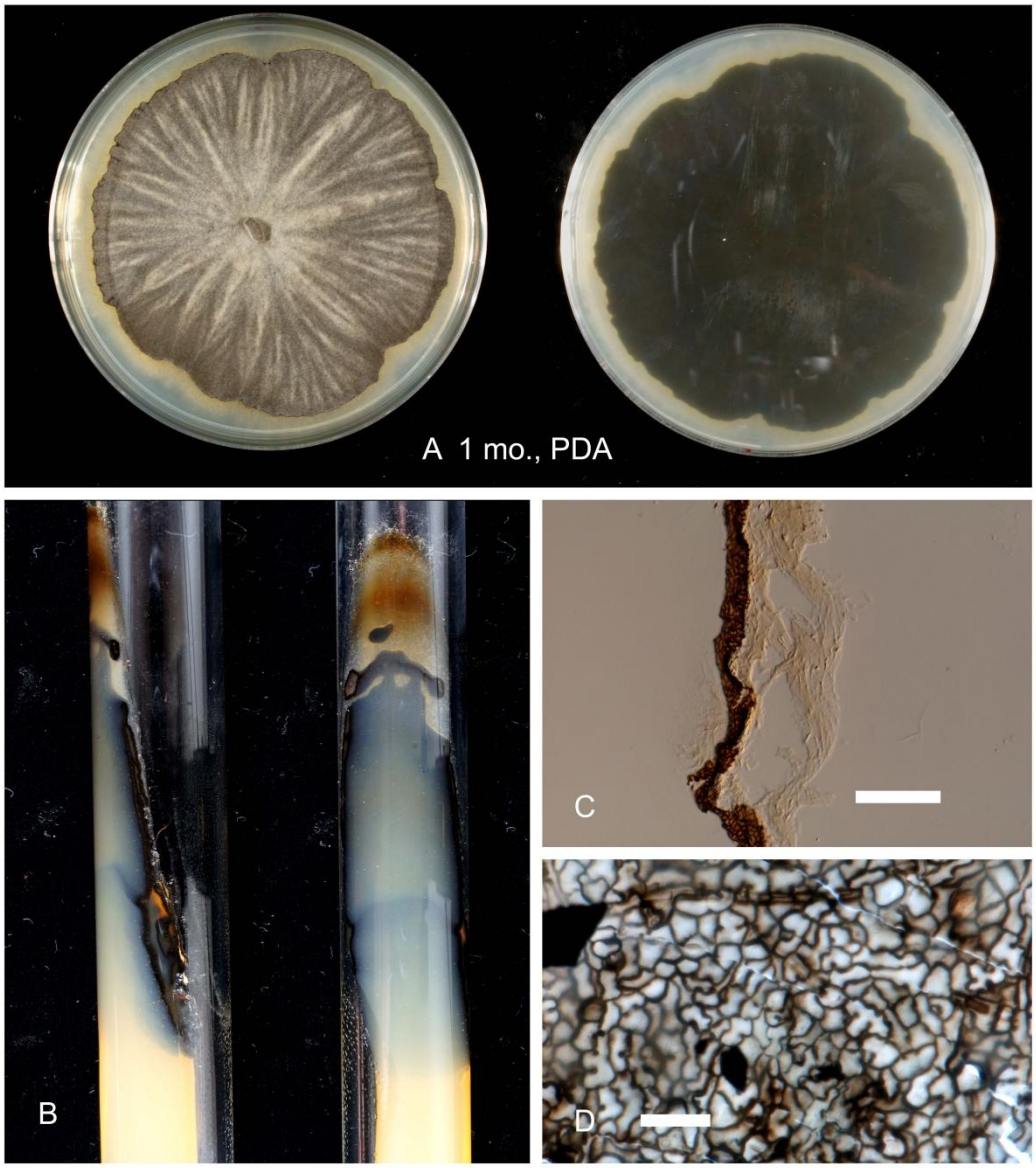

Fig. 5.53. Cultural characters of *Lambertella corni-maris* (FC-2821, Culture of TNS-F-40083). **A:** Colony on PDA (20℃, 1 mo). **B:** Colony on PDA (20℃, 3 mo). **C:** Vertical view of rind. **D:** Surface view of rind showing the textura epidermoid cells. *Bars* **C** 100 μm; **D** 20 μm.

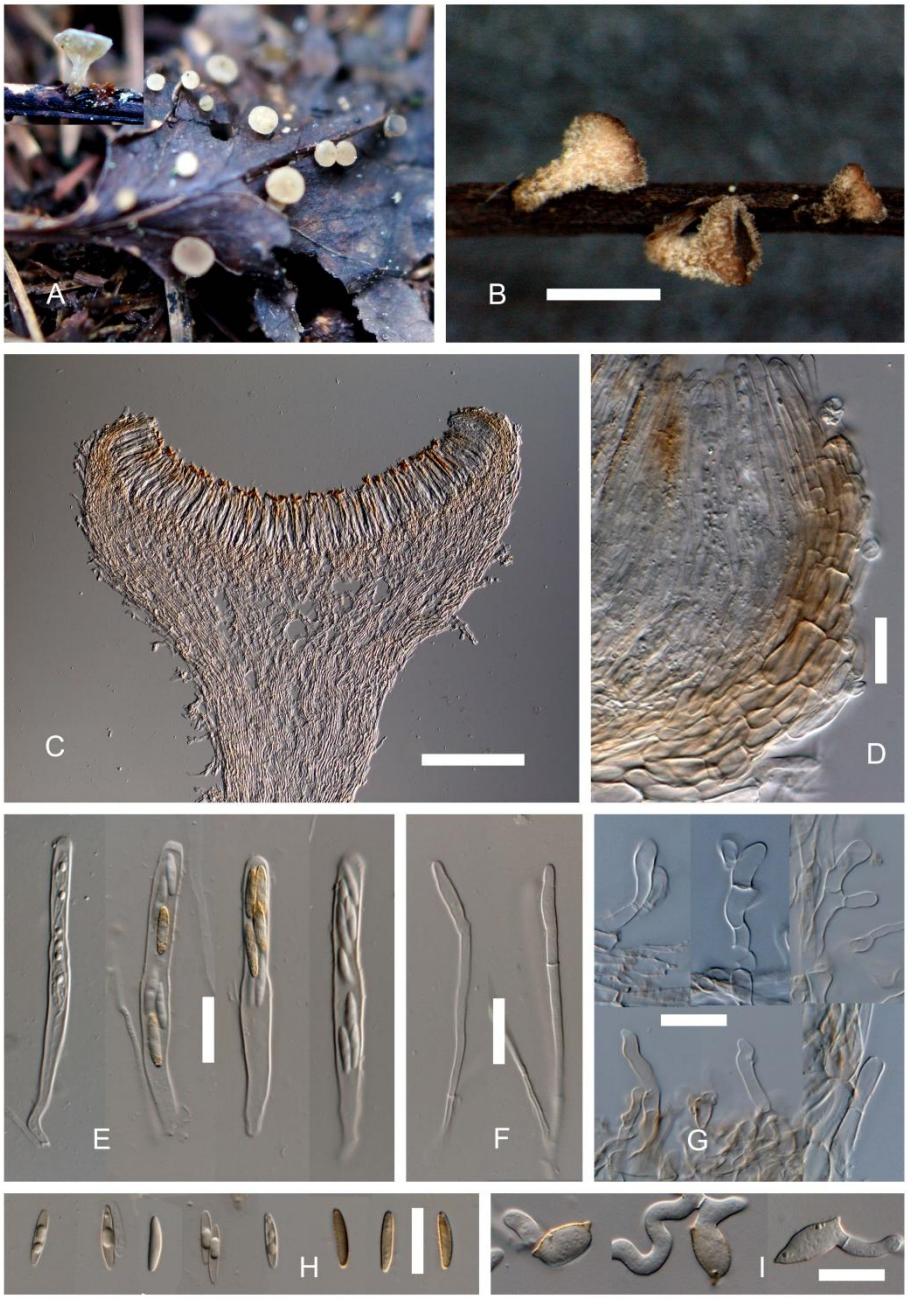

Fig. 5.54. *Lambertella pyrolae* (TNS-F-40132). **A:** Fresh apothecia on *Pyrola* leaves. **B:** Dried apothecia in a higher magnification. **C:** Vertical section of an apothecium. **D:** Structure of ectal excipulum at the margin. **E:** Asci showing the pigmented and hyaline ascospores within asci. **F:** Paraphyses. **G:** Hairs. **H:** Pigmented (pale brown and brown) ascospores. **I:** Germination of ascospores. *Bars* **B** 1mm; **C** 200 µm; **D–I** 20 µm.

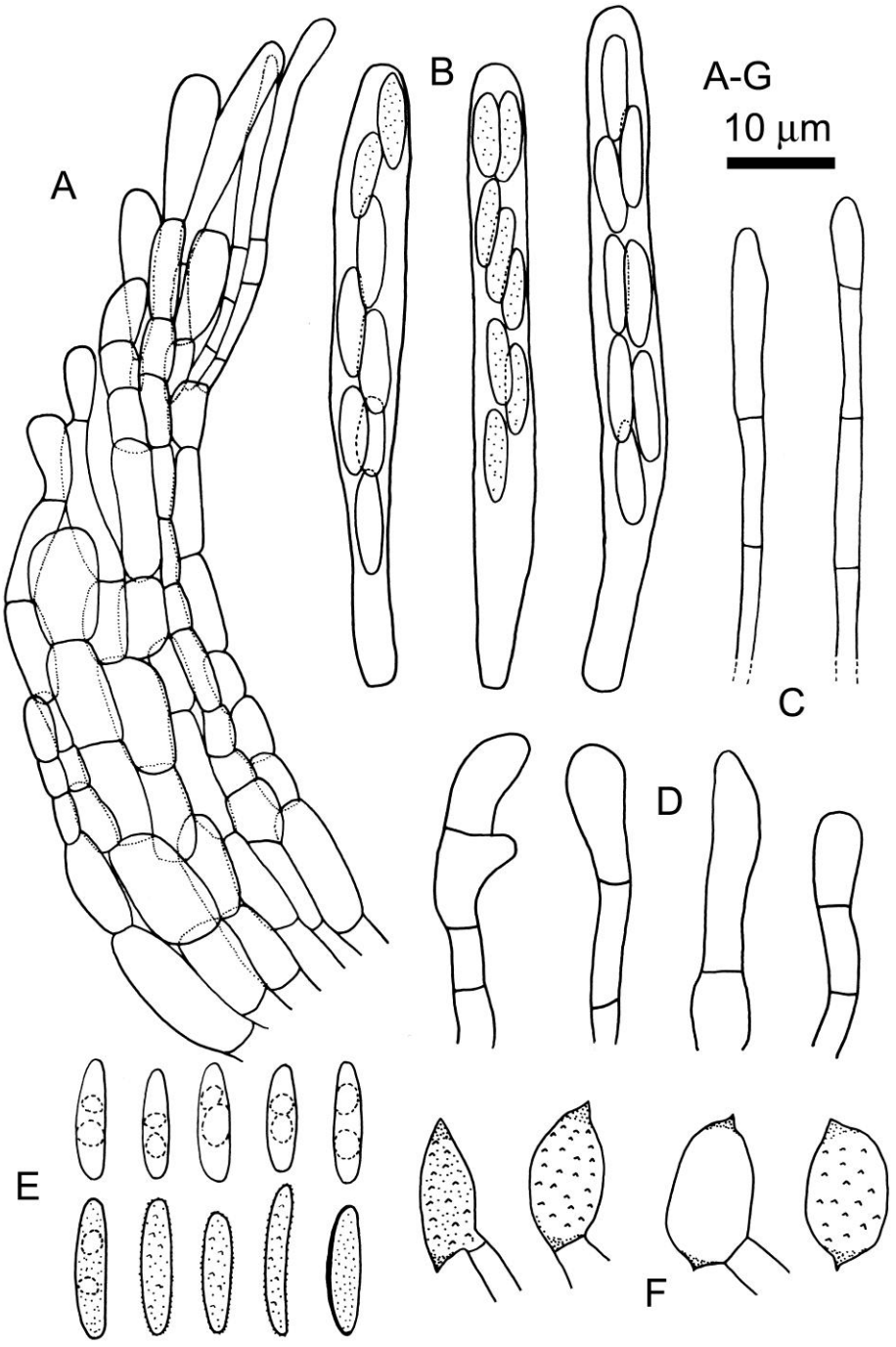

Fig. 5.55. Camera lucida illustration of *Lambertella pyrolae* (TNS-F-40132). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Hairs. **E:** Hyaline and brown ascospores. One at the bottom right shows the banded ornament. **F:** Germination of ascospores. *Bars* **A–G** 10 µm.

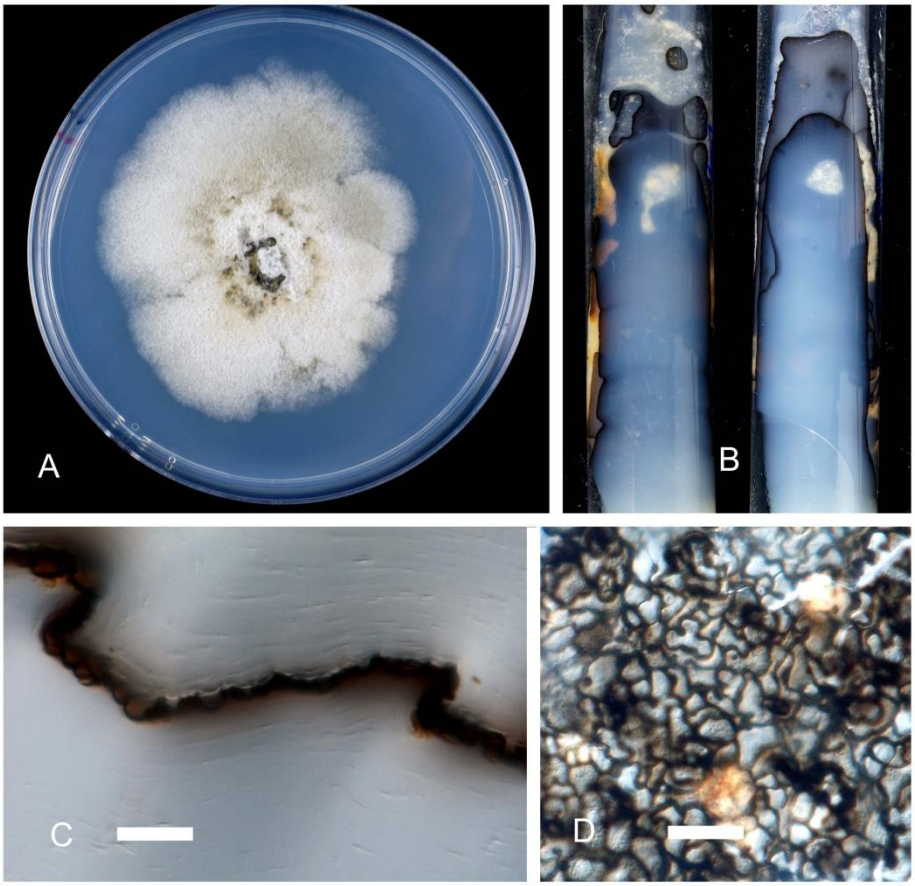

Fig. 5.56. Cultural characters of *Lambertella pyrolae* (FC-2996, Culture of TNS-F-40132). **A:** Colony on PDA (20°C, 14 days). **B:** Rind on PDA (20°C, 1 month). **C:** Vertical view of rind. **D:** Surface view of rind showing the epidermoid cells. *Bars* **C, D** 20μm.

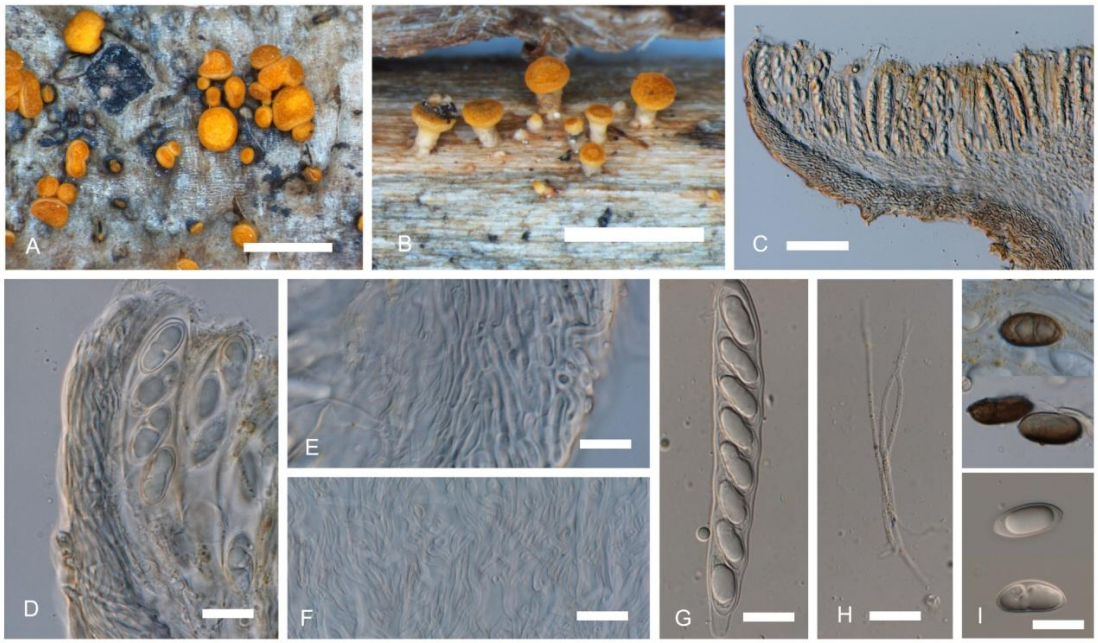

Fig. 5.57. *Hymenoscyphus yunnanense* (TNS-F-40028). **A:** Fresh apothecia on decaying branch. **B:** Fresh apothecia, showing the short stipe. **C:** Vertical section of an apothecium. **D:** Close up of ectal excipulum at the margin. **E:** Close up of ectal exipulum, showing the outermost thick-walled cells. **F:** Close up of medullay excipulum. **G:** Ascus. **H:** Paraphyses. **I:** Ascospores. *Bars* **A, B** 20 mm; **C** 100 µm; **D–I** 20 µm.

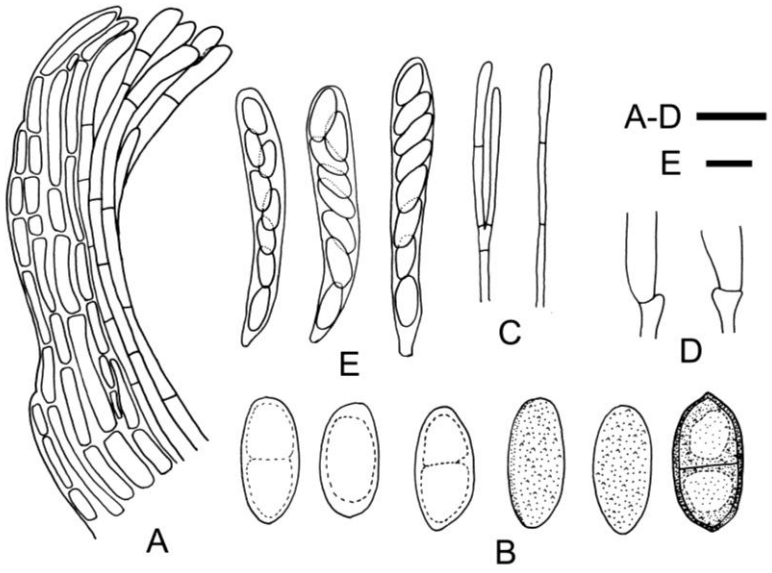

Fig. 3.58. Camera lucida illustration of *Hymenoscyphus yunnanense* (TNS-F-40028). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Croziers at the base of asci. **D:** Ascospores. *Bars* **A–D** 10 µm; **E** 20 µm.

