

FULL PAPER

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Pleosporales in Japan (5): *Pleomassaria*, *Asteromassaria*, and *Splanchnonema*

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Abstract Eight species in Pleomassariaceae are described and illustrated. They are *Pleomassaria maxima*, *Pl. swidae*, *Pl. siparia* “type A,” *Prosthemium canba*, *Asteromassaria macroconidica*, *A. pulchra*, *Splanchnonema mori*, and *S. argus*. Of these, *Pl. swidae* on twigs of *Swida controversa*, *Pr. canba* on *Betula ermanii*, and *A. macroconidica* on twigs of *Prunus ×yedoensis* and an unknown woody plant are new. Three species, *Pl. siparia* “type A,” *A. pulchra*, and *S. argus*, are reported from Japan for the first time. A new combination, *S. mori* (\equiv *Massaria mori*), is proposed.

Key words Ascomycetes · *Corynespora* · *Myxocyclus* · *Shearia* · Taxonomy

Introduction

The present article is the fifth in a series describing pleosporalean fungi in Japan. Eight species in Pleomassariaceae including three new species and one new combination are reported here.

The family Pleomassariaceae comprises taxa with medium- to large-sized sphaeroid or conoid ascomata, rounded or short papillate beak with a lysigenous ostiole, pseudoparenchymatous ascomal wall, usually with a weft of brown hyphae, narrowly cellular pseudoparaphyses embedded in gel matrix, and oblong to clavate bitunicate asci. The most characteristic feature in this family is found in the ascospores. They are large-sized, reddish- to dark brown, obovoid with bipolar asymmetry or fusoid to oblong with bipolar symmetry, one to several transversely septate or

muriform, and usually surrounded by a conspicuous gelatinous sheath.

Species in the family are considered to be saprophytes on woody substrate, but their hosts are mostly restricted. *Asteromassaria verruculosa* Sivan. is only known as a foliicolous species (Sivanesan 1988), although Barr (1993b) suggested that it belongs to Venturiaceae. Two *Prosthemium* Kunze species that are anamorph-states of *Pleomassaria siparia* (Berk. & Broome) Sacc. complex are reported as endophytes or phelloglyphs (Kowalski and Holdenrieder 1996; Kowalski and Kehr 1992).

There are several opinions about the members in this family. For example, Barr (1993b) recognized five genera in this family, but in the *Dictionary of the Fungi* (Kirk et al. 2001), three genera are listed in Pleomassariaceae. At present, we consider that the following seven genera may have relationships to *Pleomassaria* Speg., the type genus of the family: these are *Asteromassaria* Höhn., *Endotryblidium* Petr., *Kirschsteiniiothelia* D. Hawksw., *Peridiothelia* D. Hawksw., *Pleomassaria*, *Splanchnonema* Corda, and *Splanchospora* Lar. N. Vassiljeva. Among these, *Pleomassaria*, *Asteromassaria*, and *Splanchnonema* are major groups in terms of species number, and they are treated here.

Pleomassaria, lectotypified by *P. siparia*, is characterized by large-sized (mostly more than $35 \times 11 \mu\text{m}$), ellipsoid to obovoid ascospores with both transverse and longitudinal septa (Barr 1982; Shoemaker and LeClair 1975). Barr (1993b) treated this genus as a synonym of *Splanchnonema* based on the similarities in most respects except for the ascospore septation. In this article, however, we consider that the differences in ascospores are enough to separate them as a natural group respectively. The ascospores of *Pleomassaria* species are dictyosporous and more nearly symmetrical than those in *Splanchnonema*. In *Splanchnonema*, by contrast, those are phragmosporous and mostly have a strongly submedian primary septum. Barr (1982, 1990) recognized seven North American species and one European species in *Pleomassaria*. Furthermore, some species previously described in *Splanchnonema*, such as *S. arbuti* M.E. Barr, *S. vaccinii* M.E. Barr (Barr 1993a), *S. noliae* A.W. Ramaley & M.E. Barr (Ramaley and Barr

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1995), and *S. dasyllirionis* A.W. Ramaley (Ramaley 1995), are obviously related to *Pleomassaria* and must be transferred to this genus.

Asteromassarina, typified by *A. macrospora* (Desm.) Höhn., is composed of 11 species (Barr 1982, 1993b; Boise 1985; Mehrotra and Sivanesan 1989; Sivanesan 1988). The ascospores of *Asteromassarina* are somewhat similar to those of *Splanchnonema*. In the former genus, those are symmetrically septate in both hemispheres and with nearly median primary septum, whereas in the latter those are mostly asymmetrically septate and/or with more submedian primary septum.

Splanchnonema was established by Corda taking *S. pustulatum* Corda [now a synonym of *S. foedans* (Fr.) Kuntze] as the type species (Barr 1982; Shoemaker and LeClair 1975). Twelve species are described as *Splanchnonema* by Barr (1982), and later more than 10 species are additionally reported (Aptroot 1998; Barr 1993a,b; Hsieh et al. 1997; Leroy et al. 2000; Ramaley 1995; Ramaley and Barr 1995; Subramanian and Sekar 1987). These species, however, probably include *Pleomassaria* or *Splanchospora* elements, and rearrangements are required. *Splanchnonema* can be distinguished from *Splanchospora* by its phragmospores, rather than didymospores with a strongly submedian primary septum.

Barr (1982) stated that if information about the anamorphs is known for more of the taxa, this will clarify relationships among the species in the family. However, such knowledge is probably still insufficient. *Shearia* Petr. (Barr 1982; Sutton 1980) and *Prosthemium* (Hantula et al. 1998; Paavolainen et al. 2000) are reported as anamorphs of *Pleomassaria* species. In *Asteromassarina*, those are *Scolicosporium* Lib. ex Roum. (Sivanesan 1984; Spooner and Kirk 1982) and *Macrodiplodiopsis* Petr.-like (Barr 1993b). *Myxocyclus* Riess, *Stegosporium* Corda (Sivanesan 1984), *Macrodiplodiopsis* (Glawe 1985), and *Helminthosporium* Link (Subramanian and Sekar 1987) are known for anamorphs of *Splanchnonema*. The anamorphs in this family are generally coelomycetous, having holoblastic large conidia with somewhat complex morphology. However, there are also species having hyphomycetous anamorphs with tretic conidia, such as *Splanchnonema kalakadense* Subram. & Sekar (Anam.: *Helminthosporium*) and *Kirschsteiniothelia aethiops* (Berk. & M.A. Curtis) D. Hawksw. (Anam.: *Dendryphiopsis* S. Hughes) (Subramanian and Sekar 1987).

The floristic work of Pleomassariaceae in Japan is only poorly known. *Shearia fusa* (Berk. & M.A. Curtis) M.E. Barr [an anamorphic state of *Pleomassaria maxima* Ellis & Everh. (Tubaki et al. 1983)], *Splanchnonema phoricoides* (I. Miyake) Leroy, Gauthier & M.E. Barr (Kimura 1979; Miyake 1916), and *Kirschsteiniothelia elasterascus* Shearer (Tsui et al. 2003) have been reported to date. *Massaria moricola* I. Miyake (Miyake 1916) most probably is a species of *Asteromassarina*, and it is close to *A. olivaceohirta* (Schwein.) M.E. Barr (Barr 1982; Shoemaker et al. 2003) on *Morus* spp. Besides, there are some mentions of *Pleomassaria* in the list of Hara (1954), but their occurrences in Japan are doubtful.

The methods of microscopic observation, single spore isolation and induction of anamorph/teleomorph formation in culture are the same as those described in Tanaka and Harada (2003). Specimens cited in this article were mainly kept at the Herbarium of Hirosaki University, Fungi (HHUF), and some isotype or paratype materials were preserved at TNS-F. All culture strains were deposited at the culture collections of MAFF and JCM.