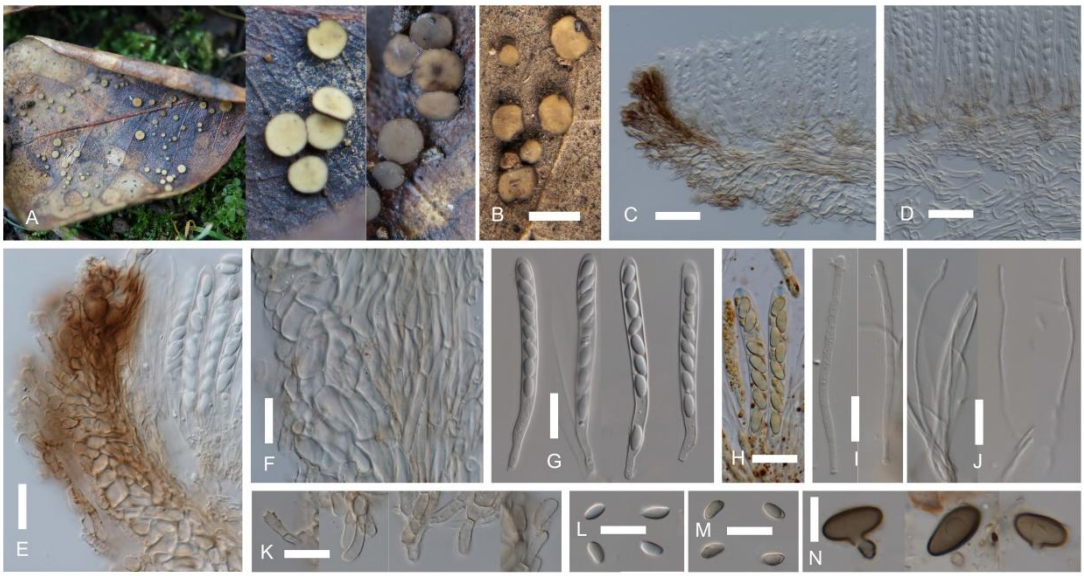

Fig. 5.59. *Brunneimargo camelliae* (TNS-F-40027). **A:** Fresh apothecia on decaying leaves of *Camellia japonica*. **B:** Dried apothecia. **C:** Ectal excipulum. **D:** Close up of medullay excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Close up of ectal exipulum in flank, showing the layered structure. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Clavate paraphyses. **J:** Filiform paraphyses. **K:** Hairs. **L:** Hyaline ascospores. **M:** Pale brown ascospores. **N:** Germinating ascospores. *Bars* **B** 2 mm; **C–D** 40 µm; **E–M** 20 µm; **N** 8 µm.

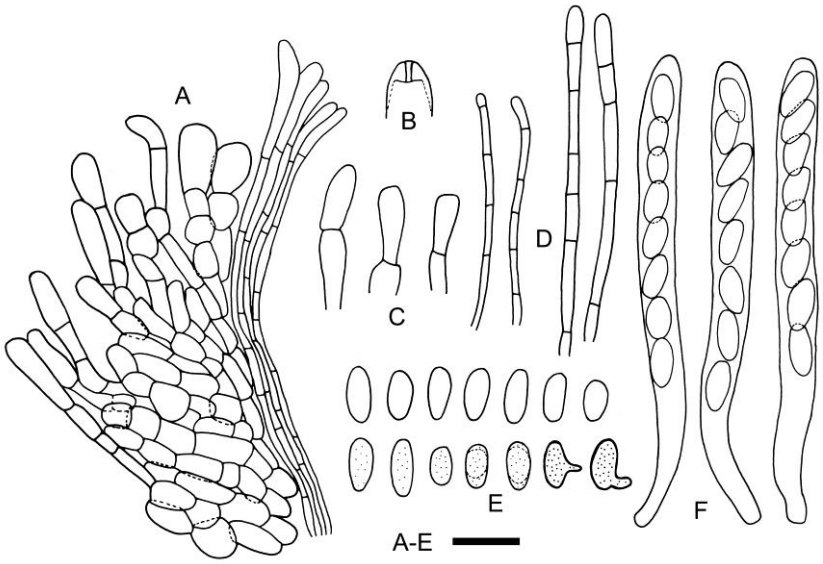

Fig. 5.60. Camera lucida illustration of *Brunneimargo camelliae* (TNS-F-40027). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Reaction of ascal apex to MLZ. **C:** Hairs. **D:** Two types of paraphyses. **E:** Asci. **F:** Ascospores. *Bars* **A–E** 10 µm.

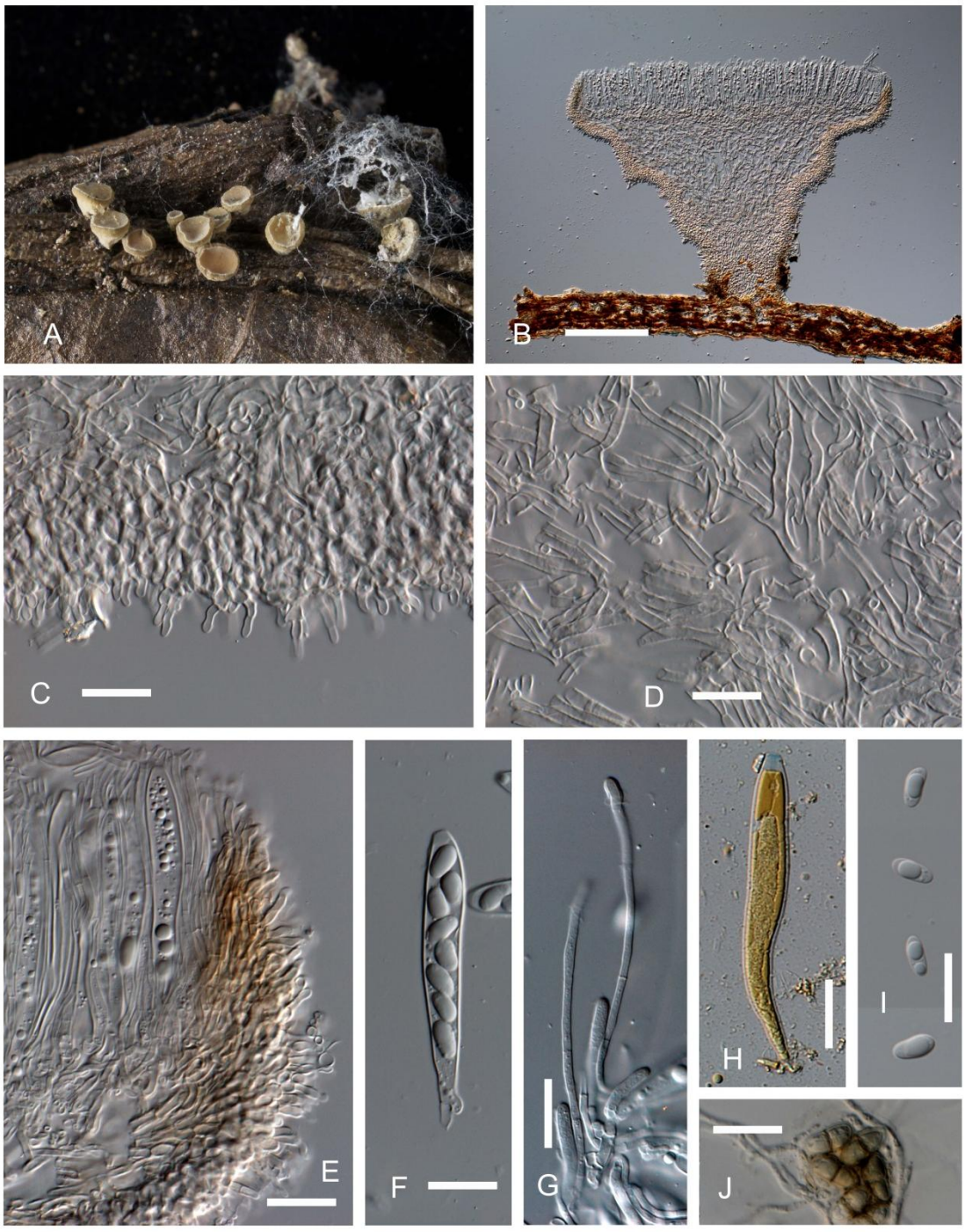

Fig. 5.61. *Crassitunica tsubakii* (TNS-F-44021). **A:** Fresh apothecia on decaying leaves of *Aucuba japonica*. **B:** Vertical section of an apothecium. **C:** Close up of ectal exipulum in flank. **D:** Close up of medullay excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Ascus. **G:** Paraphyses. **H:** Reaction of ascal apex to MLZ. **I:** Ascospores. **J:** Germinating ascospores. *Bars* **B–C** 200 μm; **D–I** 20 μm.

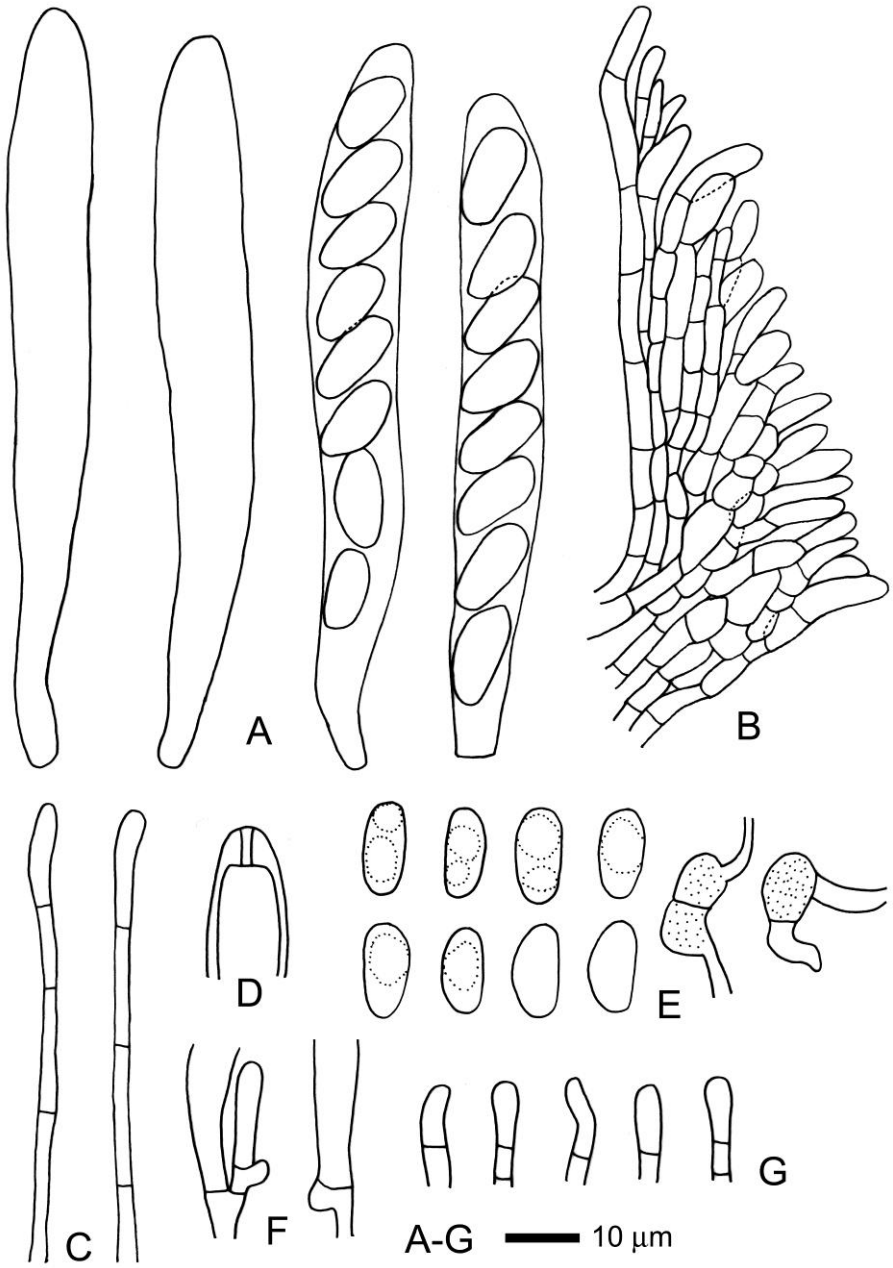

Fig. 5.62. Camera lucida illustration of *Crassitunica tsubakii* (TNS-F-44021). **A:** Asci. **B:** Vertical section of an apothecium through the margin showing the ectal excipulum. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. **F:** Croziers at the base of asci. **G:** Hairs. *Bars* **A–G** 10 µm.

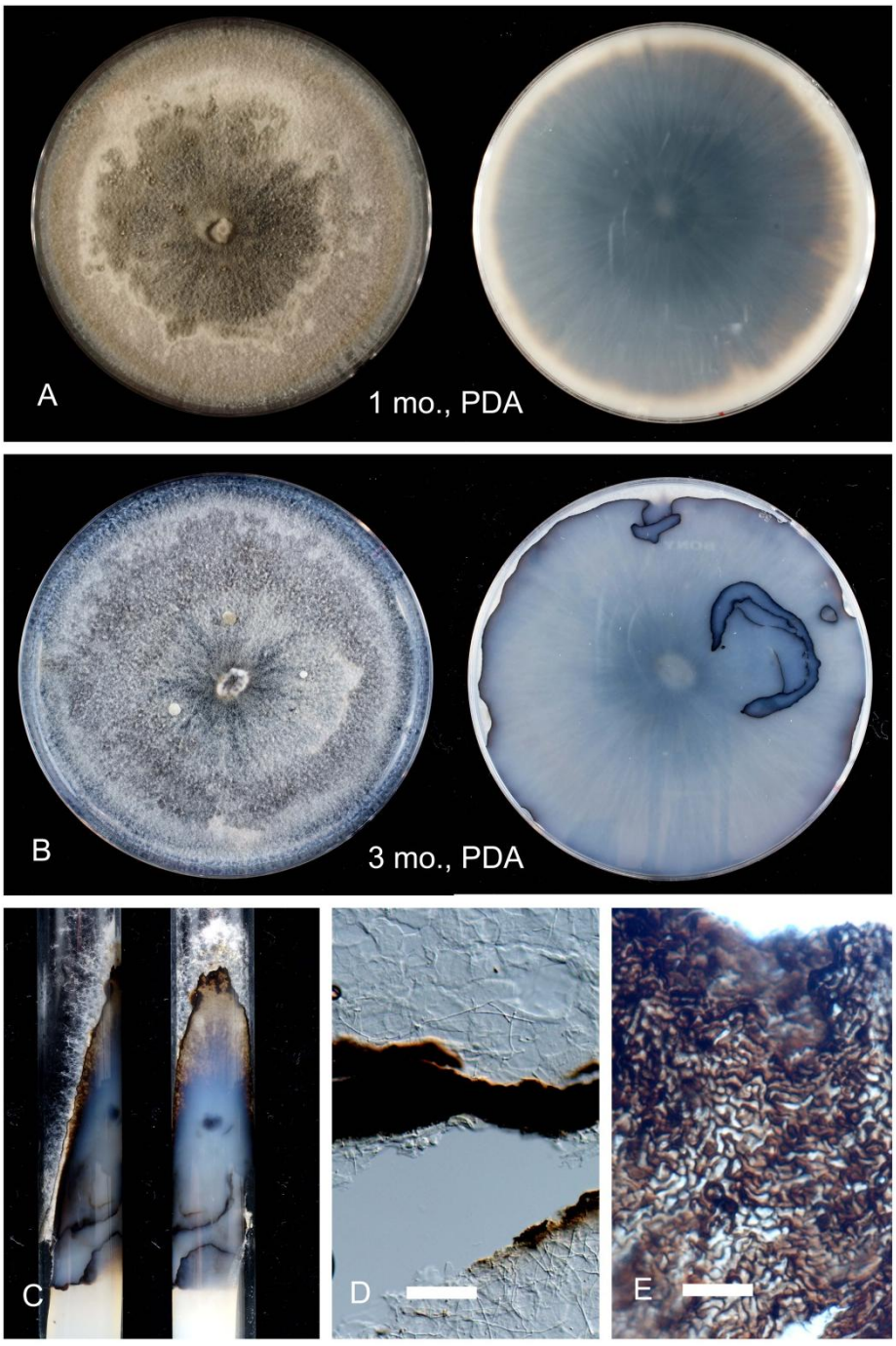

Fig. 5.63. Cultural characters of *Crassitunica tsubakii* (FC-2834, Culture of TNS-F-44021). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Rind on PDA (20°C, 6 month). **D:** Vertical view of rind. **E:** Surface view of rind showing the epidermoid cells. *Bars* **D, E** 20µm.

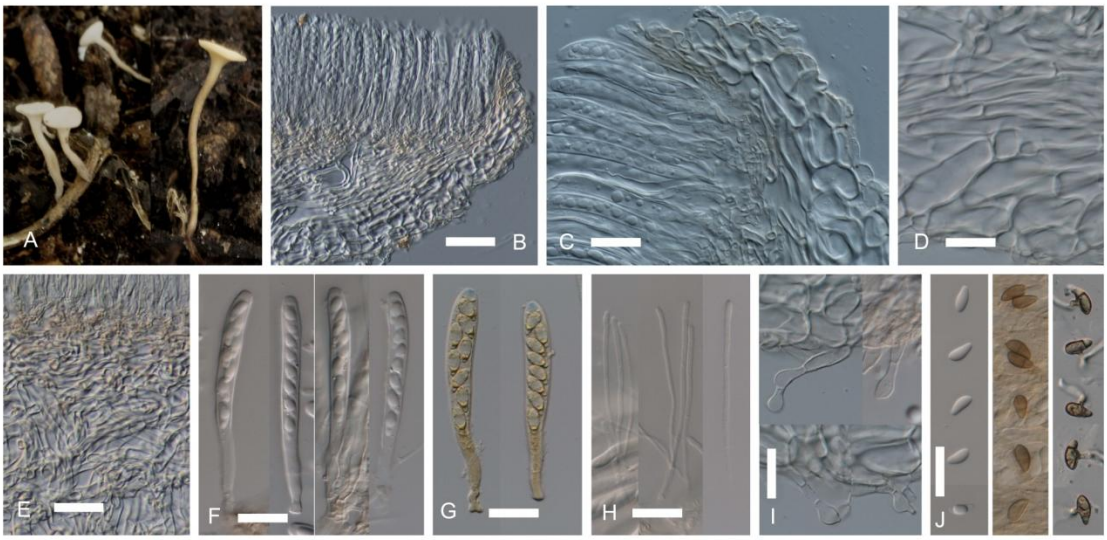

Fig. 5.64. *Lanzia longipes* (TNS-F-40082). **A:** Fresh apothecia on unknown petioles. **B:** Vertical section of an apothecium. **C:** Close up of ectal excipulum at the margin. **D:** Close up of ectal exipulum. **E:** Structure of medullay excipulum. **F:** Ascus. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Hairs. **J:** Ascospores (Hyaline ascospores, brown ascospores and germinating ascospores with spermatia). *Bars* **B, E** 40 µm; **C–D, F–J** 20 µm.

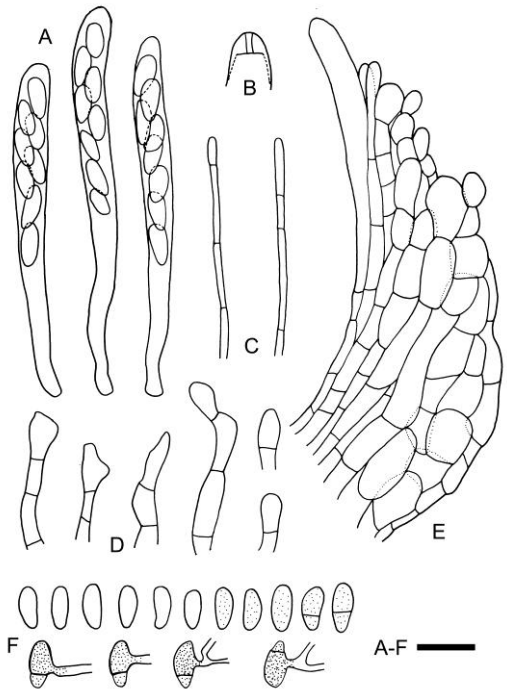

Fig. 5.65. Camera lucida illustration of *Lanzia longipes* (TNS-F-40082). **A:** Asci. **B:** Reaction of ascal apex to MLZ. **C:** Paraphyses. **D:** Hairs. **E:** Vertical section of an apothecium through the margin showing the ectal excipulum. **F:** Ascospores. *Bars* **A–F** 10 µm.