Taxonomy

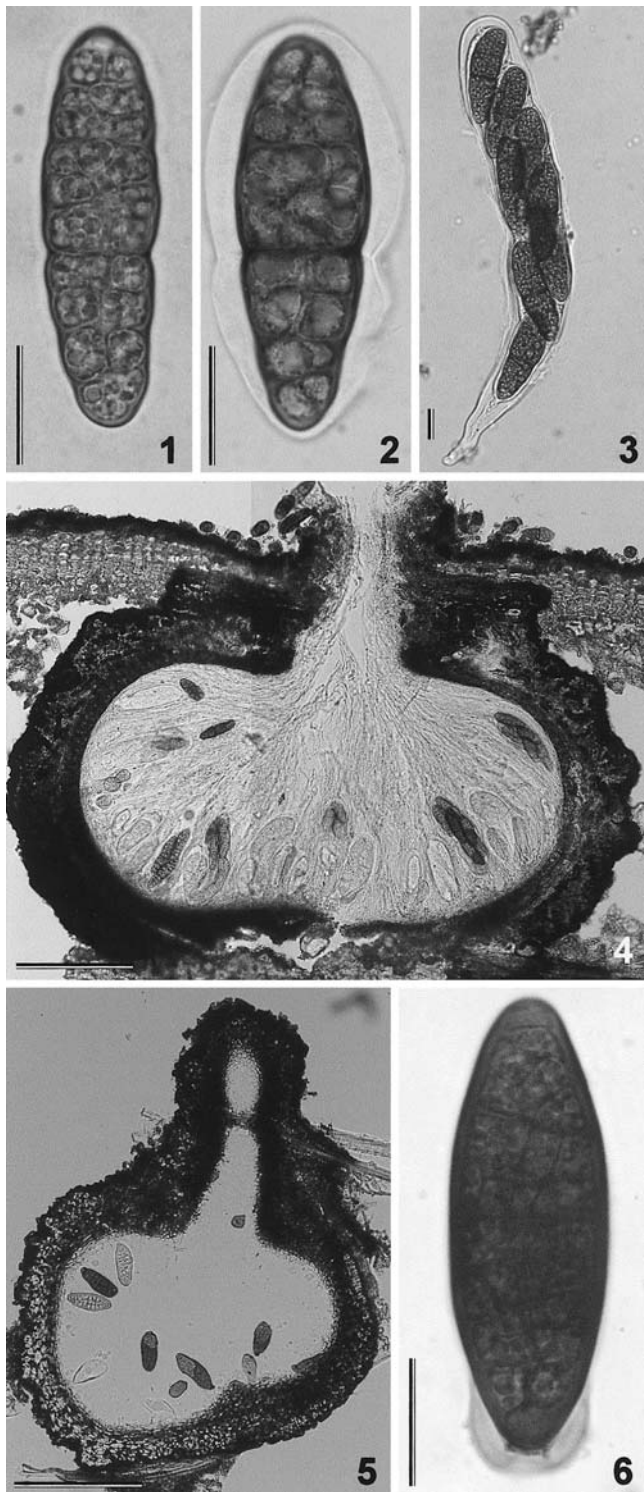
1. *Pleomassaria maxima* Ellis & Everh., Bull. Torrey Bot. Club. 25: 505, 1898.

Anamorph: *Shearia fusa* (Berk. & M.A. Curtis) M.E. Barr, Mycotaxon 15: 373, 1982. Figs. 1–6, 74

Ascomata 550–850 µm high, 650–1150 µm in diameter, immersed, globose, with a papillate beak 150–250 µm long. Ascomal wall composed of 2 zones; outer zone 42–100 µm thick, composed of globose to rectangular cells 5–20 µm in diameter; inner zone 22–33 µm thick, composed of 5–7 layers of prismatic cells of 10–20 × 3.5–6.5 µm. Pseudoparaphyse 1–2 µm thick, septate, branched and anastomosed, with slime coating. Asci (185.5–)207.5–283(–308) × 41.5–51(–55) µm (mean = 253.0 × 47.6 µm, *n* = 28), fissitunicate, clavate, 8-spored. Ascospores (51–)59.5–75(–83) × (18–)19.5–25 µm (mean = 66.5 × 22.3 µm, *n* = 80), L/W 2.6–3.5 (mean = 3.0, *n* = 80), fusiform to cylindrical, muriform, 7–11-transversely septate, with a primary septum submedian (0.52–0.57; mean = 0.54, *n* = 80), pale brown to dark brown, smooth, with a sheath 2–7 µm thick.

Cultural characteristics: Colonies on potato dextrose agar (PDA; Difco, Detroit, MI, USA) 39–43 mm in diameter after 4 weeks at 20°C in the dark, Orange Grey (5B2; Kornerup and Wanscher 1978) with Black to Grey (1C1) dots of conidiomata, with White (1A1) irregular margin; reverse Greyish Orange (5B5) to Orange White (5A2); no pigment produced. On rice straw agar (RSA; Tanaka and Harada 2003), the *Shearia fusa*-state formed within 2 months. Conidiomata 500–750 µm high, 500–620 µm in diameter, subglobose. Beak central, 220–380 µm long. Wall 65–115 µm thick at sides, composed of 2 zones; outer zone of angular cells 5–20 µm in diameter, inner zone composed of 3–4 layers of rectangular cells of 12–23 × 5–8 µm. Conidiogenous cells holoblastic, annellidic, 5–30 × 4–8.5 µm, cylindrical to doliform. Conidia 67–98 × 22–27(–30.5) µm (mean = 81.5 × 25.2 µm, *n* = 50), L/W 2.9–3.6 (mean = 3.2, *n* = 50), fusiform, 11–17-septate, truncate (5–6.5 µm wide) at the base, with a basal sheath.

Specimens examined: On twigs of *Magnolia praecocissima* Koidz. var. *borealis* (Sarg.) Koidz.: Campus of Hokkaido University, Sapporo, Hokkaido, 41°20.5' E, 43°04.0' N, Apr. 19, 2000, KT. 104 (HHUF 28525); 171 (HHUF 28526); Apr. 20, 2000, KT. 172 (HHUF 28527); Apr. 24, 2000, KT. 110 (HHUF 28528); May 1, 2000, KT. 138–140 (HHUF 28529–28531); May 11, 2000, KT. 175 (HHUF 28532); May 26, 2000, KT. 216 (HHUF 28533); May



Figs. 1–6. *Pleomassaria maxima*. **1, 2** Ascospores. **3** Ascus. **4** Ascoma in longitudinal section. **5** Conidioma in longitudinal section. **6** Conidium. (**1–4** from HHUF 28546; **5, 6** from culture MAFF 239510 = JCM 12842). Bars **1–3, 6** 20 μm ; **4, 5** 200 μm

31, 2000, KT. 237 (HHUF 28534); June 12, 2000, KT. 282 (HHUF 28535); Sept. 2, 2001, KT. 744 (HHUF 28536). On twigs of *Magnolia praecocissima* Koidz. var. *praecocissima*: Campus of Tsukuba University, Tennodai, Tsukuba, Ibaraki, 140°06.2'E, 36°06.3'N, Mar. 1, 2002, YH. 845

(HHUF 28537); Campus of Hirosaki University, Hirosaki, Aomori, 140°28'E, 40°35'N, Apr. 25, 2003, SH. 1064–1070 (HHUF 28538–28544); Apr. 26, 2004, KT. 1670–1672 (HHUF 28546–28548); Yagami, Towada, Aomori, 141°08'E, 40°35'N, July 17, 2003, SH. 1282 (HHUF 28545). On twigs of *Magnolia* sp.: Nagasaki Glover Garden, Minamiyamanote, Nagasaki, 129°52.2'E, 32°43.5'N, May 30, 2004, KT. and SH. 1722 (HHUF 28549). On twigs of *Magnolia heptapeta* (Buc'hoz) Dandy: Hokkaido University Botanical Garden, Sapporo, Hokkaido, 141°20.4'E, 43°03.4'N, June 6, 2004, KT. 1723 (HHUF 28550). Single ascospore culture: isolated from HHUF 28546 (MAFF 239510 = JCM 12842).