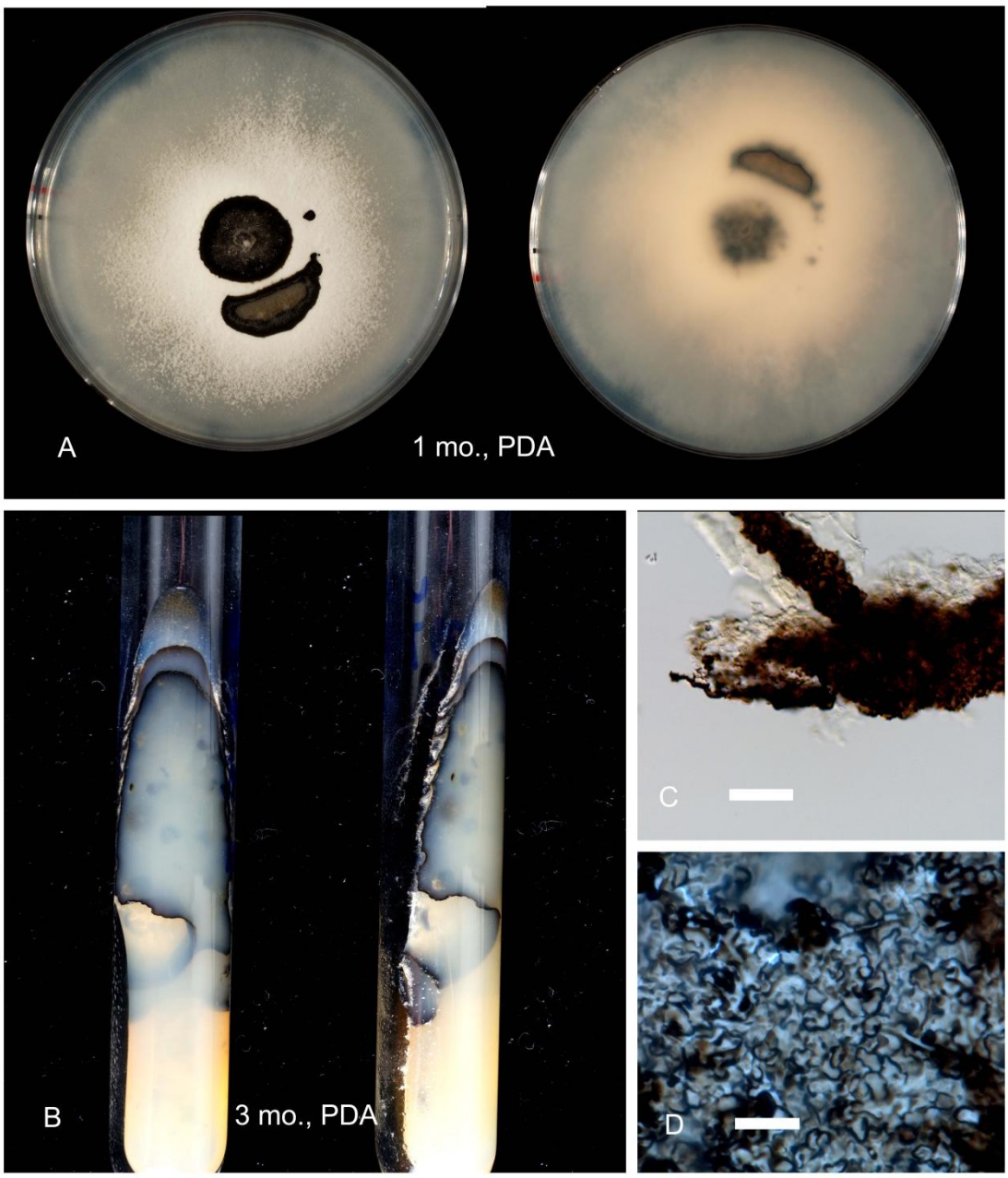

Fig. 5.66. Cultural characters of *Lanzia longipes* (FC-2832, Culture of TNS-F-40097). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:**Vertical view of rind. **D:**Surface view of rind showing the epidermoid to irregular cells. *Bars* **C** 40µm**; D** 20µm.

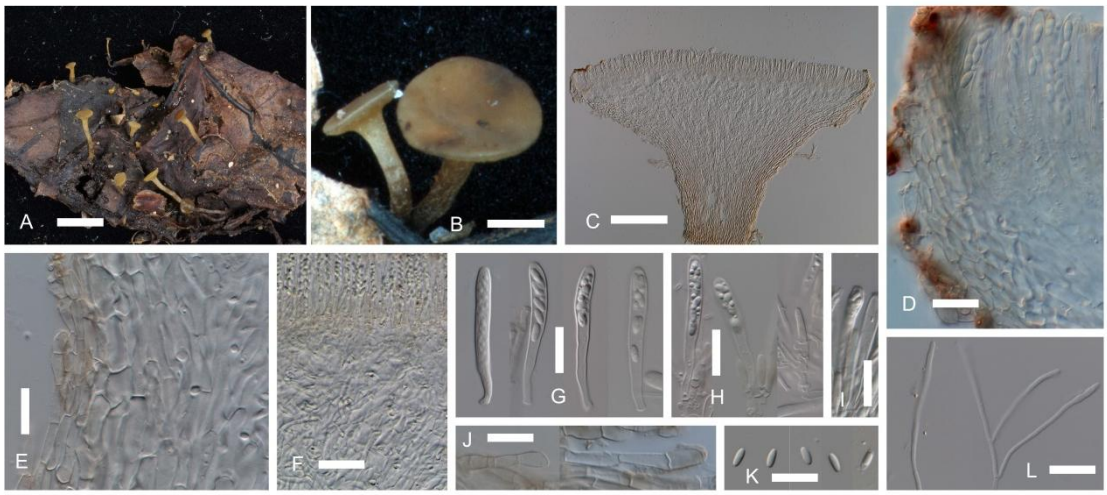

Fig. 5.67. *Lanzia pruni-serotinae* (TNS-F-40119). **A:** Fresh apothecia on leaves of *Prunus grayana.* **B:** Fresh apothecia in a higher magnification. **C:** Vertical section of an apothecium. **D:** Close up of ectal excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Structure of medullay excipulum. **G:** Ascus. **H:** Croziers at the base of asci. **I:** Reaction of ascal apex to MLZ. **J:** Hairs. **K:** Ascospores. **L:** Paraphyses. *Bars* **A** 4 mm; **B** 1 mm**; C** 200 µm; **D–L** 20 µm.

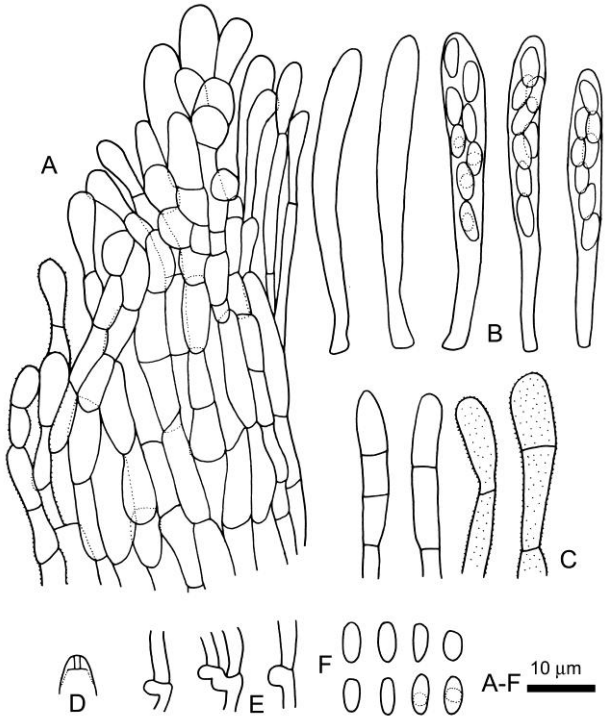

Fig. 5.68. Camera lucida illustration of *Lanzia pruni-serotinae* (TNS-F-40119). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Hairs. **D:** Reaction of ascal apex to MLZ. **E:** Croziers. **F:** Ascospores. *Bars* **A–F** 10 µm.

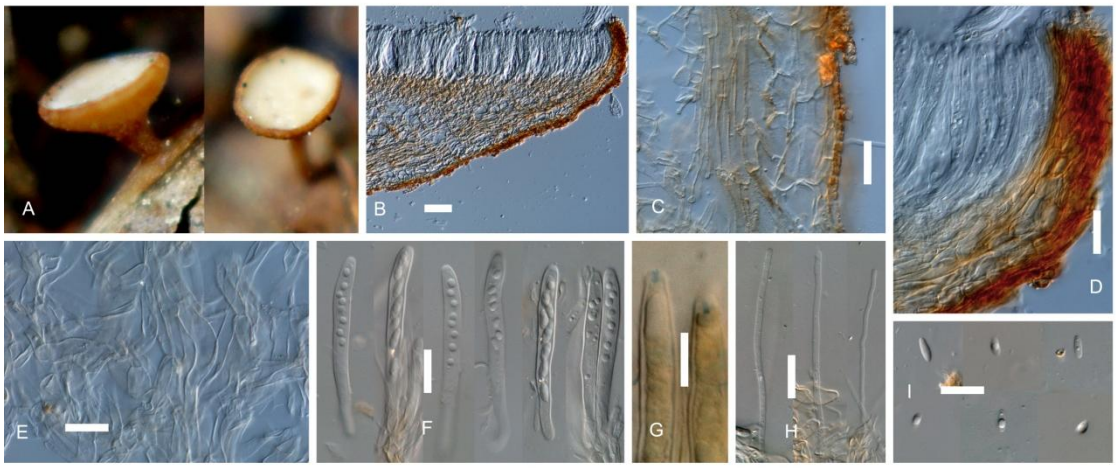

Fig. 5.69. *Lanzia* sp.1 (TNS-F-40038). **A:** Fresh apothecia on decaying leaves of unknown plant**. B:** Vertical section of an apothecium. **C:** Close up of ectal exipulum. **D:** Close up of ectal excipulum at the margin. **E:** Structure of medullay excipulum. **F:** Ascus. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. *Bars* **B** 50 µm; **C–F, H–I** 20 µm; **G** 10 µm.

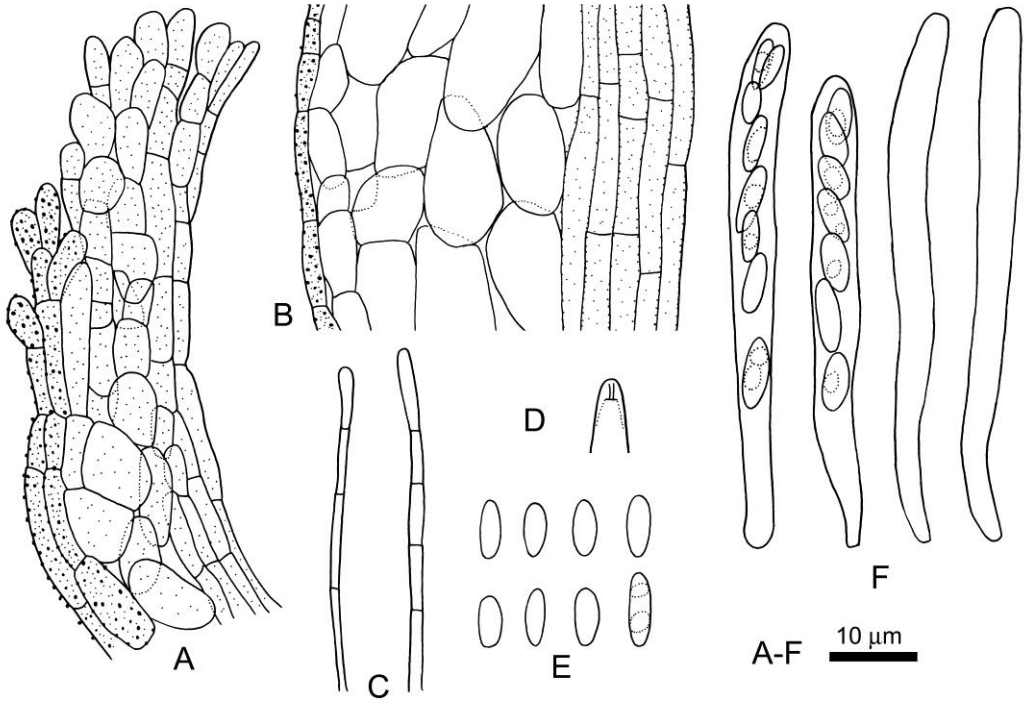

Fig. 5.70. Camera lucida illustration of *Lanzia* sp.1 (TNS-F-40038). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Ectal exipulum in the middle flank, showing the layered structure. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. **F:** Asci. *Bars* **A–F** 10 µm.

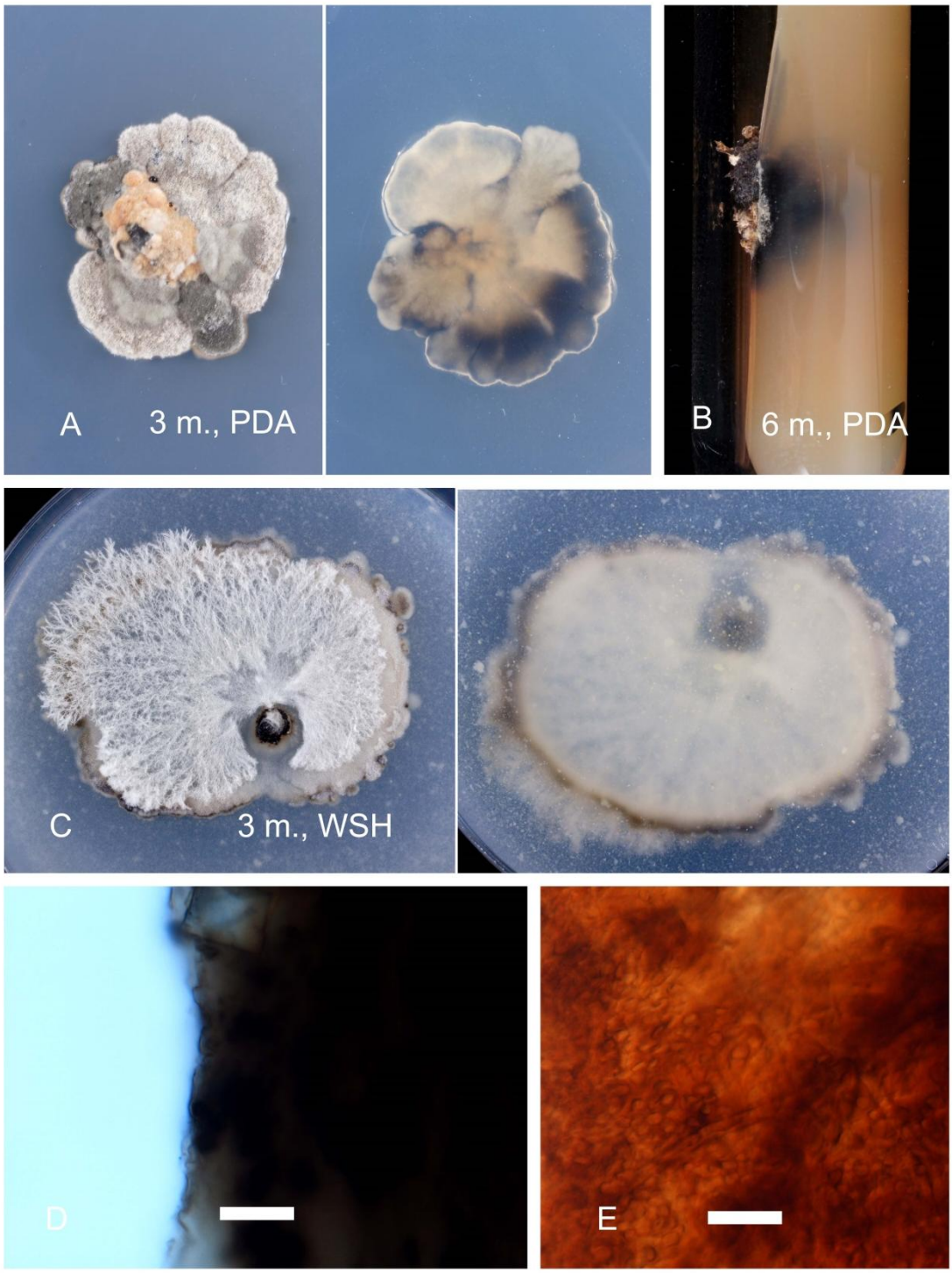

Fig. 5.71. Cultural characters of *Lanzia* sp.1 (FC-2790, Culture of TNS-F-40038). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 6 mo.). **C:** Colony on WSH (20°C, 3 mo.). **D:** Vertical view of rind. **E:** Surface view of rind showing the globulose cells. *Bars* **D, E** 20µm.

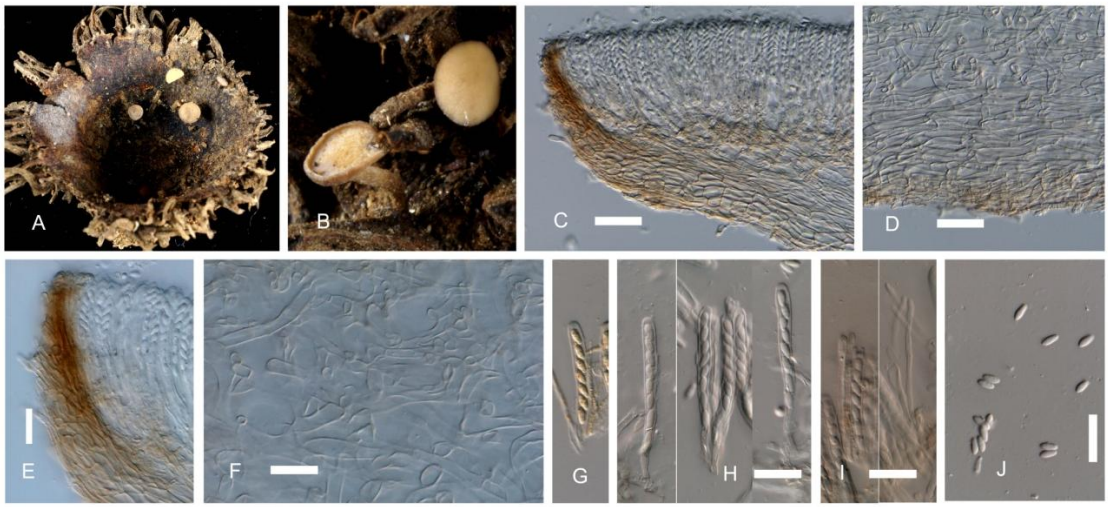

Fig. 5.72. *Lanzia* sp.2 (TNS-F-40081). **A:** Fresh apothecia on fallen fruits of *Quercus* sp. **B:** Fresh apothecia in a higher magnification. **C:** Vertical section of an apothecium. **D:** Close up of ectal exipulum in flank, showing the arrangement of cells. **E:** Close up of ectal excipulum at the margin. **F:** Structure of medullay excipulum. **G:** Reaction of ascal apex to MLZ. **H:** Asci. **I:** Paraphyses. **J:** Ascospores. *Bars* **C–D** 40 µm; **E–J** 40 µm

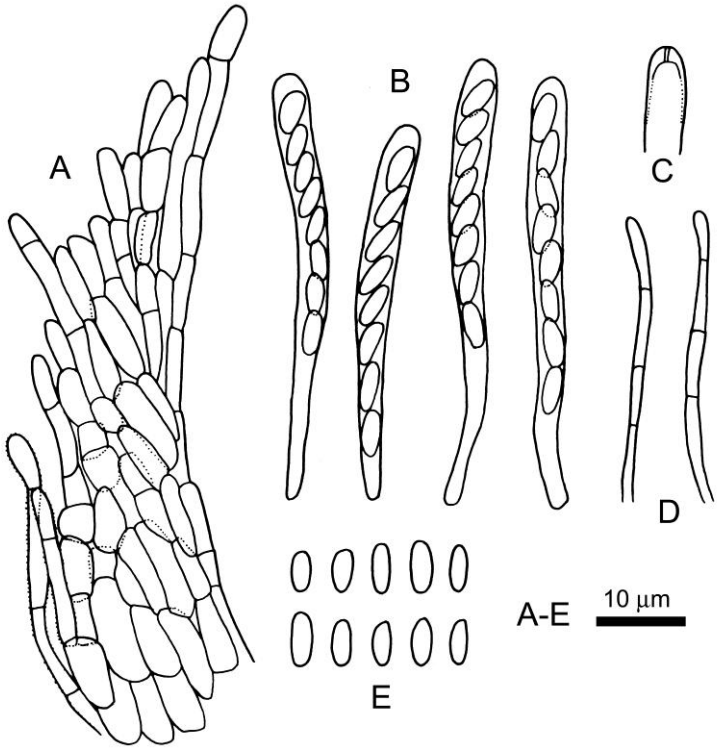

Fig. 5.73. Camera lucida illustration of *Lanzia* sp.2 (TNS-F-40081). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10 µm.