Notes: The fungus on *Magnolia* spp. appears to be common in eastern North America (Barr 1982) and it is also reported from China (Teng 1934). In Japan, the anamorphic state of this fungus has been recorded as *Shearia formosa* (Ellis & Everh.) Petr. (Tubaki et al. 1983), but the teleomorph state was first recognized only recently in Japan.

2. *Pleomassaria swidae* Kaz. Tanaka, Y. Harada & M.E. Barr, sp. nov.

Anamorph: *Corynespora* sp.

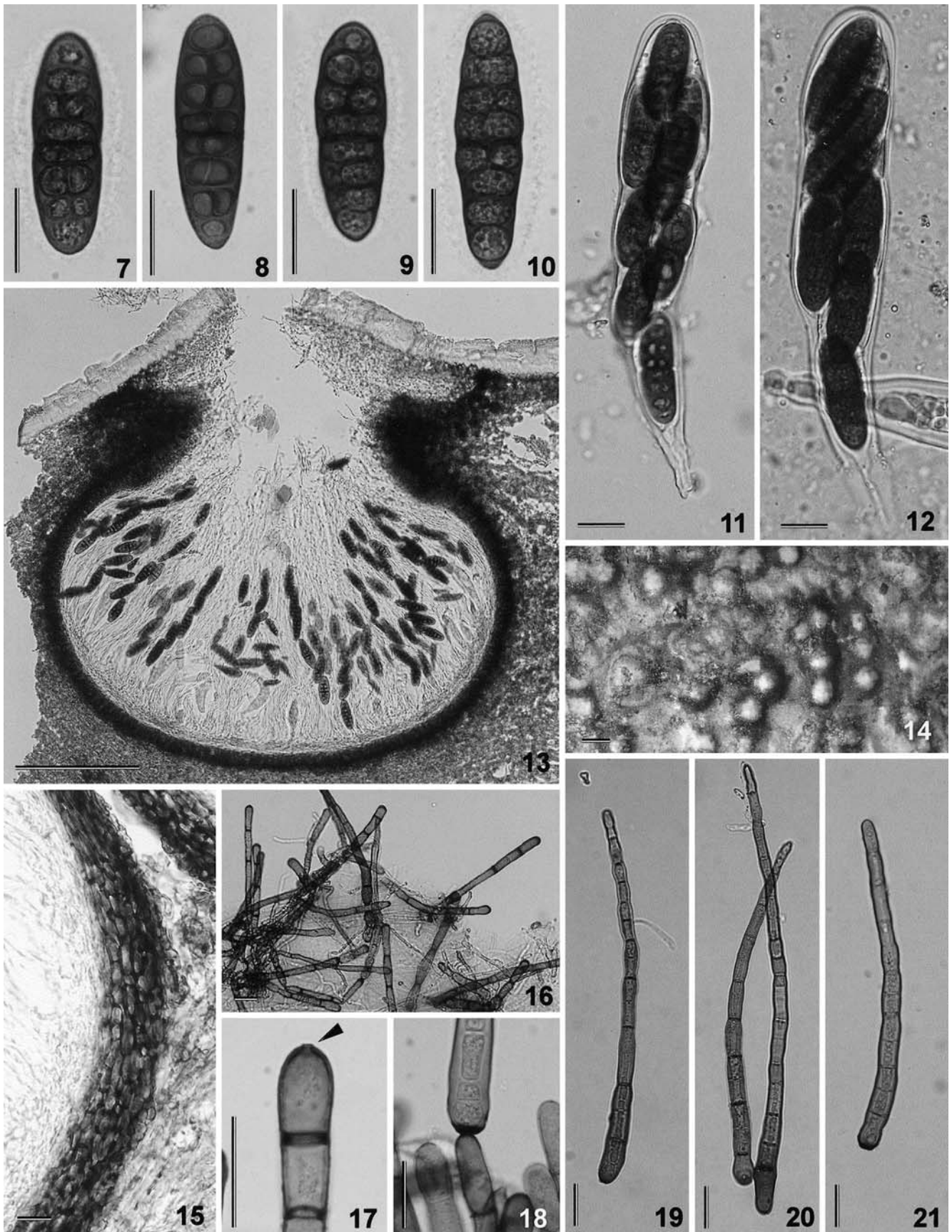
Figs. 7–21, 75

Ascomata 500–730 μm alta, 400–950 μm diametro, sparsa vel gregaria, immersa, globosa vel depresso-globosa. Rostrum 120–200 μm longum, centrale. Paries ascomatis 20–30 μm crassus, ex cellulis polygonalibus 6–10-stratosis compositus. Pseudoparaphyses 2–4 μm latae, numerosae, septatae, ramosae, mucosae circumcinctae. Asci 195–244(–285) \times (35–)37–42.5(–47.5) μm , fissitunicati, clavati, apice rotundati et loculo vadoso praediti, breviter stipitati, octospori in superiore bi- vel inferiore uni-seriebus. Ascosporae 48–60(–66) \times 14.5–19 μm , fusiformes vel ellipsoideae, transversaliter 7–8-septatae et longitudinaliter 1–2-septatae, leviter constrictae ad septum primum submedianum, non constrictae ad septa alia, fuscidulae, echinulatae vel verruculosae, strato mucoso 2–7 μm lato circumcinctae.

Holotypus: HHUF 28520.

Etymology: In reference to the host genus name *Swida*.

Ascomata 500–730 μm high, 400–950 μm in diameter, scattered to crowded, immersed, globose to depressed globose. Beak 120–200 μm long, central, with black clypeus-like structure, filled with pseudoparaphysis tips. Ascomal wall uniformly 20–30 μm thick, composed of 6–10 layers of brown polygonal cells of 3.5–15 \times 2–6.5 μm . Pseudoparaphyses 2–4 μm thick, numerous, septate, branched, with slime coating. Asci 195–244(–285) \times (35–)37–42.5(–47.5) μm (mean = 222.3 \times 39.2 μm , n = 60), fissitunicate, basal and lateral, clavate, rounded at the apex, with shallow apical chamber, short-stalked (20–52 μm long; mean = 35.5 μm , n = 28), with 8 ascospores biserial above uniseriate below. Ascospores 48–60(–66) \times 14.5–19 μm (mean = 54.1 \times 16.5 μm , n = 100), L/W 2.9–3.7 (mean = 3.3, n = 100), fusiform to ellipsoidal, rounded at the apex, slightly tapered at the base, mostly straight, with 7–8 transverse septa (septa of upper hemisphere + the primary septum + septa of lower



Figs. 7–21. *Pleomassaria swidae*. 7–10 Ascospores. 11, 12 Asci. 13 Ascoma in longitudinal section. 14 Ascomata on host. 15 Ascomal wall. 16 Conidiophores. 17 Conidiophore apex with a conidiogenous pore (arrowhead). 18 Conidium with conidiophore. 19–21 Conidia. (7, 8, 11, 13, 15 from HHUF 28520; 9, 10, 12, 14 from HHUF 28521; 16, 19, 20 from culture MAFF 239507 = JCM 12839; 17, 18, 21 from culture MAFF 239508 = JCM 12840). Bars 7–12, 15–21 20µm; 13 200µm; 14 500µm

hemisphere = 3 + 1 + 3, 4 + 1 + 3) and 1–2 longitudinal septa, with a primary septum submedian (0.50–0.56; mean = 0.52, $n = 100$) and slightly constricted, not constricted at other septa, dark brown, echinulate to verrucose, with a conspicuous sheath 2–7 μm thick (later expanding up to 50 μm thick).

Cultural characteristics: The colony color and growth rate are variable depending on the isolate. In MAFF 239507 (=JCM 12839), colonies on PDA 78 mm in diameter after 4 weeks at 20°C in the dark, Olive (2E3) with entire White (1A1) margin; reverse similar; no pigment produced. In MAFF 239508 (=JCM12840), colonies attaining 58 mm in same condition, Bluish Grey (22C2) with White (1A1) to Yellowish White (3A2) irregular margin; reverse Olive Brown (4D3); slightly yellowish pigment produced. On RSA, a *Corynespora* anamorph formed within 2 months. Conidiophores 85–280(–400) \times 7–10 μm , macronematous, mononematous, straight, septate, brown, smooth. Conidiogenous cells monotretic, terminal, with a single apical conidiogenous pore. Conidia 80–210(–255) \times 9–15 μm (mean = 142.6 \times 12.1 μm , $n = 51$), obclavate to rostrate, straight or slightly curved, with a thick-walled truncate base 3–7.5 μm wide, 8–20-distoseptate, pale brown, smooth.

Specimens examined: On twigs of *Swida controversa* (Hemsl.) Soják: Iwate University, Ueda, Morioka, Iwate, 141°08'E, 39°42'N, Feb. 18, 2003, YH. 1018 (HHUF 28520 holotype; TNS-F 11170 isotype); Kahaku Botanical Garden, Amakubo, Tsukuba, Ibaraki, 140°06.5'E, 36°05.5'N, Feb. 27, 2003, YH. 1019a (HHUF 28521); 1019b (TNS-F 11171); 1020 (HHUF 28522). Single ascospore cultures: isolated from HHUF 28520 (MAFF 239507 = JCM 12839); isolated from HHUF 28521 (MAFF 239508 = JCM 12840). Dried culture specimens of anamorph (grown on culms of *Oryza sativa* L.): made from culture MAFF 239507 (HHUF 28666–28668).

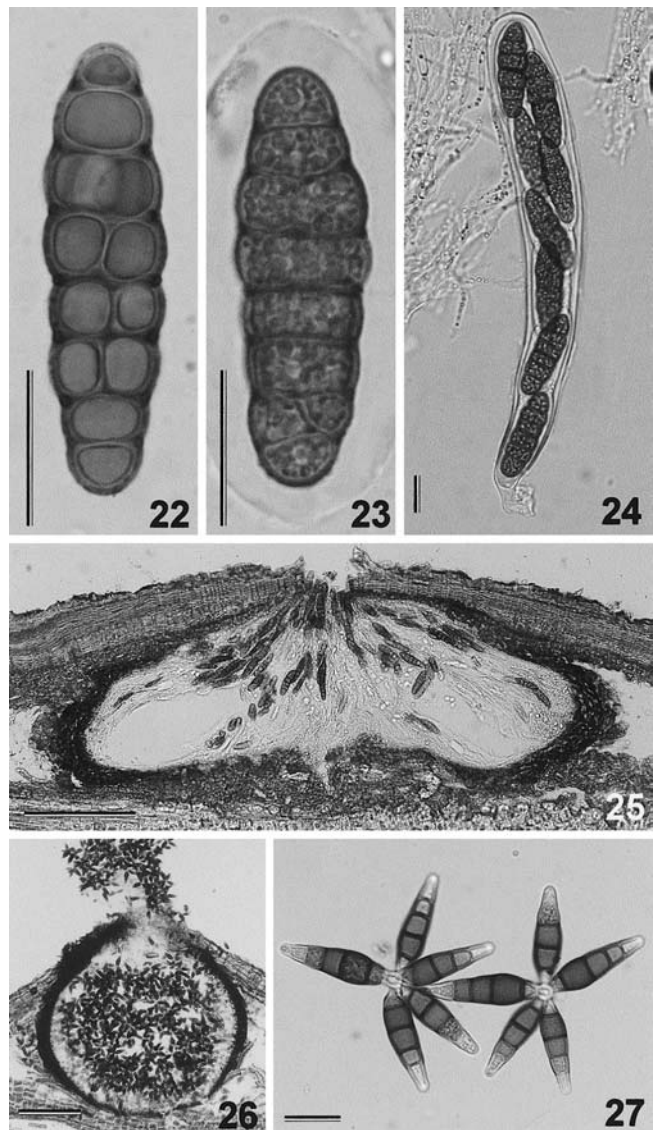
Notes: The ascospores of *P. swidae* are very similar to those of *P. siparia* in the number of septa and the dimensions. In the latter species, however, those are constricted at all transverse septa and have more yellowish pigmentation. Furthermore, the conidial state of *P. siparia* is *Prosthemium* of coleomycetous fungus.

Corynesporasca Sivan. also has a *Corynespora* anamorph (Sivanesan 1996). This genus is characterized by the cleistothecioid ascomata, the obovoid asci, and the oblong 3-septate ascospores mostly with a supramedian primary septum, and is placed in Corynesporascaceae, Pleosporales (Kirk et al. 2001; Sivanesan 1996). The pseudoparaphyses of the genus are described as trabecular. However, those appear to be cellular, and the genus can probably be close to the Pleosporaceae.

3. *Pleomassaria siparia* (Berk. & Broome) Sacc., Syll. Fung. 2: 239, 1883, “**type A**” sensu Paavolainen et al. (2000).

Anamorph: *Prosthemium asterosporum* T. Kowalski & Holdenr., Mycol. Res. 100: 1243, 1996. Figs. 22–27, 76

Ascomata 300–500 μm high, 1000–1200 μm in diameter, immersed, subglobose, with a central ostiole. Ascomal wall 40–50 μm thick at sides, composed of pale brown polygonal



Figs. 22–27. *Pleomassaria siparia* “type A.” **22, 23** Ascospores. **24** Ascus. **25** Ascoma in longitudinal section. **26** Conidioma in longitudinal section. **27** Conidia. (**22–25** from HHUF 28524; **26, 27** from culture MAFF 239509 = JCM 12841). Bars **22–24, 27** 20 μm ; **25, 26** 200 μm

cells of 10–30 \times 3–10 μm . Pseudoparaphyses 1.5–2 μm thick. Asci 242.5–305 \times 32.5–37.5 μm , clavate, 8-spored. Ascospores (46–)51.5–61(–63) \times 13–18 μm (mean = 56.2 \times 15.9 μm , $n = 50$), L/W 3.1–3.9 (mean = 3.5, $n = 50$), fusiform, muriform, (5–)7-transversely septate, with a primary septum mostly submedian (0.49–0.54; mean = 0.52, $n = 39$), constricted at each septum, yellow to brown, finely echinulate, with a mucilaginous sheath 5–8 μm thick.

Cultural characteristics: Colonies on PDA 56 mm in diameter after 4 weeks at 20°C in the dark, Olive (3F3) with White (1A1) irregular margin; reverse White (1A1) with numerous black dots of conidiomata; no pigment produced. On RSA, the *Prosthemium asterosporum*-state formed within 2 months. Conidiomata 350–550 μm high, 400–750 μm in diameter, globose to depressed globose. Wall

25–50 µm thick. Conidia staurosporous, (66–)73–99 µm in diameter (mean = 85.4 µm, $n = 49$), with 4–5 (up to 10) arms equally developed; arms (35–)37–50.5 × 9.5–14 µm (mean = 43.2 × 11.8 µm, $n = 50$), L/W 3.1–4.2 (mean = 3.7, $n = 50$), 4–5-septate, brown at middle three to four cells, hyaline to pale brown at both end cells, adjoined at the central cell of 6–8 µm diameter.

Specimens examined: On dead twigs of *Betula verrucosa* Ehrh.: Tomita, Hirosaki, Aomori, 140°28.3'E, 40°35.3'N, Apr. 11, 2004, KT. and YO. 1665 (HHUF 28523); 1669 (HHUF 28524). Single ascospore culture: isolated from HHUF 28524 (MAFF 239509 = JCM 12841).

Notes: *Pleomassaria siparia*, a common fungus on birch, has been described many times (Barr 1982; Dennis 1978; Ellis and Ellis 1997; Eriksson 1981; Shoemaker and LeClair 1975; Sivanesan 1984). The anamorph has been known as *Prosthemium betulinum* Kunze (Hantula et al. 1998; Tonolo 1956). Recently, however, another fungus, *Pr. asterosporum*, with similar conidia was described from birch (Kowalski and Holdenrieder 1996). Furthermore, it was revealed that *Pl. siparia* is composed of two species, which are distinguishable by the anamorphs but not by teleomorph morphology (Paavolainen et al. 2000). Our collections of *Pl. siparia* producing *Pr. asterosporum* anamorph are certainly similar to the teleomorph of *Pr. betulinum* reported by Hantula et al. (1998), and we could not find differences between the two ascomal states. Paavolainen et al. (2000) proposed referring these two teleomorphs as *Pl. siparia* “type A” (anam.: *Pr. asterosporum*) and “type B” (anam.: *Pr. betulinum*), and we followed their opinion.