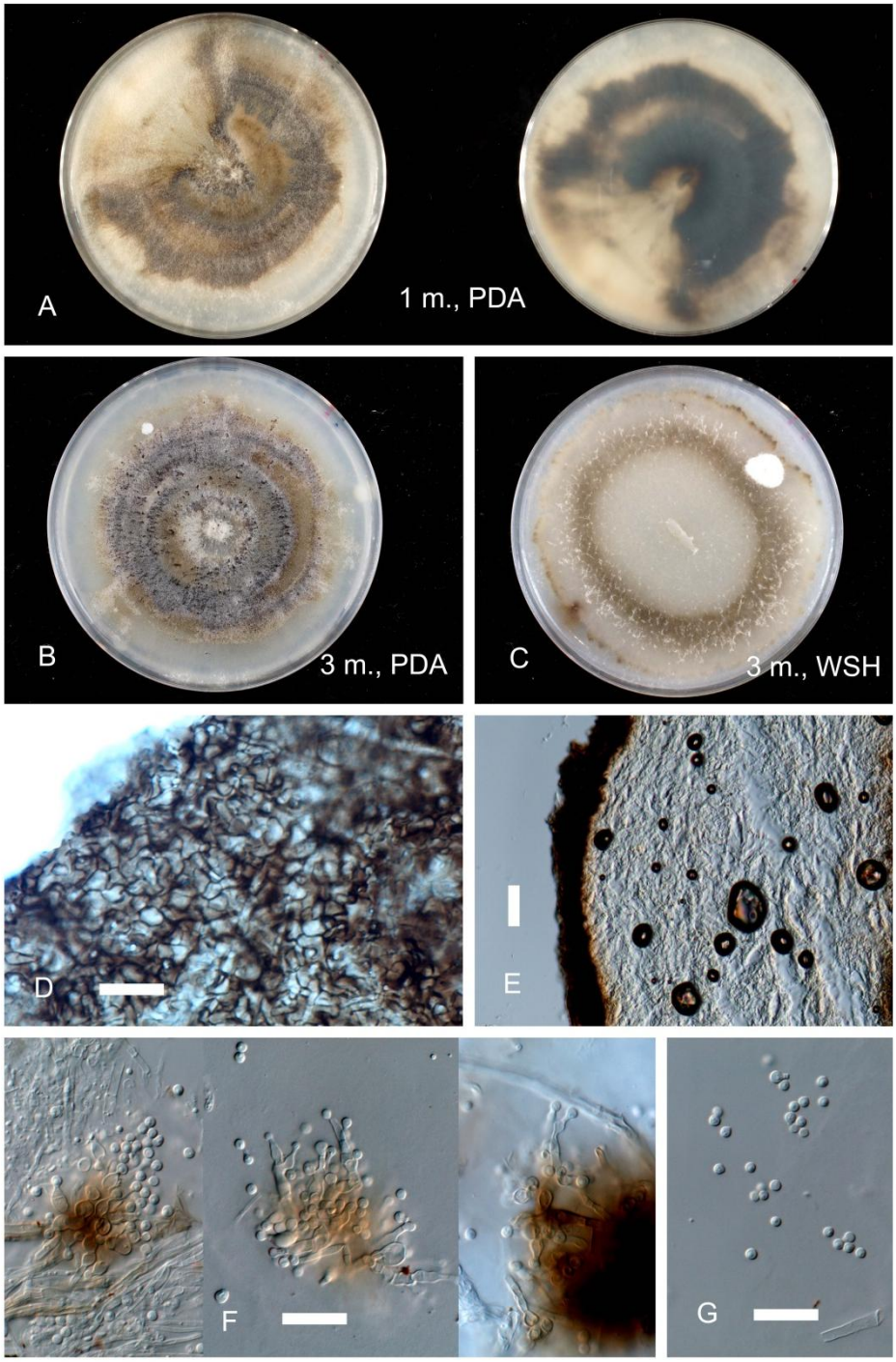

Fig. 5.74. Cultural characters of *Lanzia* sp.2 (FC-2820, Culture of TNS-F-40081). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 6 mo.). **C:** Colony on WSH (20°C, 3 mo.). **D:** Surface view of rind showing the angular to epidermoid cells. **E:**Vertical view of rind**. F:** Spermatia and spermatiophores. **G:** Spermatia. *Bars* **D, F–G** 20µm; **E** 50 µm.

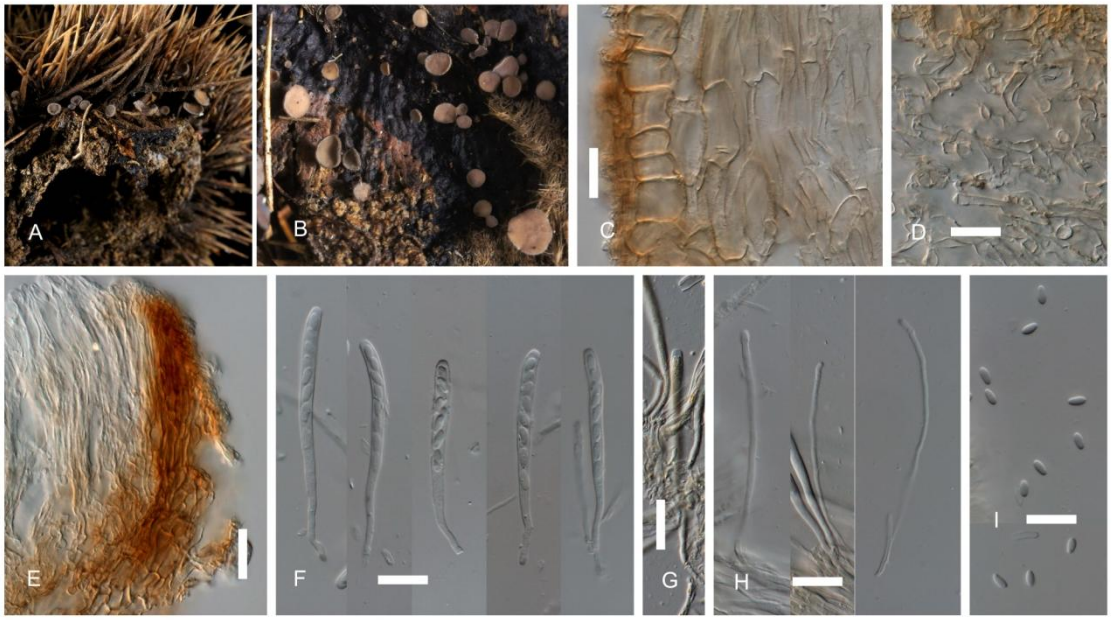

Fig. 5.75. *Lanzia* sp.3 (TNS-F-40096). **A:** Fresh apothecia on decaying involucres and spines of *Castanea crenata*. **B:** Fresh apothecia in a higher magnification, showing the blackened area on host substrate. **C:** Close up of ectal exipulum in flank, showing the arrangement of cells. **D:** Structure of medullay excipulum. **E:** Close up of ectal excipulum at the margin. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. *Bars* **C–I** 20 μm.

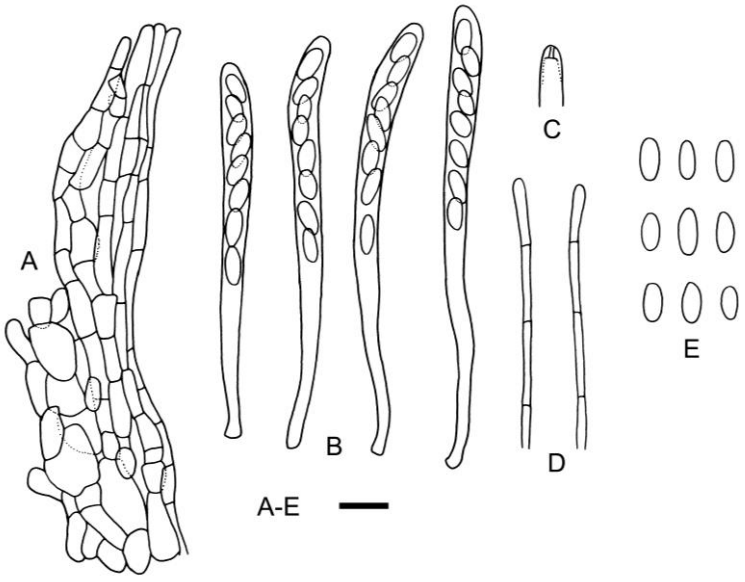

Fig. 5.76. Camera lucida illustration of *Lanzia* sp.3 (TNS-F-40096). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 20 μm.

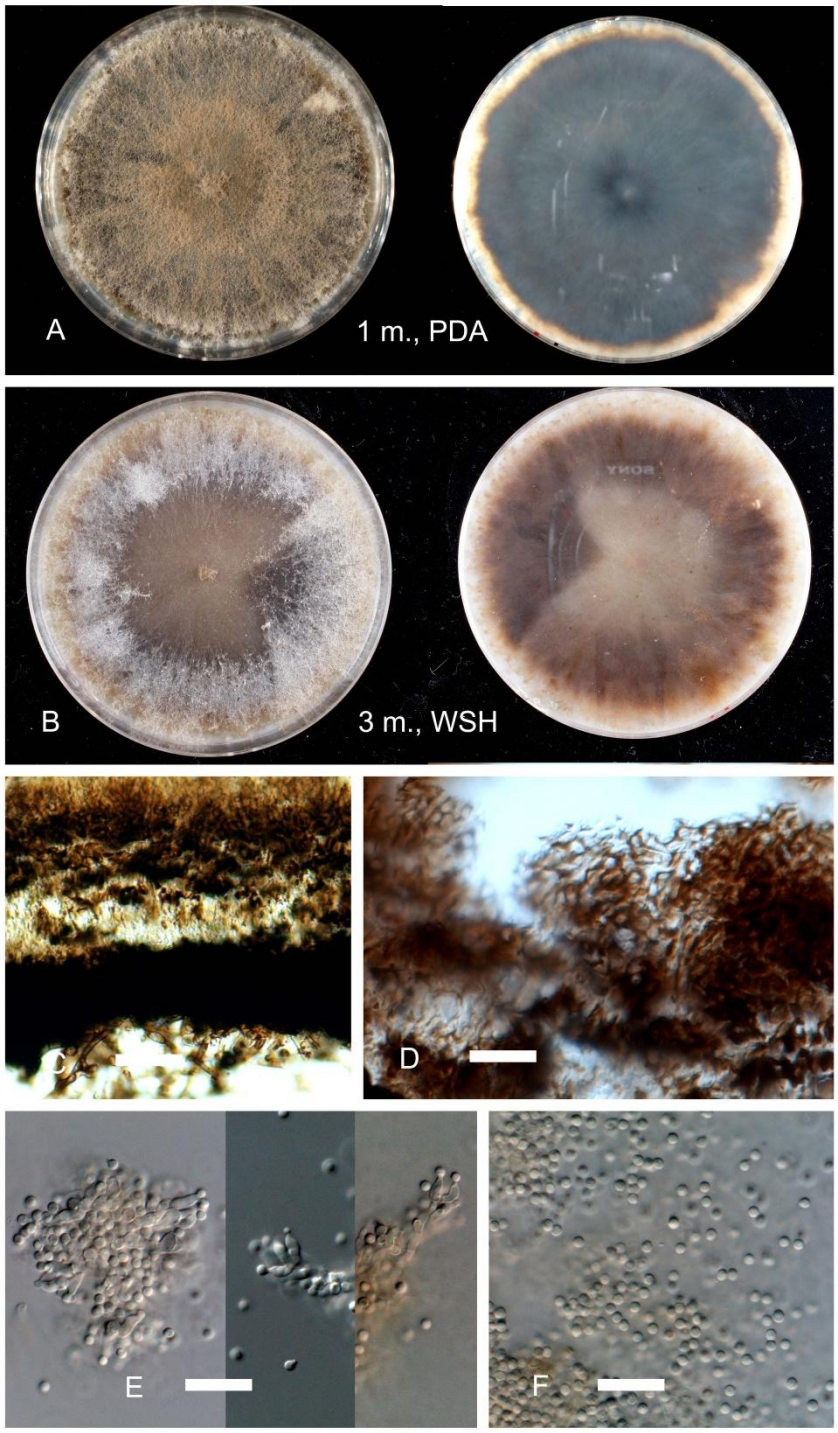

Fig. 5.77. Cultural characters of *Lanzia* sp.3 (FC-2831, Culture of TNS-F-40096). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on WSH (20°C, 3 mo.). **C:** Vertical view of rind. **D:** Surface view of rind showing the globose to angular cells. **E:** Spermatia and spermatiophores. **F:** Spermatia. *Bars* **C–F** 20μm.

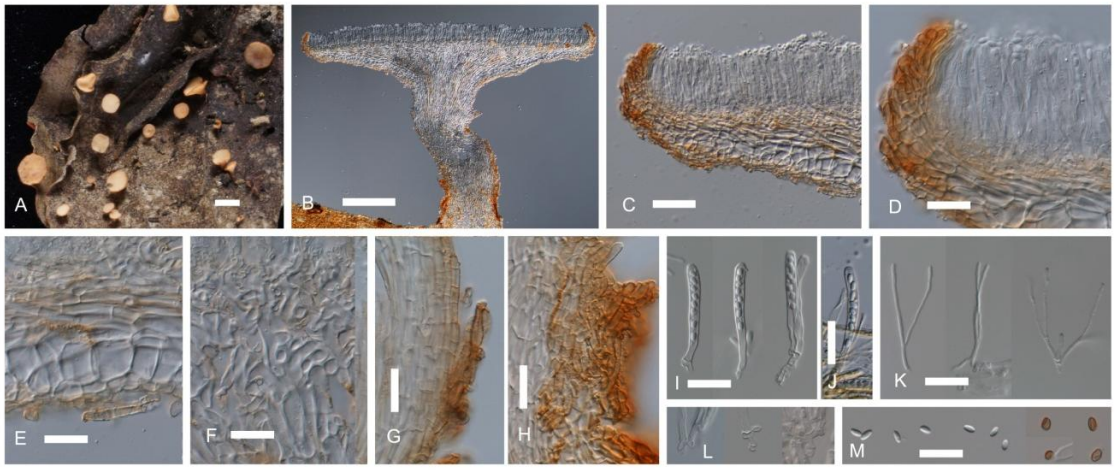

Fig. 5.78. *Lanzia* sp.4 (TNS-F-40136). **A:** Fresh apothecia on decaying leaf of *Swida controversa*. **B:** Vertical section of an apothecium. **C:** Close up of ectal excipulum**. D:** Close up of ectal excipulum at the margin. **E:** Close up of ectal exipulum in the middle flank. **F:** Structure of medullay excipulum. **G**, **H:** Roughened hyphae protruded from the covering layer. **I:** Asci. **J:** Reaction of ascal apex to MLZ. **K:** Paraphyses. **L:** Croziers in the base of asci. **M:** Ascospores. *Bars* **A** 2 mm; **B** 200 µm; **C** 40 µm; **D–M** 20 µm.

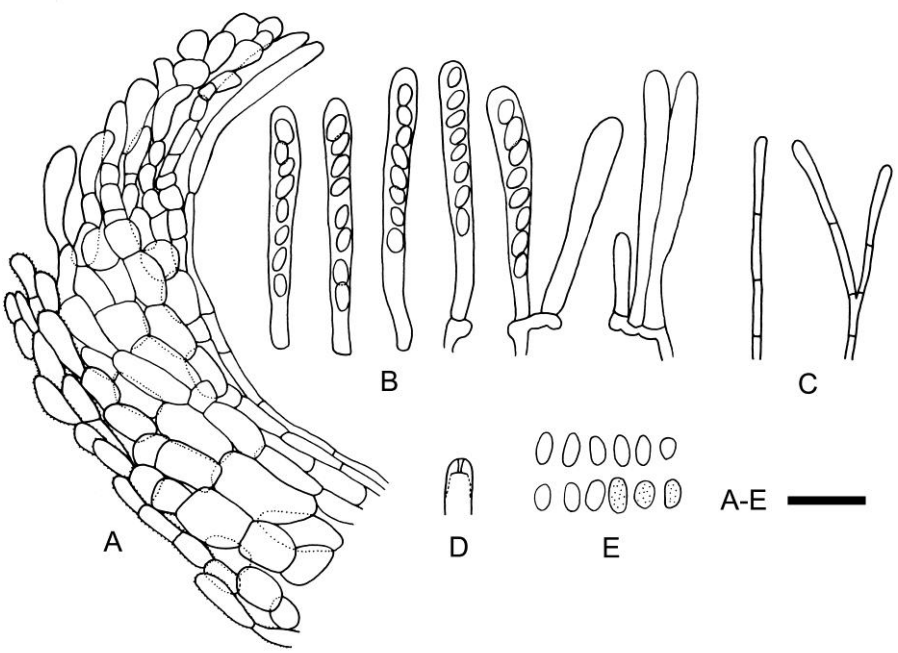

Fig. 5.79. Camera lucida illustration of *Lanzia* sp.4 (TNS-F-40136). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10µm.