4. *Prosthemium canba* Kaz. Tanaka, Y. Harada & M.E. Barr, sp. nov.

Teleomorph: unknown.

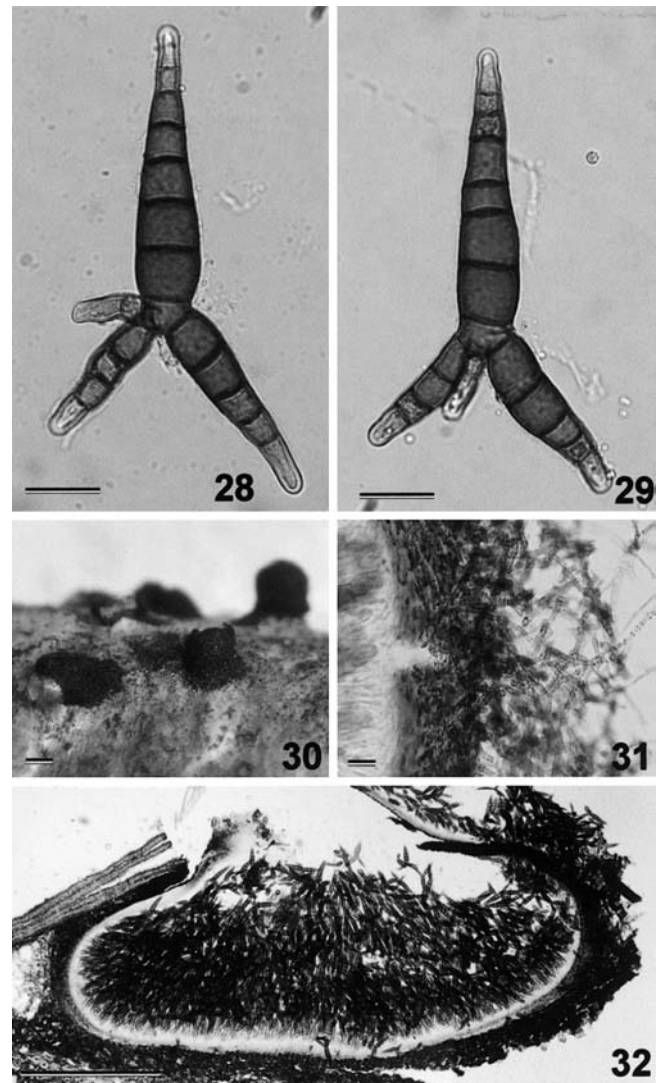
Figs. 28–32, 77

Pycnidia 450–880 µm alta, 750–2100 µm diametro, unilocularia, immersa, rostro erumpentia, sparsa vel gregaria, depresso-globosa vel globosa. Rostrum 300–500 µm longum, rectum, centrale. Paries pycnidii ad latus 30–75 µm crassus ex cellulis rectangularibus parallele 8–12-stratosis 7–25 × 2–3 µm et ad basim 10–18 µm crassus ex cellulis polygonalibus 2.5–13 × 2–5 µm compositus. Conidiophora 2–4 µm lata, ad 150 µm longa, simplicia, septata, hyalina. Cellulae conidiogenae holoblasticae. Conidia stellata, ex 3–4 brachiis obclavatis composita, echinulata, brunnea, ad cellulam apicales pallidiora; brachium primigenum (63–)66.5–81(–88.5) × (14–)15–19(–20) µm; brachium secundum 35–51.5(–55) × (10–)12–16(–18.5) µm; brachium tertium 23–44 × 6–12 µm; brachium quartum 15–35 × 5.5–10 µm.

Holotypus: HHUF 27340.

Etymology: From the Japanese common name “Kanba (Canba)” meaning birch, in reference to the host genus.

Conidiomata 450–880 µm high, 750–2100 µm in diameter, unilocular, immersed, erumpent at the beak, scattered to crowded, depressed globose to globose, with numerous brown hyphae at sides. Beak 300–500 µm long, terete, central. Wall at sides 30–75 µm thick, composed of 8–12 parallel



Figs. 28–32. *Prosthemium canba*. 28, 29 Conidia. 30 Conidiomata on host. 31 Conidiomatal wall. 32 Conidioma in longitudinal section. (All from HHUF 27340). Bars 28, 29, 31 20 µm; 30, 32 500 µm

rows of rectangular cells of 7–25 × 2–3 µm; at the base 10–18 µm thick, composed of polygonal cells of 2.5–13 × 2–5 µm. Conidiophores up to 150 µm long, 2–4 µm thick, unbranched, septate, hyaline, smooth. Conidiogenous cells holoblastic. Conidia staurosporous, echinulate, brown, pale colored at the top cell, composed of obclavate 3 to 4 arms unequally developed, (88.5–)97–124 × (40.5–)51.5–75.5(–85) µm (mean = 107.8 × 64.8 µm, $n = 30$; between the longest points × widest points); largest arms (63–)66.5–81(–88.5) × (14–)15–19(–20) µm (mean = 73.2 × 16.9 µm, $n = 50$), L/W 3.6–5.0 (mean = 4.4, $n = 50$), (5–)6–7-septate, with a connecting cell of 7–11 × 11.5–14 µm; second largest arms 35–51.5(–55) × (10–)12–16(–18.5) µm (mean = 45.5 × 13.7 µm, $n = 30$), (2–)4–5-septate; third largest arms 23–44 × 6–12 µm (mean = 35.3 × 9.6 µm, $n = 30$), (1–)3–4-septate; smallest arms 15–35 × 5.5–10 µm (mean = 25.5 × 8.0 µm, $n = 30$), 1–2-septate.

Cultural characteristics: Not examined.

Specimens examined: On twigs of *Betula ermanii* Cham.: Hakkoda, Aomori, 140°52.4'E, 40°42.2'N, Apr. 14, 2002, SH. (HHUF 27340 holotype; TNS-F 11174 isotype).

Notes: The genus *Prosthemium* is characterized by pycnidial conidiomata and staurosporous conidia that consist of a central cell and some euseptate arms. *Asterosporium* Kunze also has morphologically similar conidia, but it differs from *Prosthemium* in having acervular conidiomata (Kowalski and Holdenrieder 1996; Morgan-Jones and Kendrick 1972).

Among six species accepted in *Prosthemium* (Kowalski and Holdenrieder 1996; Saccardo 1884, 1895, 1899, 1906), *Pr. canba* is somewhat similar to *Pr. betulinum* in terms of conidial morphology, which consists of one largest arm and three smaller arms. However, the dimensions of the largest arm of *Pr. canba* are considerably larger [(63–)66.5–81 (–88.5) × (14–)15–19(–20) μm] than those of *Pr. betulinum* (40–54 × 14–20 μm; Hantula et al. 1998).

Prosthemium canba is the third *Prosthemium* species on *Betula* spp. The other two species, *Pr. asterosporium* and *Pr. betulinum*, have teleomorphic state of *Pleomassaria siparia* “type A” and “type B,” respectively. We found an ascomycete resembling *Pl. siparia* in the holotype specimen of *Pr. canba*, although it was in slightly poor condition. The new species may also have still another *Pl. siparia* complex teleomorph (namely “type C”), but cultural study with further fresh material of *Pr. canba* is required to confirm this.

5. *Asteromassaria macroconidica* Kaz. Tanaka, Y. Harada & M.E. Barr, sp. nov.