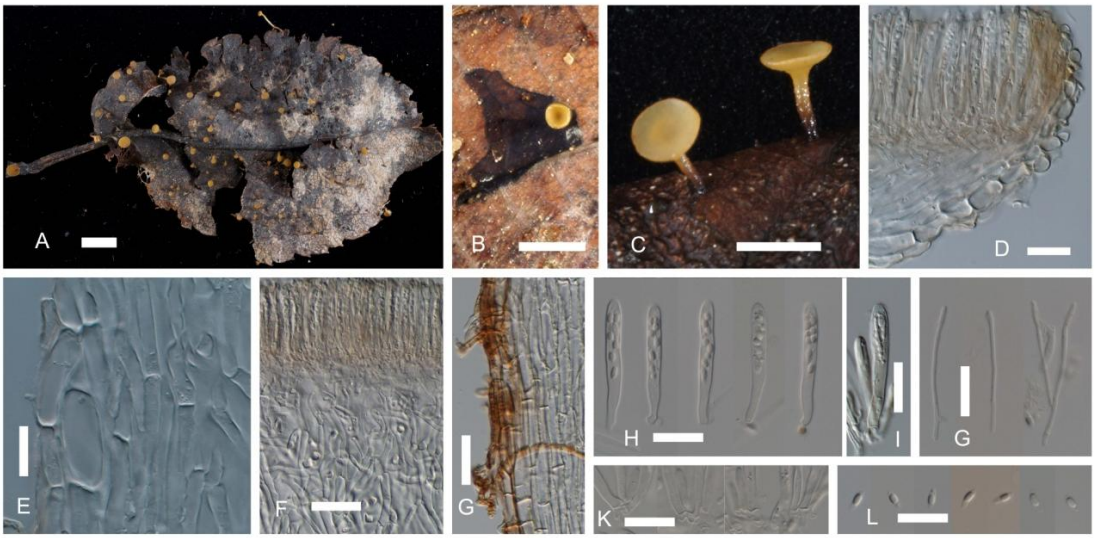

Fig. 5.80. *Lanzia* sp.5 (TNS-F-40139). **A:** Fresh apothecia on decaying leaves and petioles of *Pyrus pyrifolia*. **B:** Blackened zones delimited by rind in substrate. **C:** Fresh apothecia in a higher magnification**.** C: Vertical section of an apothecium. **D:** Close up of ectal excipulum at the margin. **E:** Close up of ectal exipulum in flank, showing the arrangement of cells. **F:** Structure of medullay excipulum. **G:** Roughened hyphae protruded from covering layer. **H:** Asci. **I:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **K:** Croziers in the base of asci. **L:** Ascospores. *Bars* **A** 5 mm**; B–C** 2 mm**; D–E, F–L** 20 µm; **F–G** 40µm.

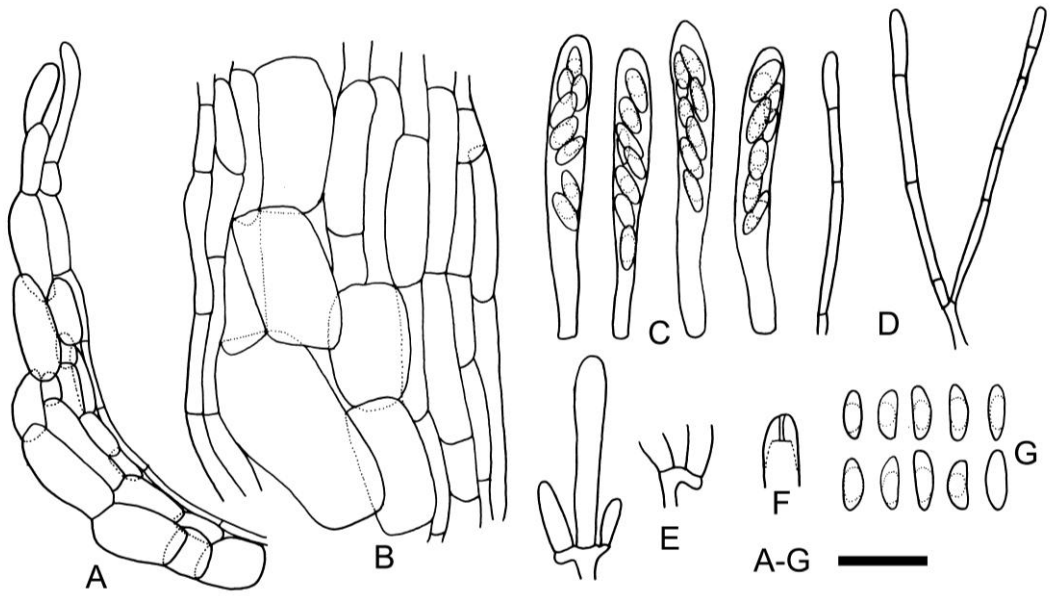

Fig. 5.81. Camera lucida illustration of *Lanzia* sp.5 (TNS-F-40139). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Ectal exipulum in flank. **C:** Asci. **D:** Paraphyses. **E:** Croziers in the base of asci. **F:** Reaction of ascal apex to MLZ. **G:** Ascospores. *Bars* **A–G** 10 µm.

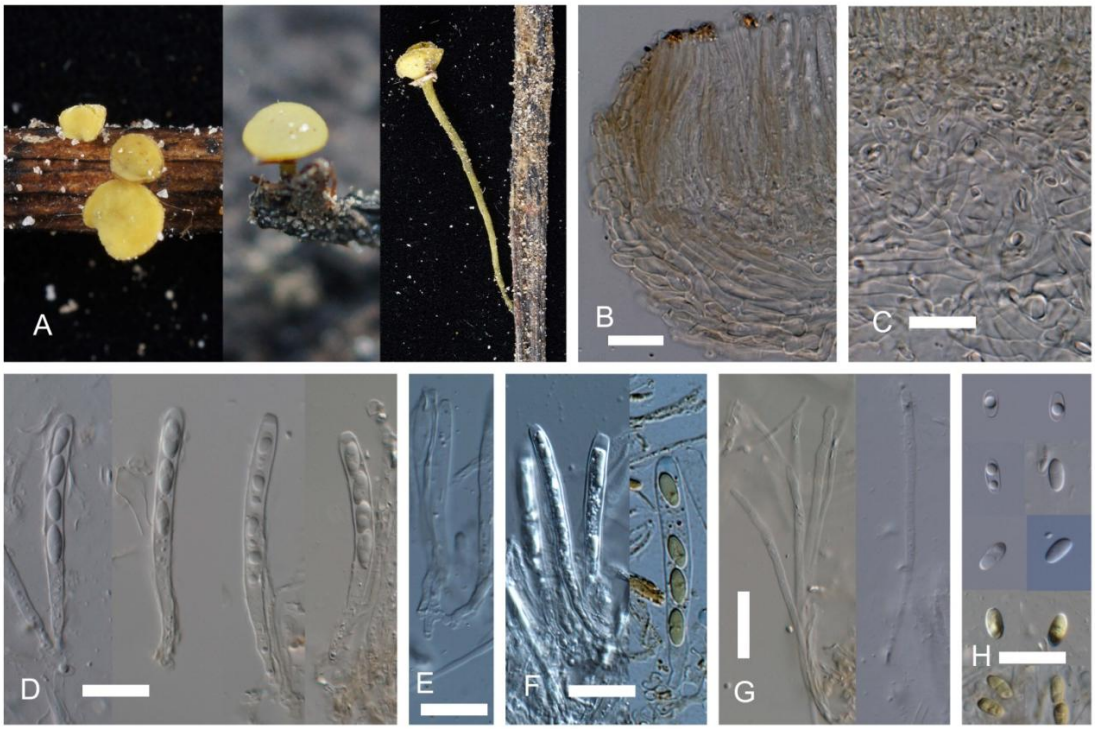

Fig. 5.82. *Lanzia* sp.6 (TNS-F-40055). **A:** Fresh apothecia on decaying herb stems. **B:** Close up of ectal excipulum at the margin. **C:** Structure of medullay excipulum. **D:** Asci. **E:** Croziers at the base of asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Ascospores. *Bars* **B–H** 20 µm.

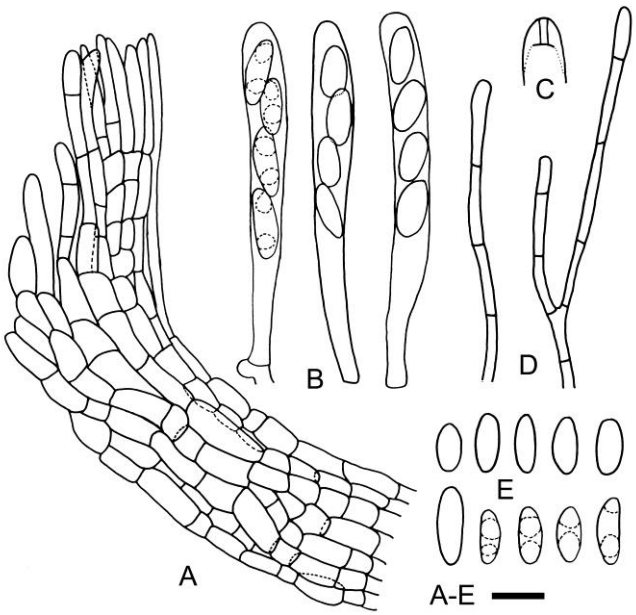

Fig. 5.83. Camera lucida illustration of *Lanzia* sp.6 (TNS-F-40055). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E** 10µm.

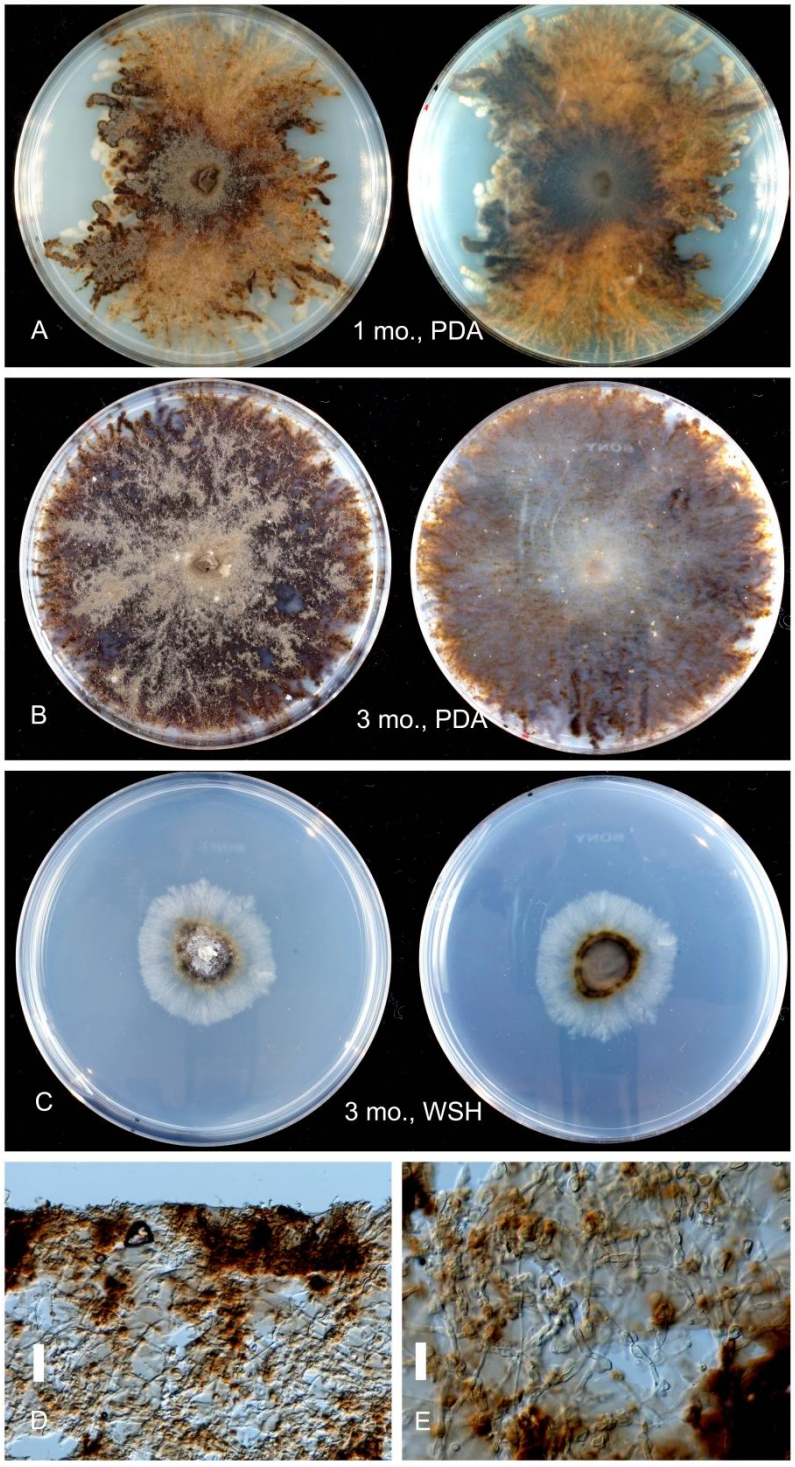

Fig. 5.84. Cultural characters of *Lanzia* sp.6 (FC-2802, Culture of TNS-F-40055). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Colony on WSH (20°C, 3 mo.). **D:** Vertical section of blackened area in PDA. **E:** Hyphae structure in PDA. *Bars* **D** 50 μm; **E** 25μm.

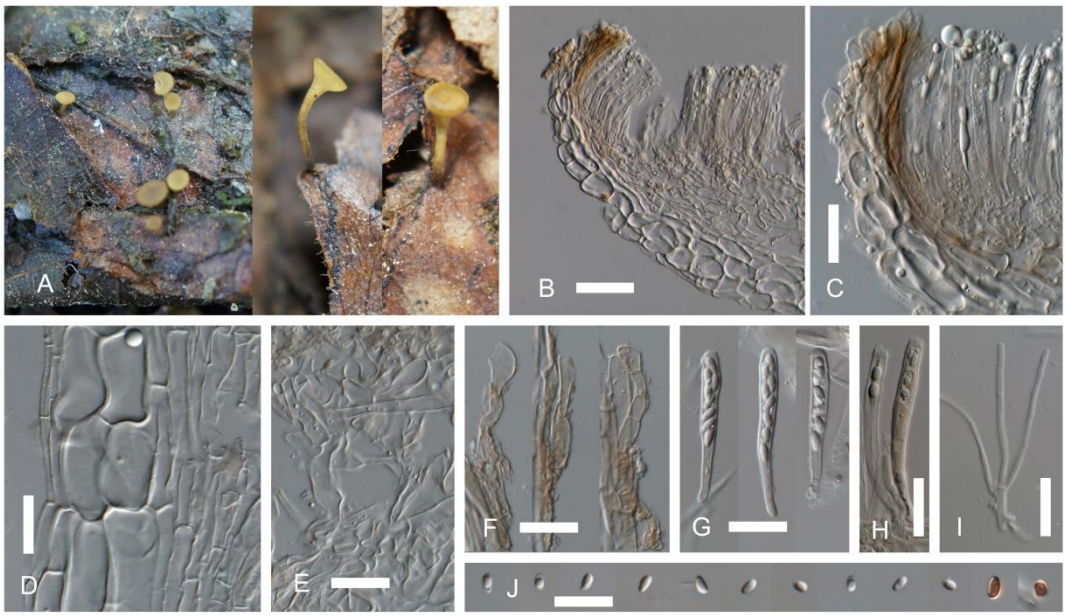

Fig. 5.85. *Lanzia* sp.7 (TNS-F-40036). **A:** Fresh apothecia on decaying leaves of *Betula* sp. **B:** Close up of ectal excipulum. **C:** Close up of ectal excipulum at the margin. **D:** Close up of ectal exipulum in the middle flank. **E:** Structure of medullay excipulum. **F:** Hairs. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Paraphyses. **J:** Ascospores. *Bars* **B** 40 μm; **C–J** 20 μm.

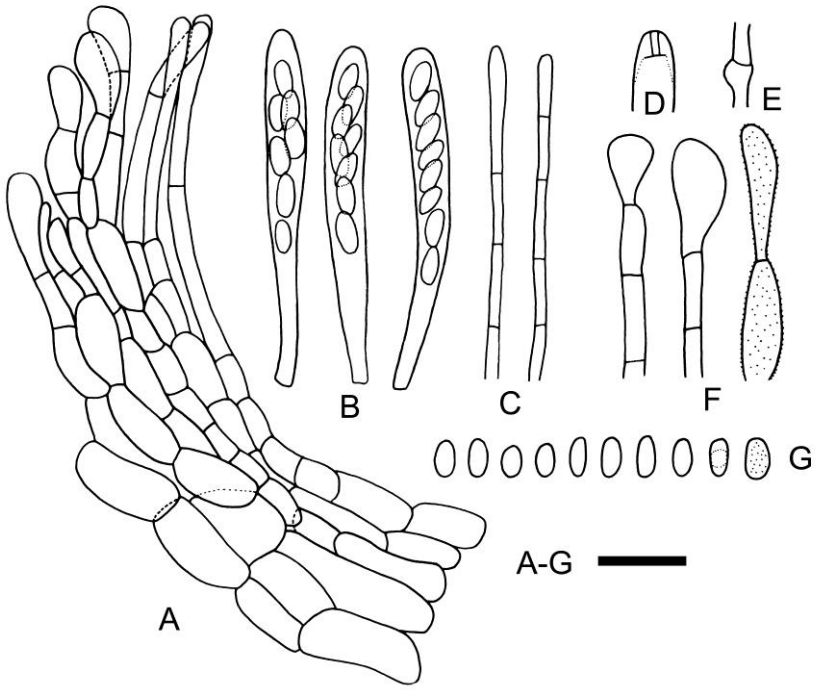

Fig. 5.86. Camera lucida illustration of *Lanzia* sp.7 (TNS-F-40036). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Crozier in the base of ascus. **F:** Hairs. **G:** Ascospores. *Bars* **A–G** 10μm.

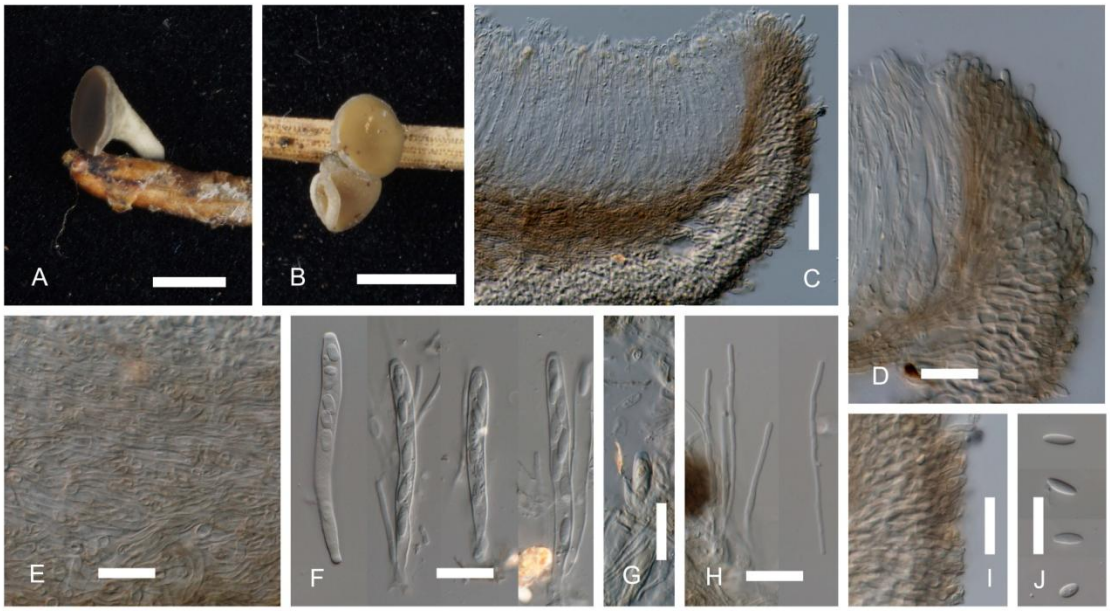

Fig. 5.87. *Rutstroemia pinicola* (TNS-F-40105). **A, B:** Fresh apothecia on fallen leaves of *Pinus luchuensis.* **C:** Vertical section of an apothecium. **D:** Close up of ectal excipulum at the margin. **E:** Structure of medullay excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Close up to cells in ectal excipulum. **J:** Ascospores. *Bars* **A–B** 2 mm**; C** 40 µm; **D– J** 20 µm.