Anamorph: unknown coelomycetous fungus similar to *Sphaeropsis* Sacc. Figs. 33–49, 78

Ascomata 700–1100 μm alta, 800–1200 μm diametro, immersa, globosa, in lateribus extus hyphis atro-brunneis obducta. Rostrum 250–350 μm longum, centrale. Parietis ascomatis bistratus, 75–125(–170) μm crassus; stratum exteriore plectenchymatico et interiore ex cellulis pallidioribus complanatis 5–18 × 2–3 μm compositum. Pseudoparaphyses 1–2 μm latae, filiformes, ramosae, mucosae agglutinatae. Asci 200–270(–300) × 36–45(–47.5) μm, clavati, fissitunicati, basi lateraque ascomatis nascentes, apice rotundati, breviter stipitati. Ascospores 50.5–69(–77) × 16–22 μm, fusiformes vel ellipsoideae, apicem versus angustatae, 1(–3)-septatae, ad septum primum fere submedianum valde constrictae, brunneae vel fuscidulae, echinulatae vel verruculosae, strato mucoso 8–11 μm lato circumdatae.

Holotypus: HHUF 26741.

Etymology: In reference to the large-sized conidia.

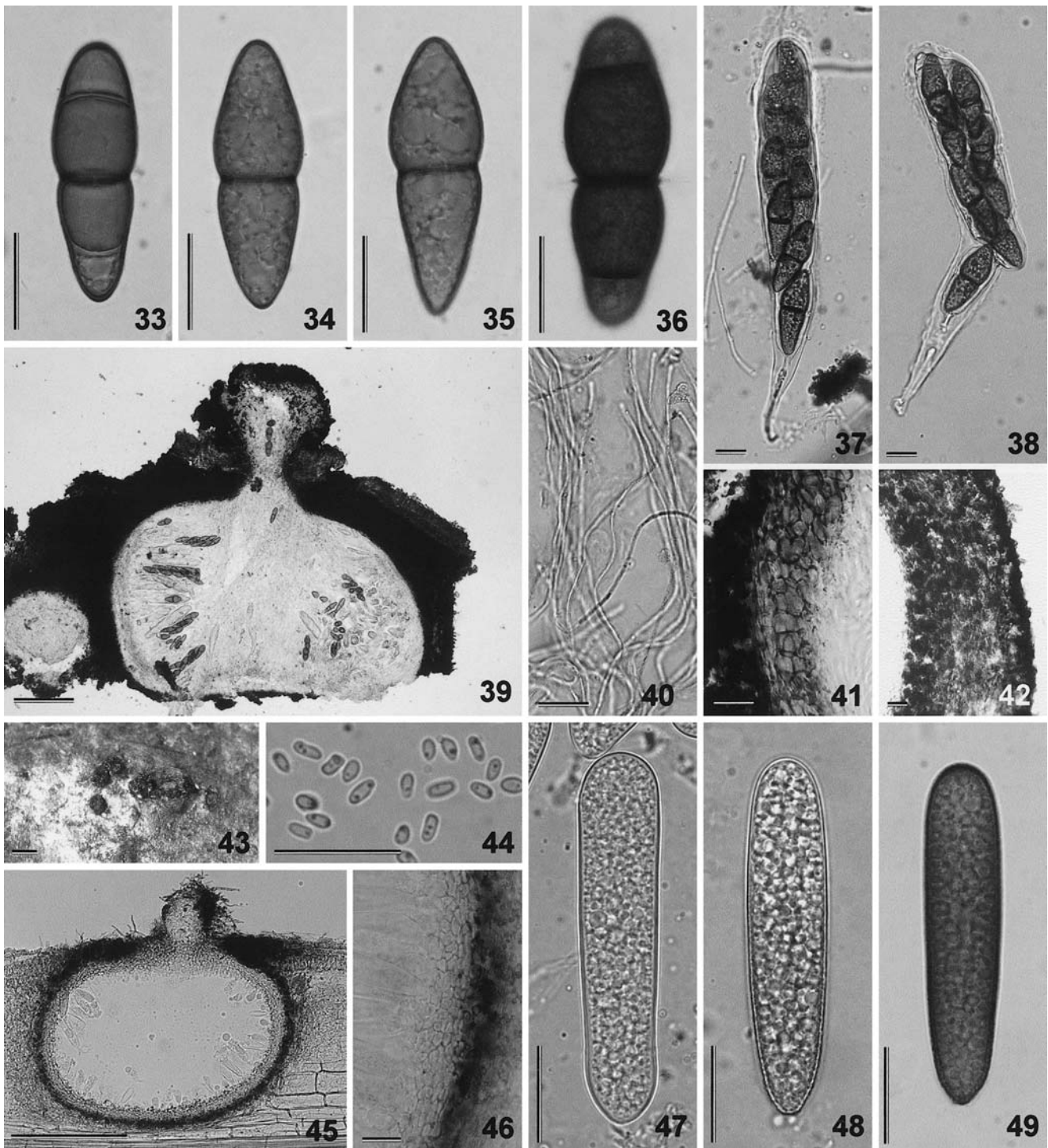
Ascomata 700–1100 μm high, 800–1200 μm in diameter, immersed, mostly crowded, globose, with dark brown hyphae at sides. Beak 250–350 μm long, cylindrical to papillate, central, composed of brown to dark brown angular cells of 5–20 μm, filled with pseudoparaphyses tips. Ascomal wall laterally 75–125(–170) μm thick of 2 layers; outer layer composed of plectenchymatic cells; inner layer composed of hyaline to pale brown flattened cells of 5–18 × 2–3 μm. Pseudoparaphyses 1–2 μm thick, branched and

anastomosed, with slime coating. Asci 200–270(–300) × 36–45(–47.5) μm (mean = 234.3 × 41.0 μm, *n* = 35), clavate, fissitunicate, basal and lateral, rounded at the apex, with an apical chamber, short-stalked (40–80 μm long), 8-spored. Ascospores 50.5–69(–77) × 16–22 μm (mean = 58.9 × 18.8 μm, *n* = 110), L/W 2.8–3.6 (mean = 3.1, *n* = 110), fusiform to ellipsoidal with narrowly rounded ends, 1(–3)-septate, with a primary septum mostly submedian (0.48–0.55; mean = 0.52, *n* = 110) and strongly constricted, brown to dark brown, echinulate to verrucose, with a sheath 8–11 μm thick (later expanding up to 20 μm thick).

Cultural characteristics: Colonies on PDA 29–31 mm in diameter after 4 weeks at 20°C in the dark. The colony color is variable depending on the isolate. In MAFF 239511 (=JCM 12843) Yellowish Grey (4B2) with White (1A1) entire margin; reverse Blond (4C4); no pigment produced. In MAFF 239512 (=JCM 12844) Greenish Grey (25E2) with White (1A1) entire margin; reverse similar; no pigment produced. On RSA, a *Sphaeropsis*-like macroconidial state and a microconidial state formed within 2 months (but the microconidial state was not formed in culture MAFF 239511). Conidiomata 340–530 μm high, 380–500 μm in diameter, pycnidial, globose, with a papillate beak. Beak 70–180 μm long, 70–130 μm in diameter, central. Conidiomatal wall uniformly 22–30 μm thick, composed of 5–6 layers of prismatic hyaline cells of 2.5–13 × 2.5–6 μm, with clypeus-like black cells at the base of beak. Conidiogenous cells 9–17 × 3.5–8.5 μm, holoblastic, doliform to cylindrical, aseptate, not branched, formed from all around the locular cavity. Conidia (62–)67–84(–88) × (13–)14.5–17.5 μm (mean = 75.4 × 15.8 μm, *n* = 110), L/W 4.3–5.4 (mean = 4.8, *n* = 110), non-septate, cylindrical, rounded at the apex, truncate at the base, with numerous small guttules, at first hyaline and smooth, later become yellowish from the base of conidia, and finally become dark brown and echinulate, without sheath. Microconidiomata 250–380 μm in diameter, globose, black. Microconidiophores up to 45 μm long, 2–4.5 μm wide. Microconidiogenous cells appearing annellidic. Microconidia 3.5–7 × 2–3 μm, globose to ellipsoid, hyaline, smooth.