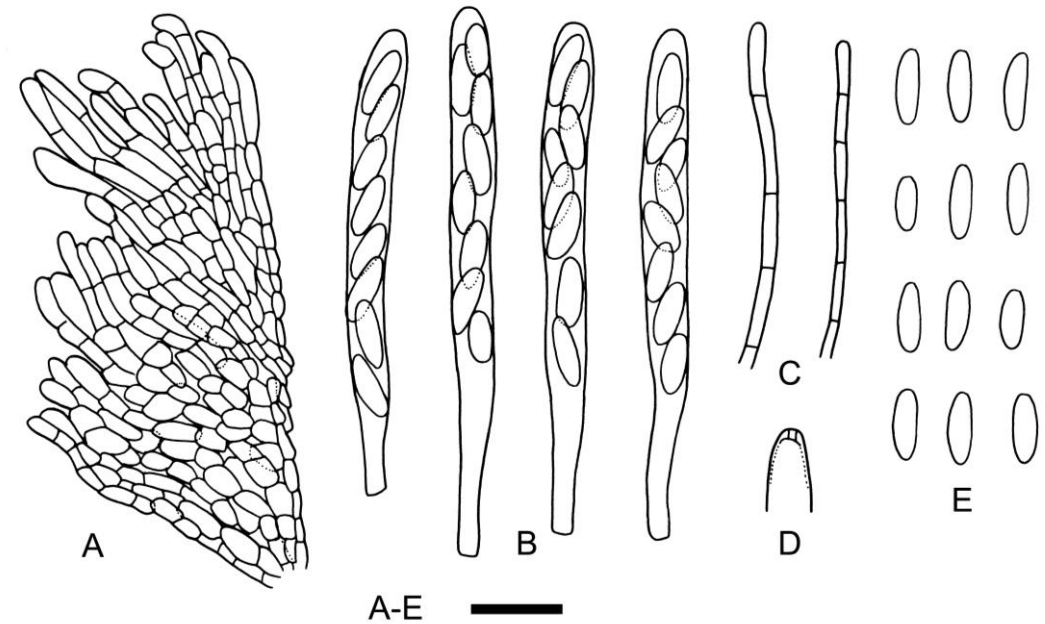

Fig. 5.88. Camera lucida illustration of *Rutstroemia pinicola* (TNS-F-40105). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

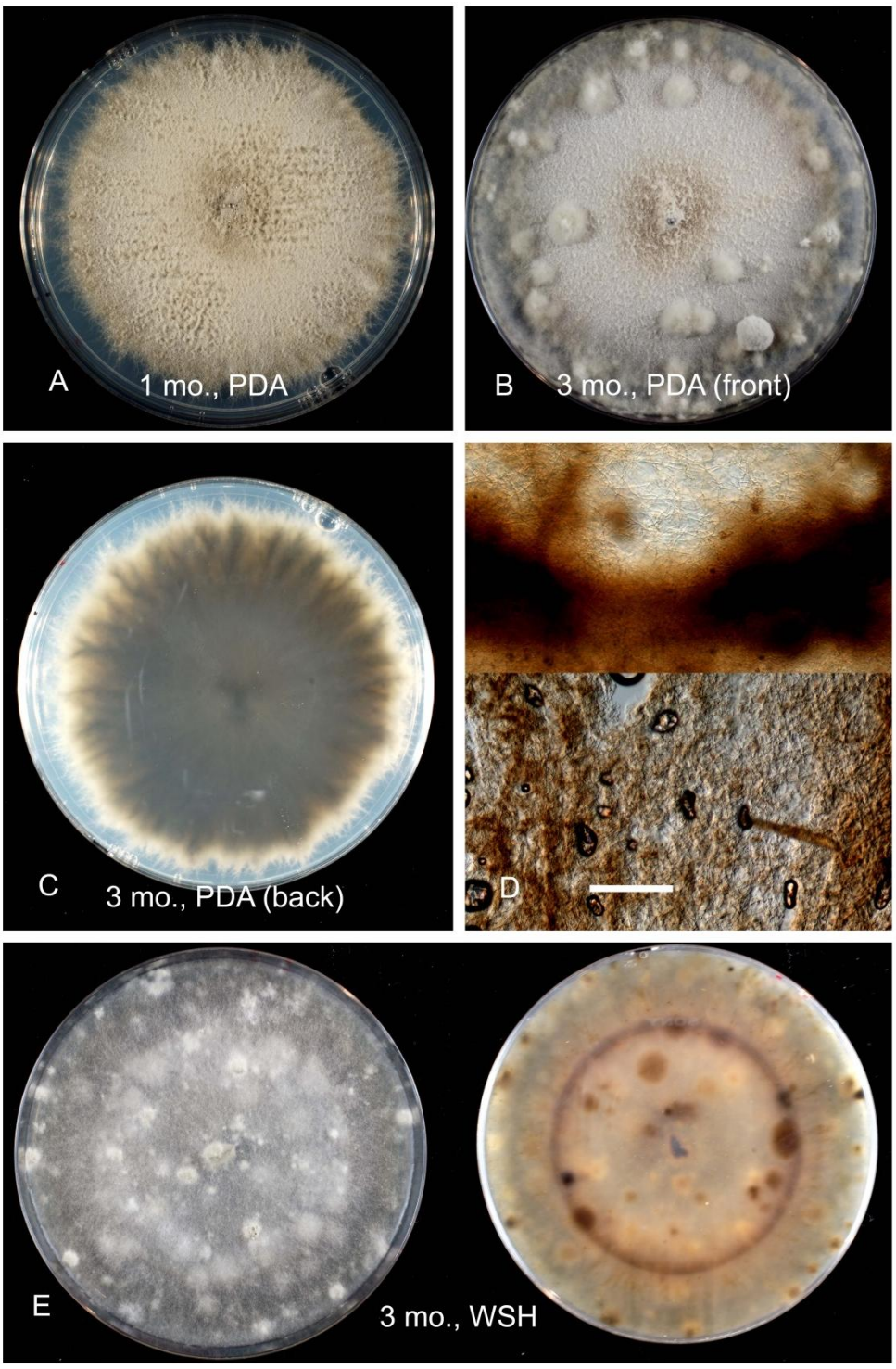

Fig. 5.89. Cultural characters of *Rutstroemia pinicola* (FC-2981, Culture of TNS-F-40105). **A:** Colony on PDA (20°C, 1 mo.). **B:** Front view of colony on PDA (20°C, 3 mo.). **C:** Back view of colony on PDA (20°C, 3 mo.). **D:** Vertical section of blackened area in PDA. **E:** Colony on WSH (20°C, 3 mo.). *Bars* **D** 200 μm.

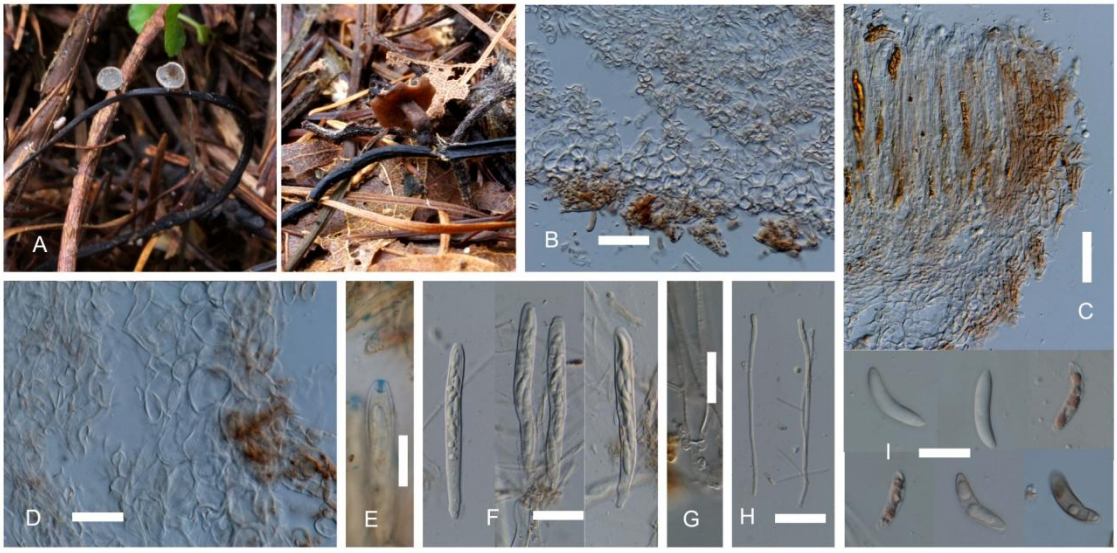

Fig. 5.90. *Moellerodiscus* sp.1 (TNS-F-40085). **A:** Fresh apothecia on decaying petioles of unknown plant. **B:** Cells in ectal excipulum. **C:** Close up of ectal excipulum at the margin. **D:** Close up of cells in ectal excipulum. **E:** Reaction of ascal apex to MLZ. **F:** Asci. **G:** Crozier in the base of ascus. **H:** Paraphyses. **I:** Ascospores. *Bars* **B, C, F, H** 40 μm; **D, E, G, I** 20 μm.

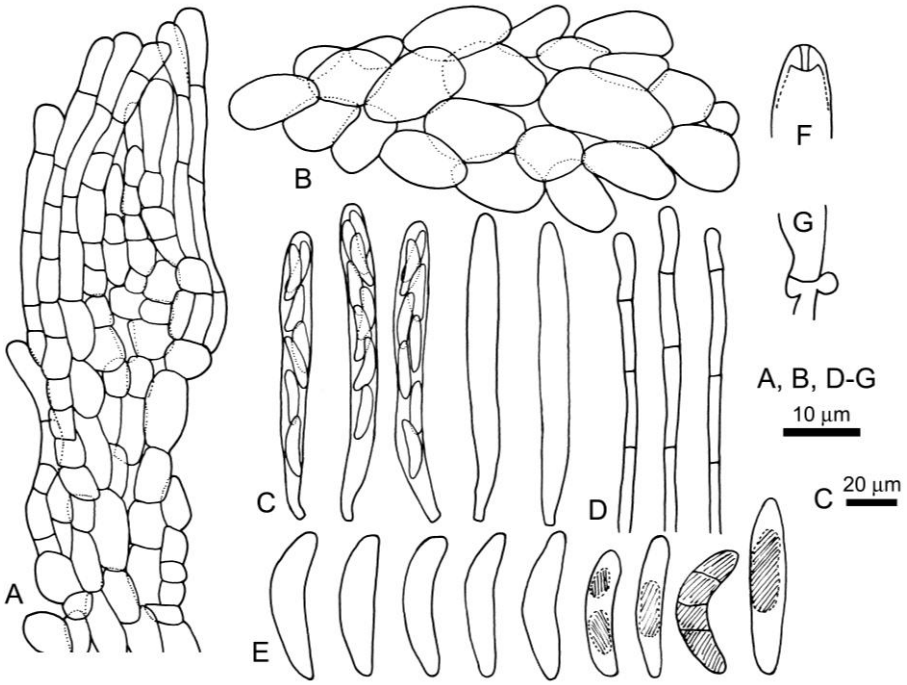

Fig. 5.91. Camera lucida illustration of *Moellerodiscus* sp.1 (TNS-F-40085). **A:** Vertical section of an apothecium through the margin. **B:** Cells in the flank of ectal excipulum. **C:** Asci. **D:** Paraphyses. **E:** Ascospores. **F:** Reaction of ascal apex to MLZ. **G:** Crozier in the base ascus. *Bars* **A, B, D–G** 10 μm; **C** 20 μm.

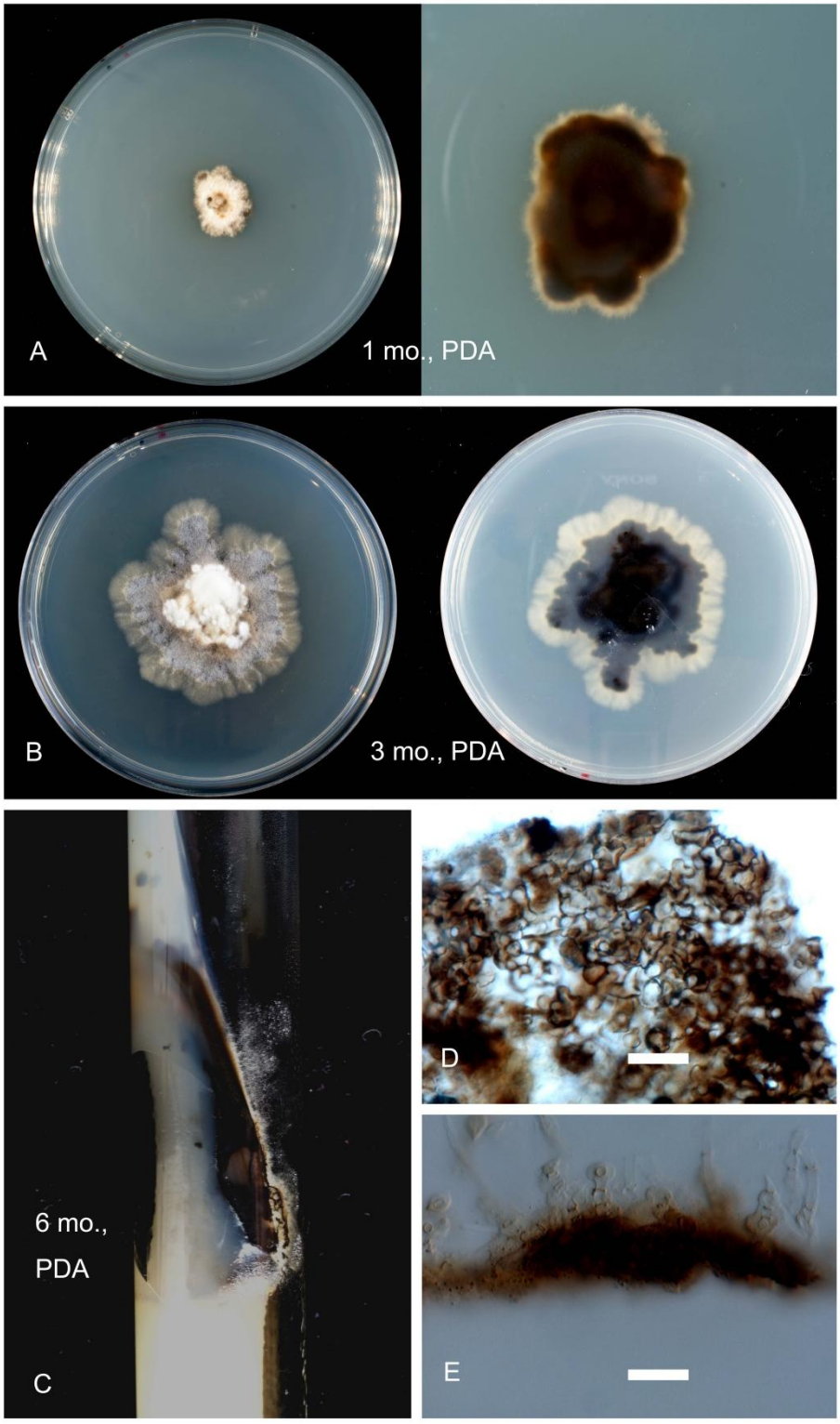

Fig. 5.92. Cultural characters of *Moellerodiscus* sp.1 (FC-2823, Culture of TNS-F-40085). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Colony on PDA (20°C, 6 mo.). **D:** Surface view of rind showing the globose cells. **E:** Vertical view of rind. *Bars* **D, E** 20μm.

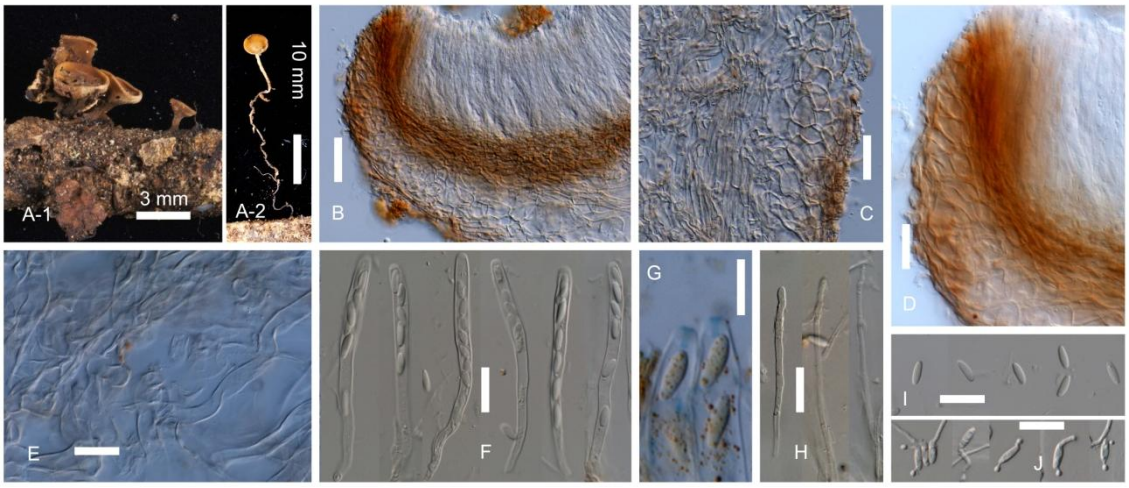

Fig. 5.93. *Moellerodiscus* sp.2 (TNS-F-40188). **A:** Fresh apothecia on decaying petioles of unknown plant. **B:** Vertical section of an apothecium. **C:** Close up of ectal excipulum in the middle flank. **D:** Close up of ectal excipulum at the margin. **E:** Structure of medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Ascospores. **J:** Germinating ascospores. *Bars* **A-1** 3 mm**; A-2** 10 mm**; B–C** 40 µm; **D–F, H–J** 20 µm; **G** 10µm.

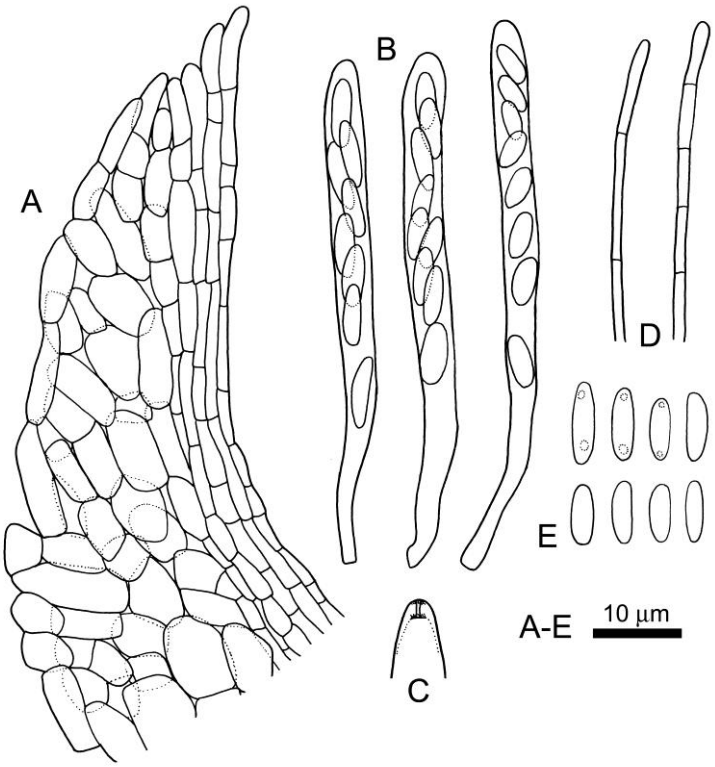

Fig. 5.94. Camera lucida illustration of *Moellerodiscus* sp.2 (TNS-F-40188). **A:** Vertical section of an apothecium through the margin. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. *Bars* **A–E**10 µm.