Specimens examined: On dead twigs of an unknown woody plant: Sakaiminami, Musashino, Tokyo, 139°32'E, 35°41'N, May 19, 2001, T. Handa (HHUF 26741 holotype; TNS-F 11172 isotype). On twigs of *Prunus xyedoensis* Matsum.: Jyonan, Aizuwakamatsu, Fukushima, 139°55.6'E, 37°29.0'N, Mar. 29, 2004, YO. 1664a (HHUF 28556); 1664b (TNS-F 11173). Single ascospore cultures: isolated from HHUF 26741 (MAFF 239511 = JCM 12843); isolated from HHUF 28556 (MAFF 239512 = JCM 12844). Dried culture specimens of anamorph (grown on culms of *Oryza sativa*): made from culture MAFF 239511 (HHUF 28669, 28670); made from culture MAFF 239512 (HHUF 28671, 28672).

Notes: This fungus has ascospores resembling those of *A. macrospora*, but their ascospore dimensions are different: the ascospores are slightly smaller in the latter [(26–)38–50(–56) × 13–20 μm; Barr 1982]. They can also be distinguished by their anamorphs. Anamorph of *A. macrospora* has been reported as *Scolicosporium* having long fusiform conidia with 7–17 transverse septa (Sivanesan 1984).



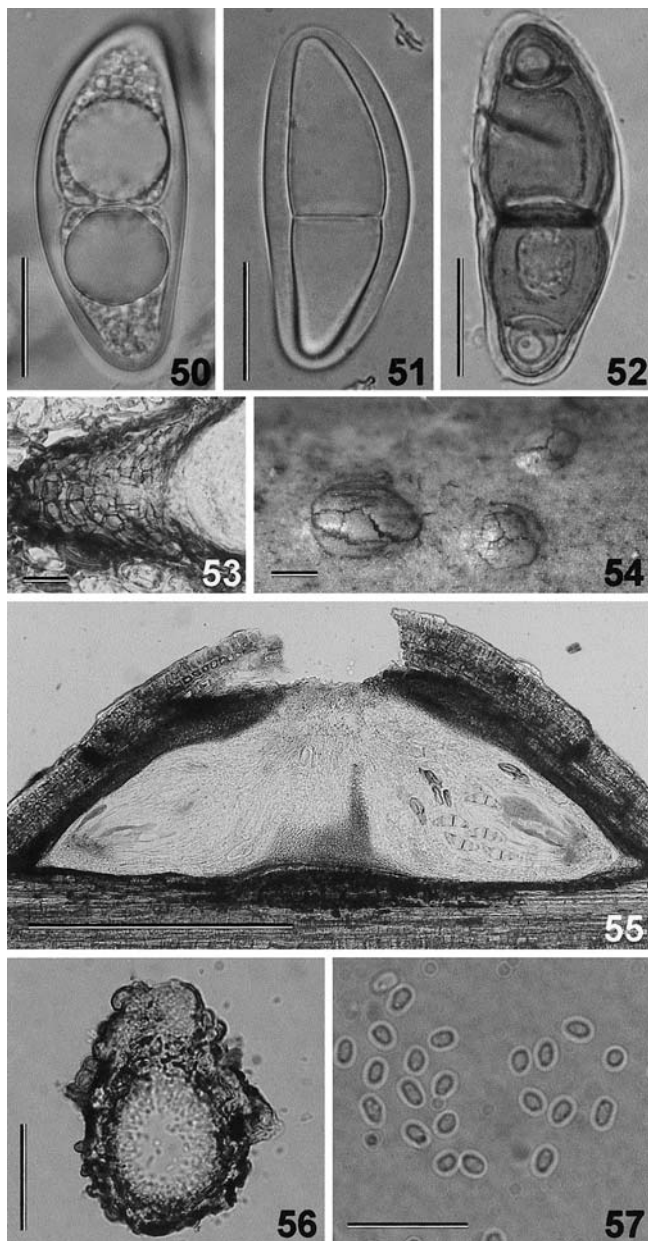
Figs. 33–49. *Asteromassaria macroconidica*. 33–36 Ascospores. 37, 38 Asci. 39 Ascus in longitudinal section. 40 Pseudoparaphyses. 41, 42 Ascomal wall. 43 Ascomata on host. 44 Microconidia. 45 Conidioma in longitudinal section. 46 Conidiomatal wall. 47–49 Conidia. (33–35, 37–

43 from HHUF 26741; 36 from HHUF 28556; 44, 47 from culture MAFF 239512 = JCM 12844; 45, 46, 48, 49 from culture MAFF 239511 = JCM 12843). Bars 33–38, 40–42, 44, 46–49 20µm; 39, 45 200µm; 43 500µm

6. *Asteromassaria pulchra* (Harkn.) Shoemaker & P.M. LeClair, Can. J. Bot. 53: 1588, 1975. Figs. 50–57, 79

Ascomata 250–380µm high, 700–1100µm in diameter, immersed in cracked epidermis, scattered to 2–4-crowded, hemispherical, with brown sparse hyphae at sides, with a

central ostiole. Ascomal wall at sides 50–90µm thick, rimlike, composed of parallel rows of brown polygonal to rectangular cells of 5–12 × 5–8µm; around the ostiole 35–55(–90)µm thick, composed of brown polygonal to subglobose cells of 4–12 × 5–9µm; at the base 5–10µm thick, flat and poorly developed. Pseudoparaphyses 1–4µm thick,



Figs. 50–57. *Asteromassaria pulchra*. **50–52** Ascospores. **53** Ascomal wall. **54** Ascomata on host. **55** Ascoma in longitudinal section. **56** Microconidioma in longitudinal section. **57** Microconidia. (**50–55** from HHUF 28393; **56, 57** from culture MAFF 239513 = JCM 12845). Bars **50–52, 57** 20 μm ; **53, 56** 50 μm ; **54, 55** 500 μm

separate, branched and anastomosed, with slime coating. Asci (145–)150–195(–215) \times (39–)41.5–50(–53) μm (mean = 176.4 \times 45.3 μm , $n = 40$), clavate, fissitunicate, rounded at the apex, with a shallow ocular chamber and faint ring, 8-spored. Ascospores (48–)51–62(–67) \times 14–22 μm (mean = 56.6 \times 17.4 μm , $n = 100$), L/W 2.8–3.6 (mean = 3.3, $n = 100$), broadly fusiform with rounded ends, mostly curved, with a submedian primary septum (0.52–0.57; mean = 0.55, $n = 100$) and constricted, hyaline to pale olivaceous, smooth, surrounded by a sheath. Sheath at first 2–5 μm thick and visible as thick wall, later the wall broken and swelling in water up to 20 μm thick. Senescent ascospores, 3-septate,

brown, echinulate. Ascospores germinate from both ends.

Cultural characteristics: Colonies on PDA 14 mm in diameter after 4 weeks at 20°C in the dark, Dull Green (28D3), with entire margin; reverse Olive Brown (4F3); no pigment produced. On RSA, a microconidial state produced on the surface of rice straws within 2 months. Microconidiomata 60–100 μm in diameter, globose, slightly papillate at the apex, scattered to 2–3-clustered, composed of 2–3 layers of brown polygonal cells 3–6 μm in diameter. Microconidiogenous cells 4–7 \times 2.5–4 μm , simple, doliform, phialidic. Microconidia 2.5–3.5 \times 1.8–2.2 μm , subglobose, hyaline, smooth.

Specimens examined: On vines of *Berchemia racemosa* Siebold: Tenosawa, Hirosaki, Aomori, 140°28'E, 40°32'N, Sept. 24, 2003, K.T. and T. Shirouzu 1472 (HHUF 28393); Nov. 27, 2003, Y.H. and N. Asama 1570–1574 (HHUF 28394–28398); Zatoishi, Ogamisawa, Hirosaki, Aomori, 140°27'E, 40°31'N, Nov. 8, 2003, K.T. and T. Shirouzu 1559 (HHUF 28400). Single ascospore culture: isolated from HHUF 28393 (MAFF 239513 = JCM 12845).