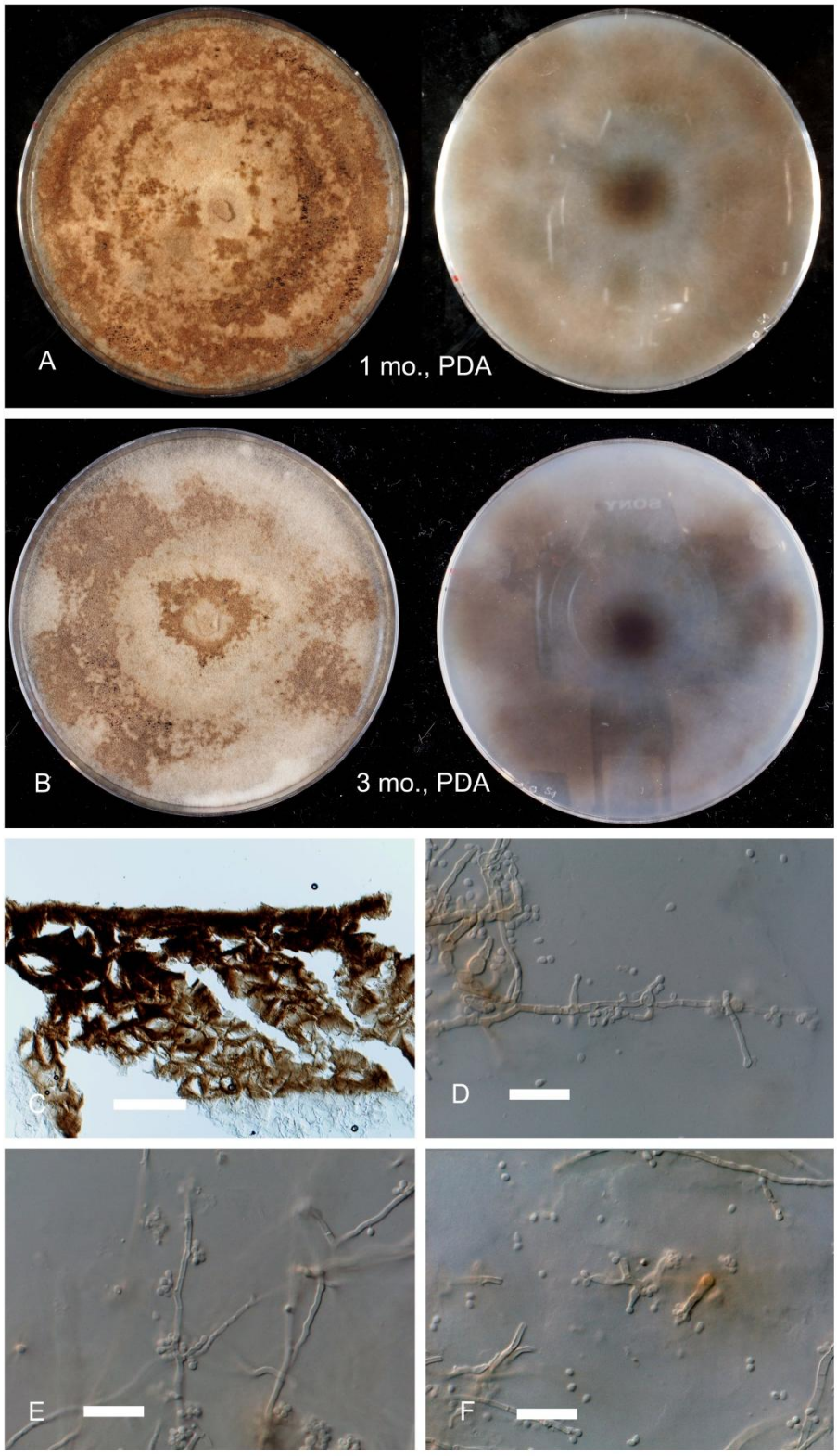

Fig. 5.95. Cultural characters of *Moellerodiscus* sp.2 (FC-5105, Culture of TNS-F-40188). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Vertical section of blackened area in PDA. **D, E, F:** Spermatia and spermatiophores. *Bars* **C** 200 μm; **D**–**F** 20μm.

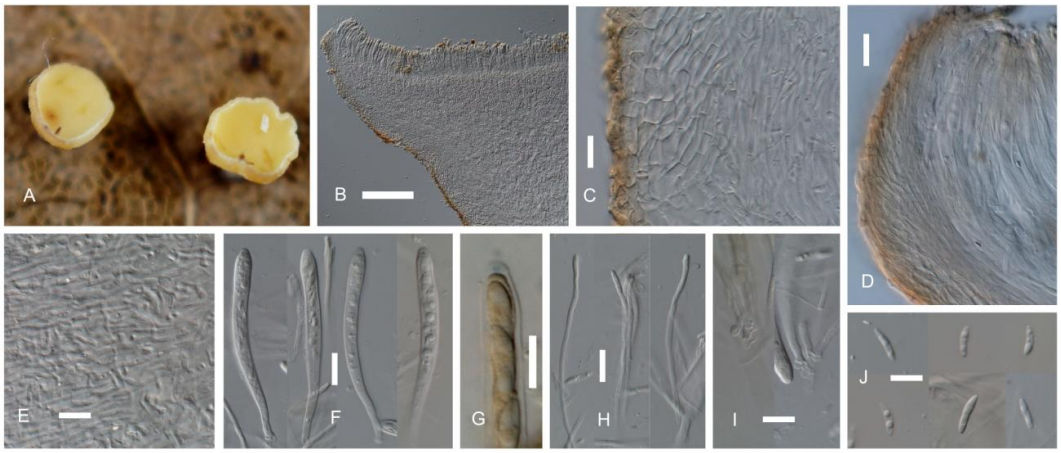

Fig. 5.96. *Phaeohelotium epiphyllum* (TNS-F-40042). **A:** Fresh apothecia on decaying leaves of *Populus maximowiczii***. B:** Vertical section of an apothecium. **C:** Close up of ectal excipulum in flank showing the layers. **D:** Close up of ectal excipulum at the margin. **E:** Structure in medullary excipulum. **F:** Asci. **G:** Reaction of ascal apex to MLZ. **H:** Paraphyses. **I:** Croziers in the base of asci. **J:** Ascospores. *Bars* **B** 200 µm; **C–F, H–J** 20 µm; **G** 10 µm.

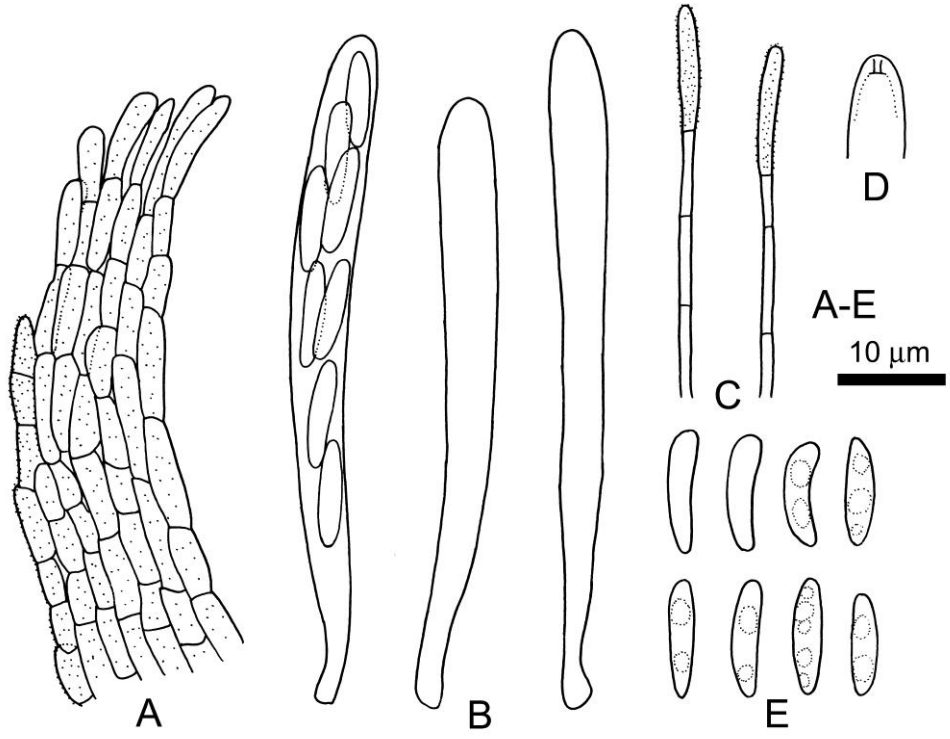

Fig. 5.97. Camera lucida illustration of *Phaeohelotium epiphyllum* (TNS-F-40042). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Asci. **C:** Paraphyses. **D:** Reaction of ascal apex to MLZ. **E:** Ascospores. *Bars* **A–E** 10 µm.

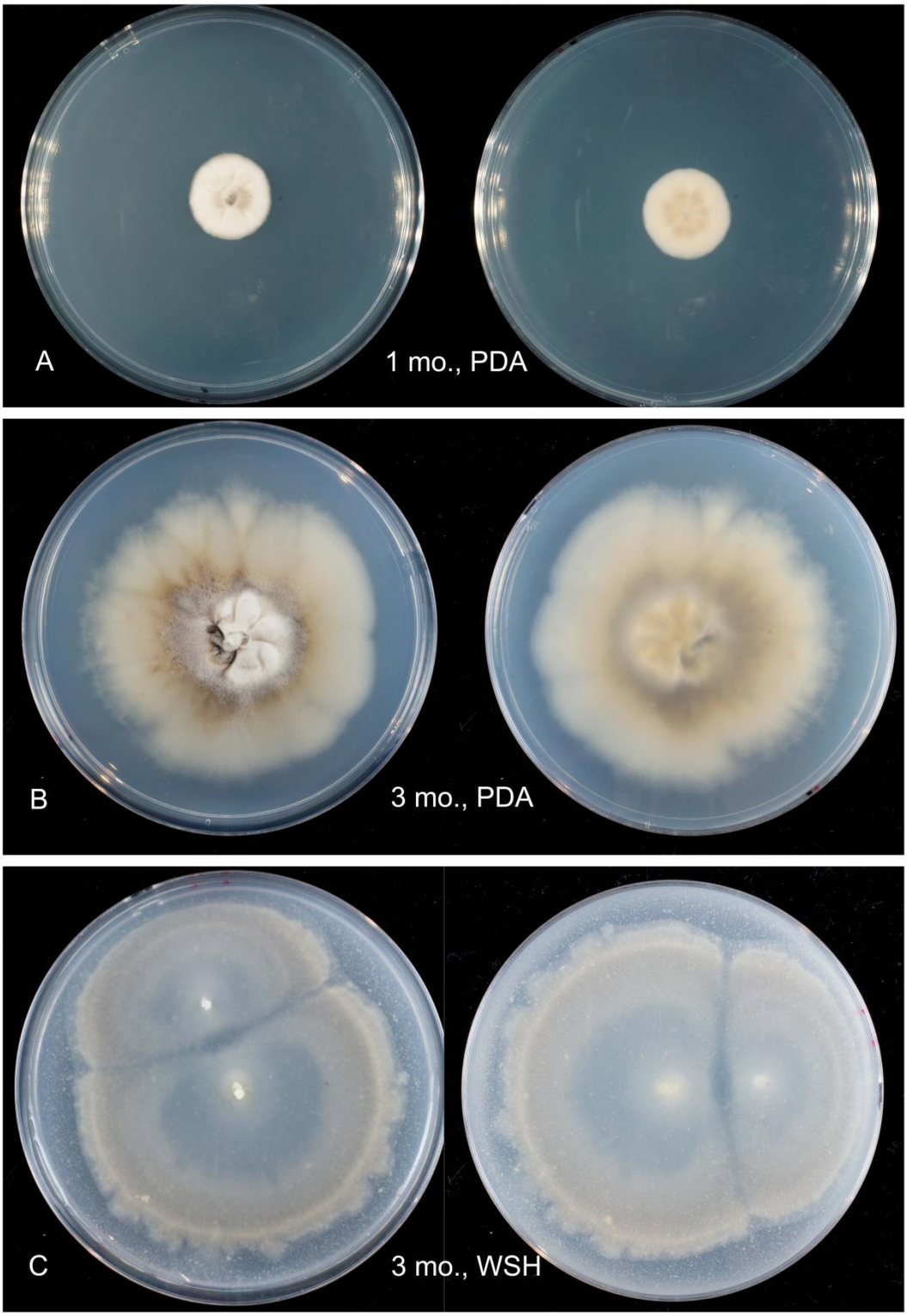

Fig. 5.98. Cultural characters of *Phaeohelotium epiphyllum* (FC-2792, Culture of TNS-F-40042). **A:** Colony on PDA (20℃, 1 mo.). **B:** Colony on PDA (20℃, 3 mo.). **C:** Colony on WSH (20℃, 3 mo.).

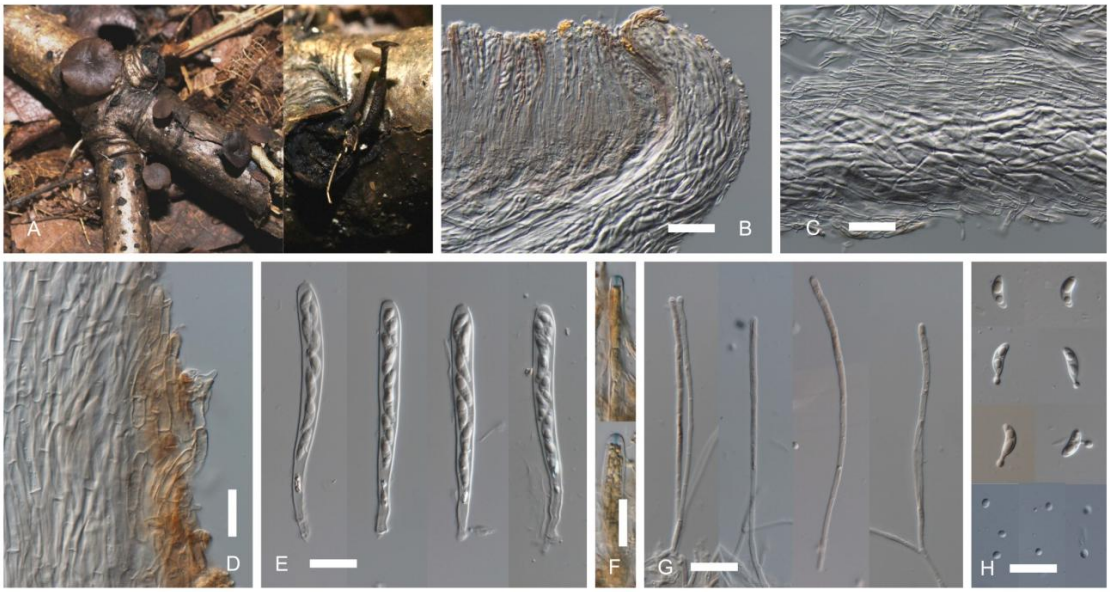

Fig. 5.99. *Poculum* sp. (TNS-F-40048). **A:** Fresh apothecia on branches of unknown plant. **B:** Close up of ectal excipulum at the margin. **C:** Close up of ectal excipulum in the middle flank. **D:** Granulate hyphae from covering layer. **E:** Asci. **F:** Reaction of ascal apex to MLZ. **G:** Paraphyses. **H:** Ascospores and spermatia protruded from ascospore. *Bars* **B–C** 40 μm; **D–H** 20 μm.

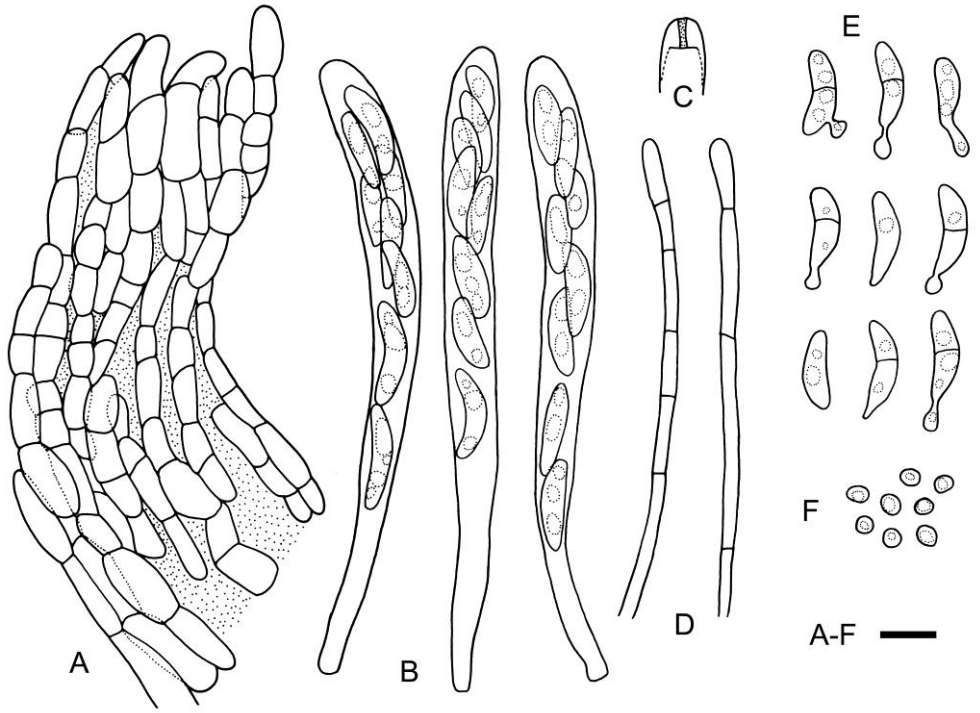

Fig. 5.100. Camera lucida illustration of *Poculum* sp. (TNS-F-40048). **A:** Vertical section of an apothecium through the margin. **B:** Asci. **C:** Reaction of ascal apex to MLZ. **D:** Paraphyses. **E:** Ascospores. **F:** Spermatica. *Bars* **A–F** 10 μm.

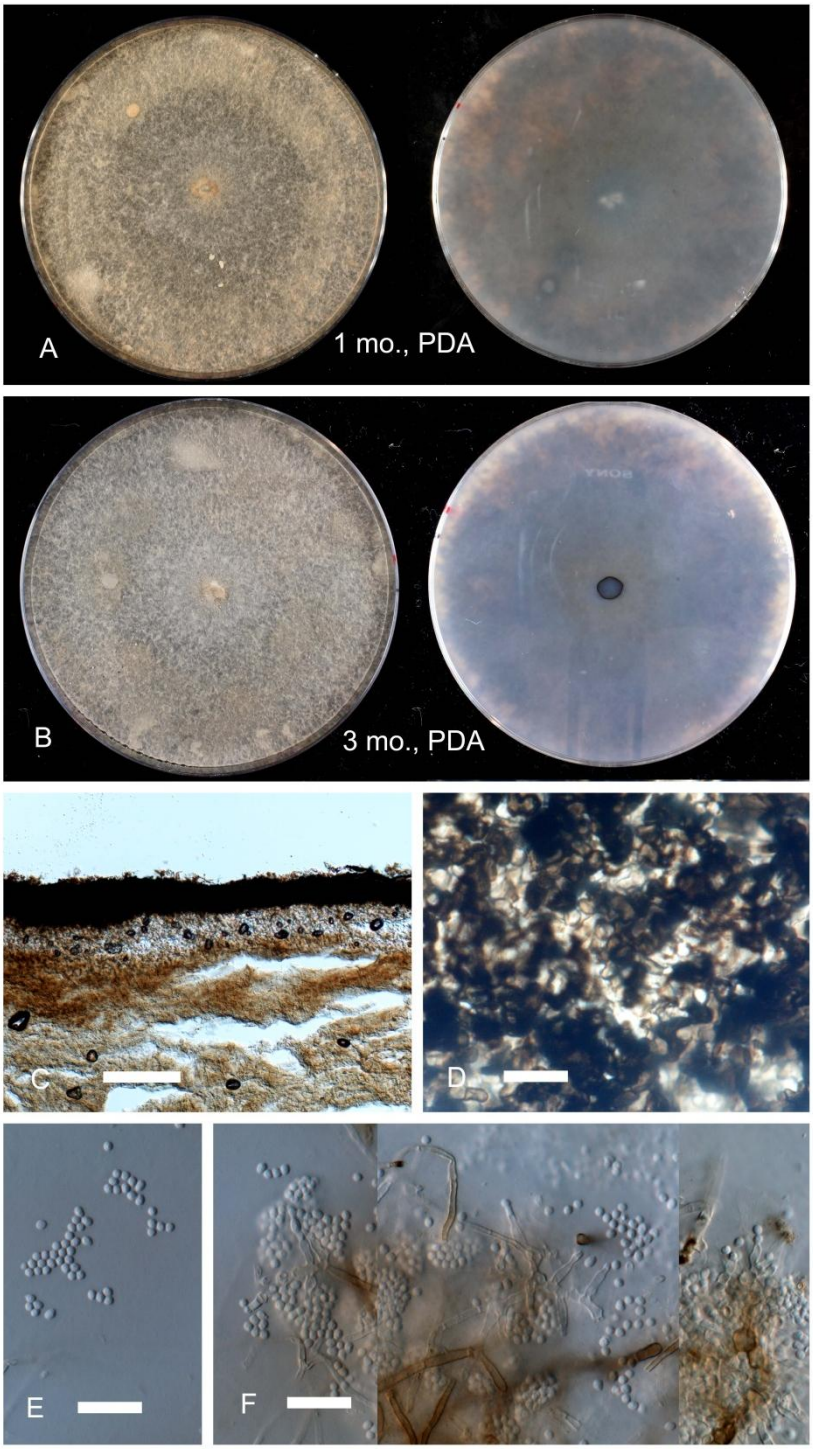

Fig. 5.101. Cultural characters of *Poculum* sp. (FC-2796, Culture of TNS-F-40048). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Vertical view of rind. **D:** Surface view of rind showing the globose to angular cells. **E:** Spermatia. **F** Spermatia and spermatiophores. *Bars* **C** 200 μm; **D–F** 20 μm.

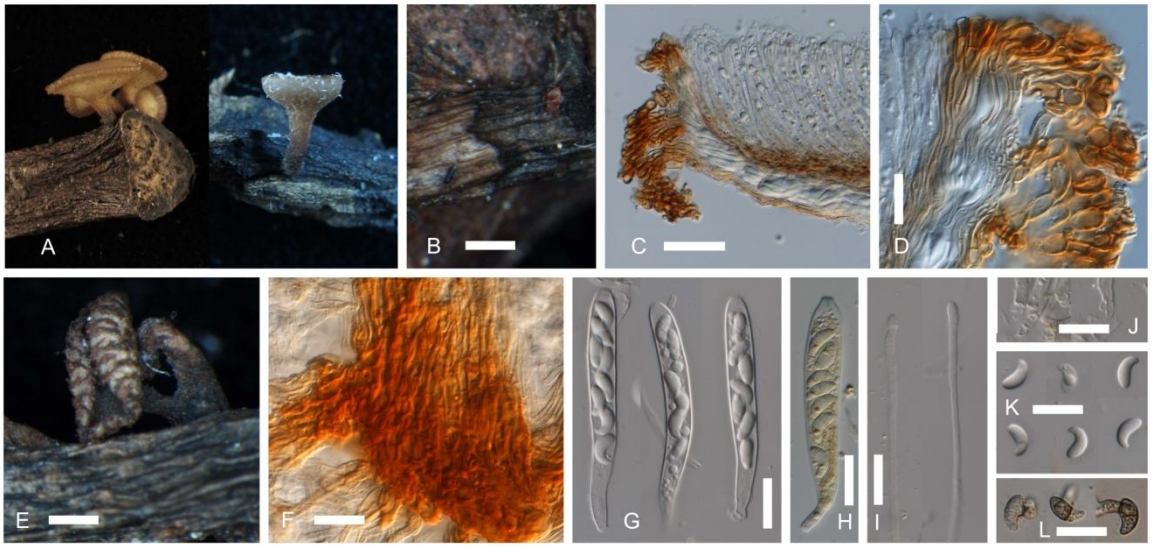

Fig. 5.102. *Poculum sydowianum* (TNS-F-40039). **A:** Fresh apothecia on *Quercus crispula* petioles. **B:** Close up of rind in substrate. **C:** Vertical section of ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Brown striation in receptacle. **F:** Close up of brown striation formed by pigmented hyphae. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Paraphyses. **J.** Croziers in the base of asci. **K.** Hyaline ascospores. **L.** Germinating ascospores. *Bars* **B, E** 1mm; **C** 100 µm; **D, F–L** 20 µm.

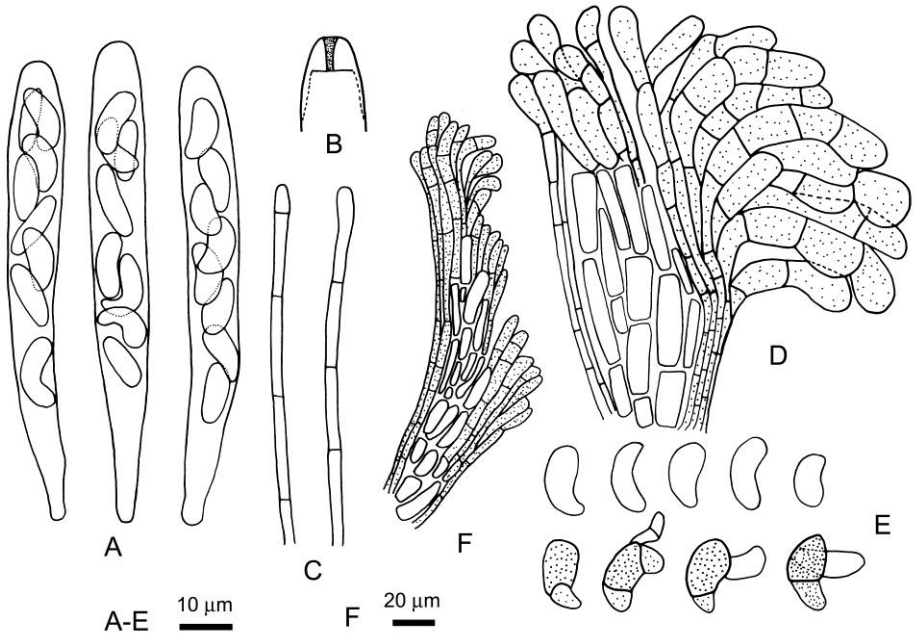

Fig. 5.103. Camera lucida illustration of *Poculum sydowianum* (TNS-F-40039). **A:** Asci. **B:** Reaction of ascal apex to MLZ. **C:** Paraphyses. **D:** Vertical section of an apothecium through the margin showing the ectal excipulum. **E:** Ascospores, the bottom shows the germinated ascospores. F: Vertical section of ectal excipulum. *Bars* **A–E** 10 µm; **F** 20 µm.

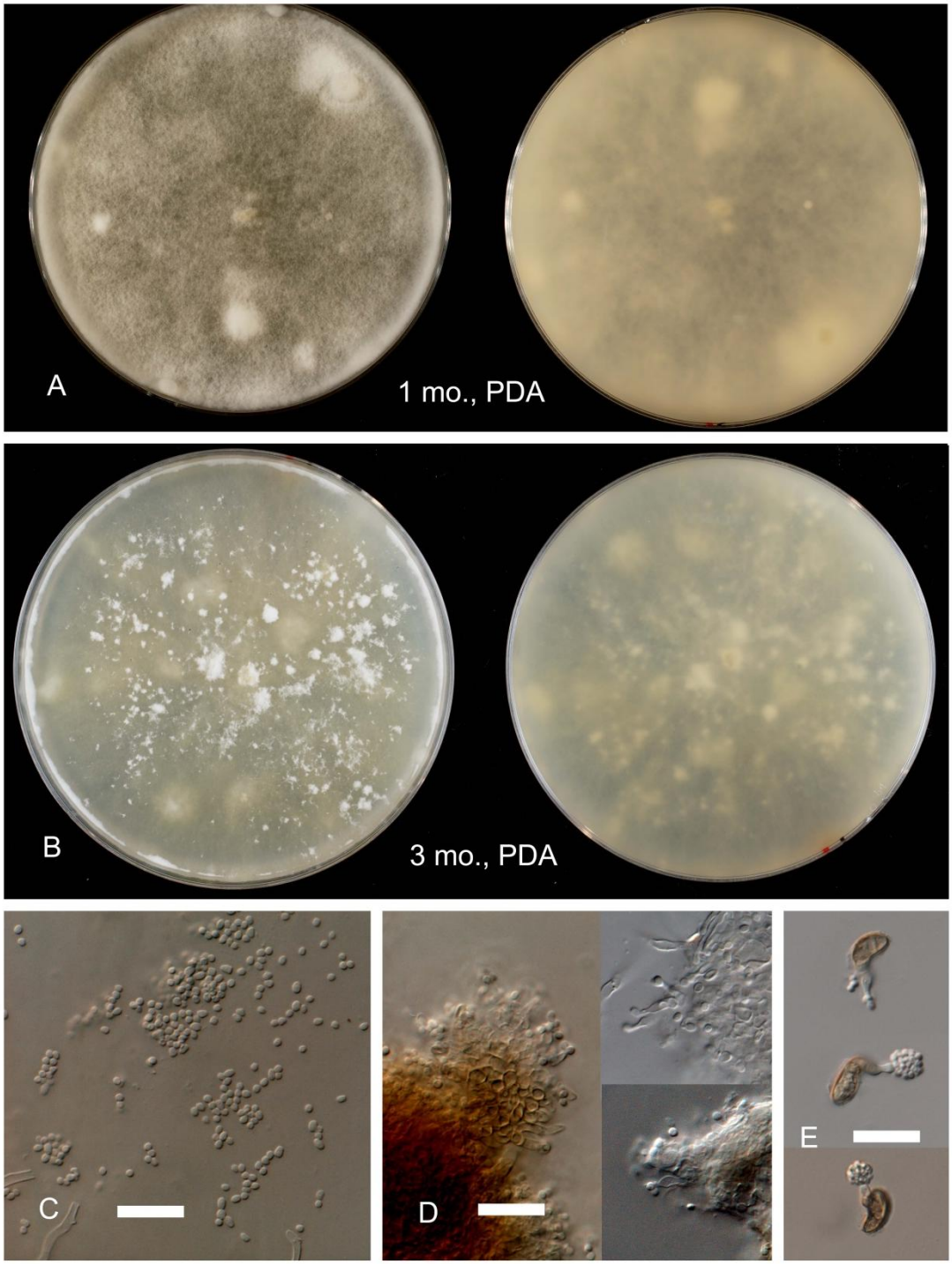

Fig. 5.104. Cultural characters of *Poculum sydowianum* (FC-2813, Culture of TNS-F-40071). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Spermatia produced in 3 mo.' PDA. **D:** Spermatia and spermatiophores. **E:** Spermatia produced in germinated ascospores. *Bars* **C–E** 20μm.

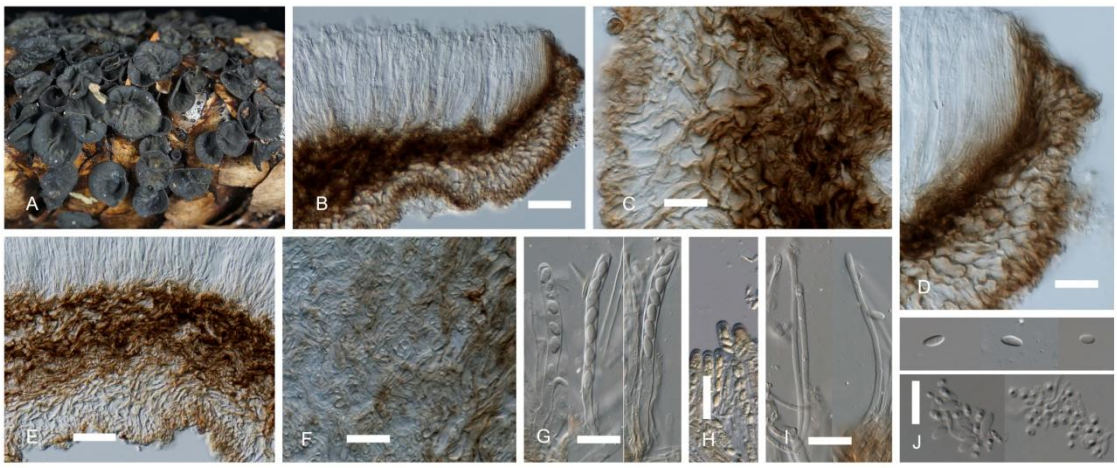

Fig. 5.105. *Rutstroemia bulgarioides* (TNS-F-40005). **A:** Fresh apothecia on decaying fruits of *Picea jegoensis*. **B:** Vertical section of ectal excipulum. **C:** Close up of cells in ectal excipulum. **D:** Close up of ectal excipulum at the margin. **E:** Close up of ectal excipulum in the flank. **F:** Structure in medullary excipulum. **G:** Asci. **H:** Reaction of ascal apex to MLZ. **I:** Paraphyses. **J.** Ascospores and spermatia protruded from ascospores. *Bars* **B, E** 40 μm; **C–D, F–J** 20 μm.

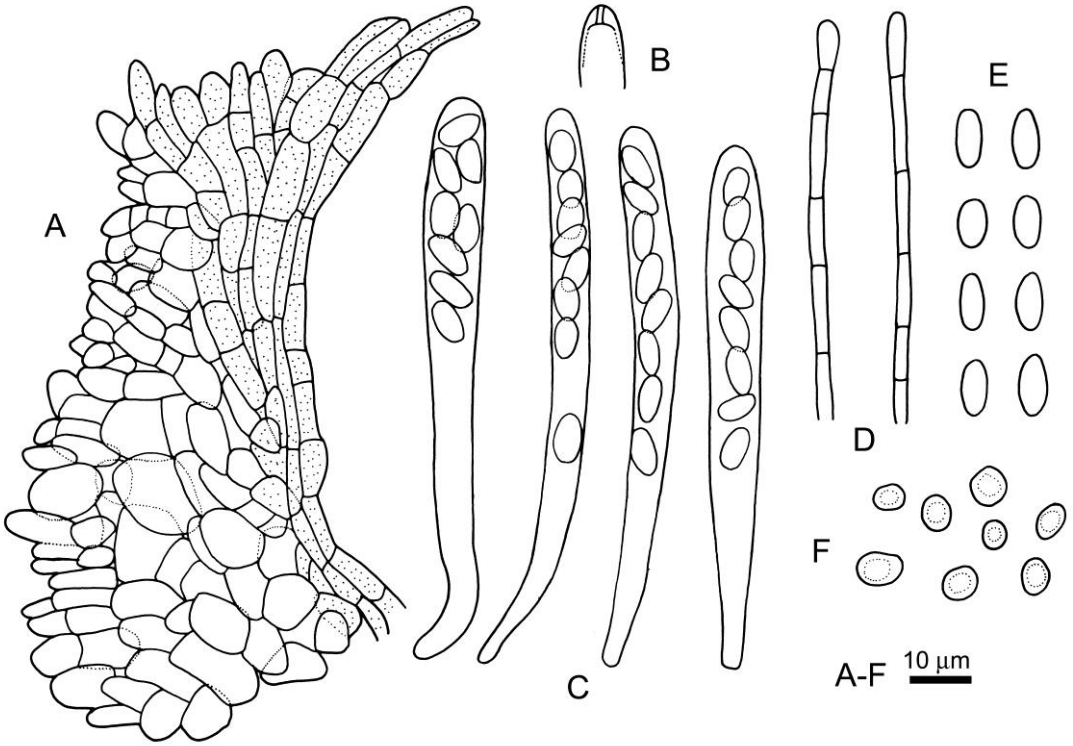

Fig. 5.106. Camera lucida illustration of *Rutstroemia bulgarioides* (TNS-F-40005). **A:** Vertical section of an apothecium through the margin showing the ectal excipulum. **B:** Reaction of ascal apex to MLZ. **C:** Asci. **D:** Paraphyses. **E:** Ascospores. **F:** Spermatia. *Bars* **A–F** 10 μm.

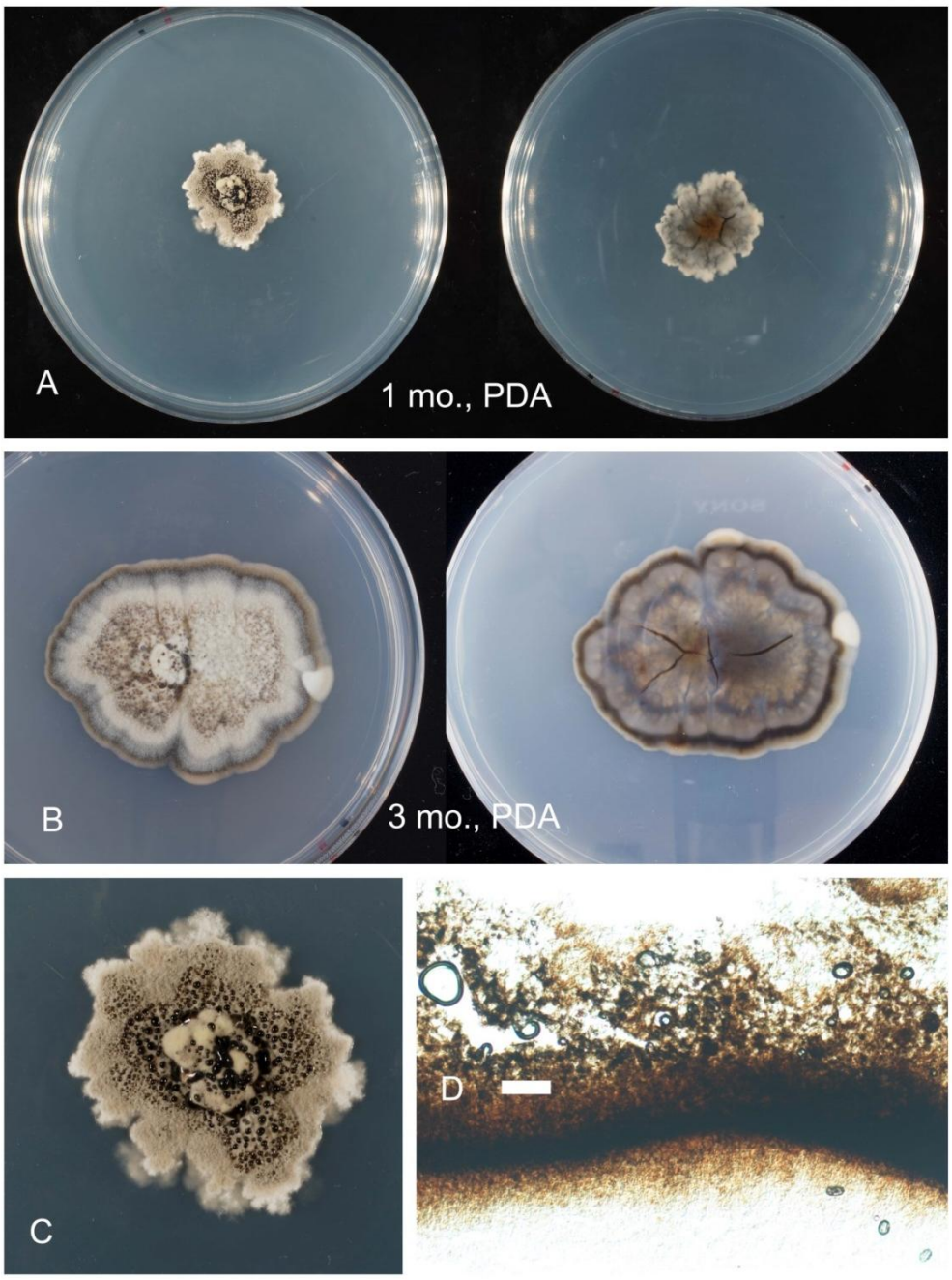

Fig. 5.107. Cultural characters of *Rutstroemia bulgarioides* (FC-2715, Culture of TNS-F-40005). **A:** Colony on PDA (20°C, 1 mo.). **B:** Colony on PDA (20°C, 3 mo.). **C:** Close up of front colony, showing the black exudates. **D:** Vertical view of blackened section from PDA culture. *Bars* **D** 50μm.