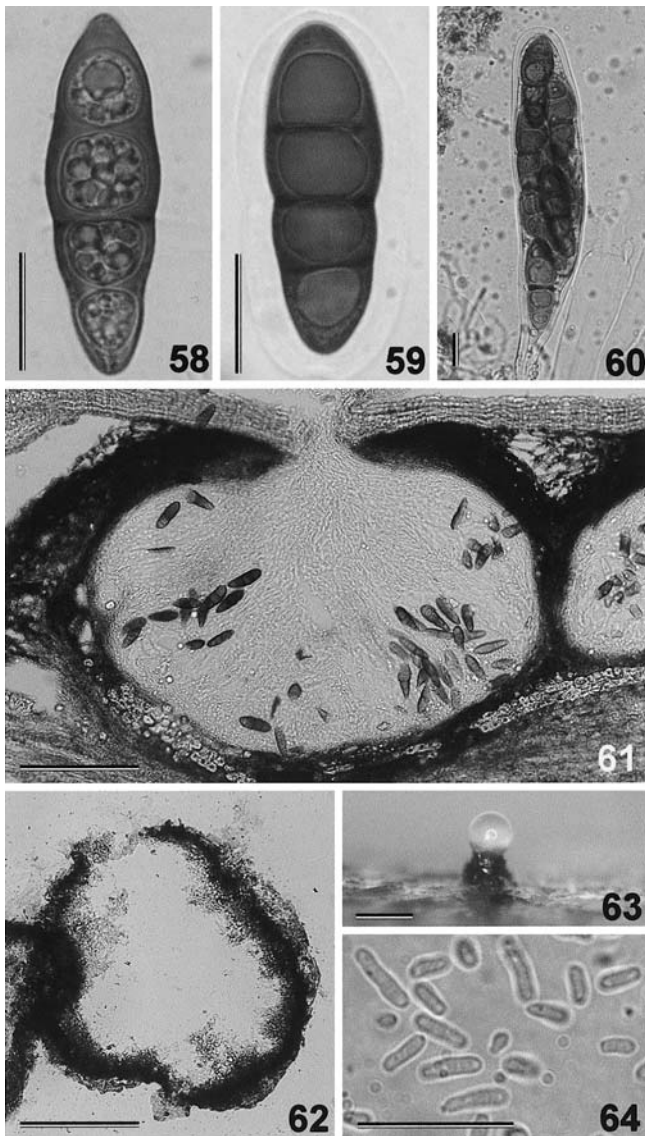
Notes: Most *Asteromassaria* species are host specific (Barr 1993b), but *A. pulchra* appears to be plurivorous. It was originally collected on *Umbellularia* (Nees) Nutt. in California, USA, but is also known from *Acer* L., *Arbutus* L., *Holodiscus* (K. Koch) Maxim., *Quercus* L. and *Salix* L. in British Columbia, Canada.

7. *Splanchnonema mori* (I. Miyake) Kaz. Tanaka, Y. Harada & M.E. Barr, comb. nov.

Basionym: *Massaria mori* I. Miyake, Bull. Seric. Exp. Sta. 1: 319, 1916. Figs. 58–64, 80

Ascomata 450–870 μm high, 720–1120 μm in diameter, immersed, scattered to crowded, globose to depressed globose, with dark brown hyphae 3 μm wide at sides. Ascomal wall 30–50 μm thick, composed of pale brown flattened cells of 3–18 \times 2–5 μm . Pseudoparaphyses 1–2.5 μm thick. Asci 183–271 \times 40–49 μm (mean = 222.4 \times 44.9 μm , $n = 30$), clavate, (4- to 6-) to 8-spored. Ascospores (48.5–)51–70(–73) \times 17–23 μm (mean = 59.7 \times 20.0 μm , $n = 80$), L/W 2.4–3.6 (mean = 3.0, $n = 80$), broadly fusiform to clavate, 3-septate, with a submedian primary septum (0.53–0.59; mean = 0.56, $n = 80$), with two globose to angular cytoplasmic inclusions in each hemisphere, pale brown to dark brown, smooth, with a sheath constricted at the primary septum.

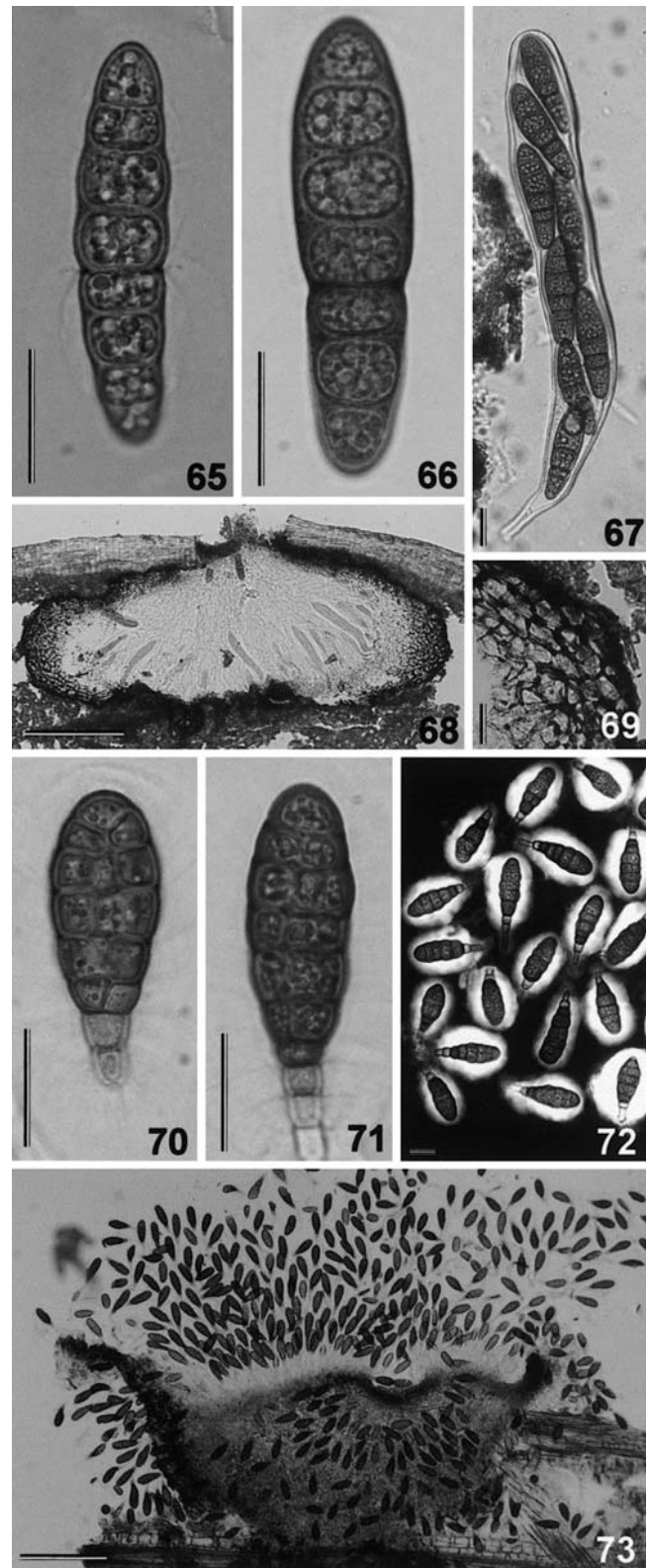
Cultural characteristics: Colonies on PDA 64–68 mm in diameter after 1 week at 20°C in the dark, after 4 weeks Greyish Yellow (4B3) to Olive Brown (4D5) with numerous White (1A1) to Black dots of microconidiomata, reverse similar; slightly yellowish pigment produced. On RSA, a microconidial state formed within 1 month. Microconidiomata 200–400 μm in diameter, globose to subglobose. Wall 35–50 μm thick, composed of brown angular cells 5–10 μm in diameter. Microconidiophores up to 40 μm long, 0- to 3-septate, branched. Microconidiogenous cells 10–20 \times 3–4 μm , appear phialidic. Microconidia 3.5–8 \times 2–3 μm , mostly cylindrical, hyaline. Sometimes an ascomatal state also formed. Ascospores 54–63.5 \times 19–



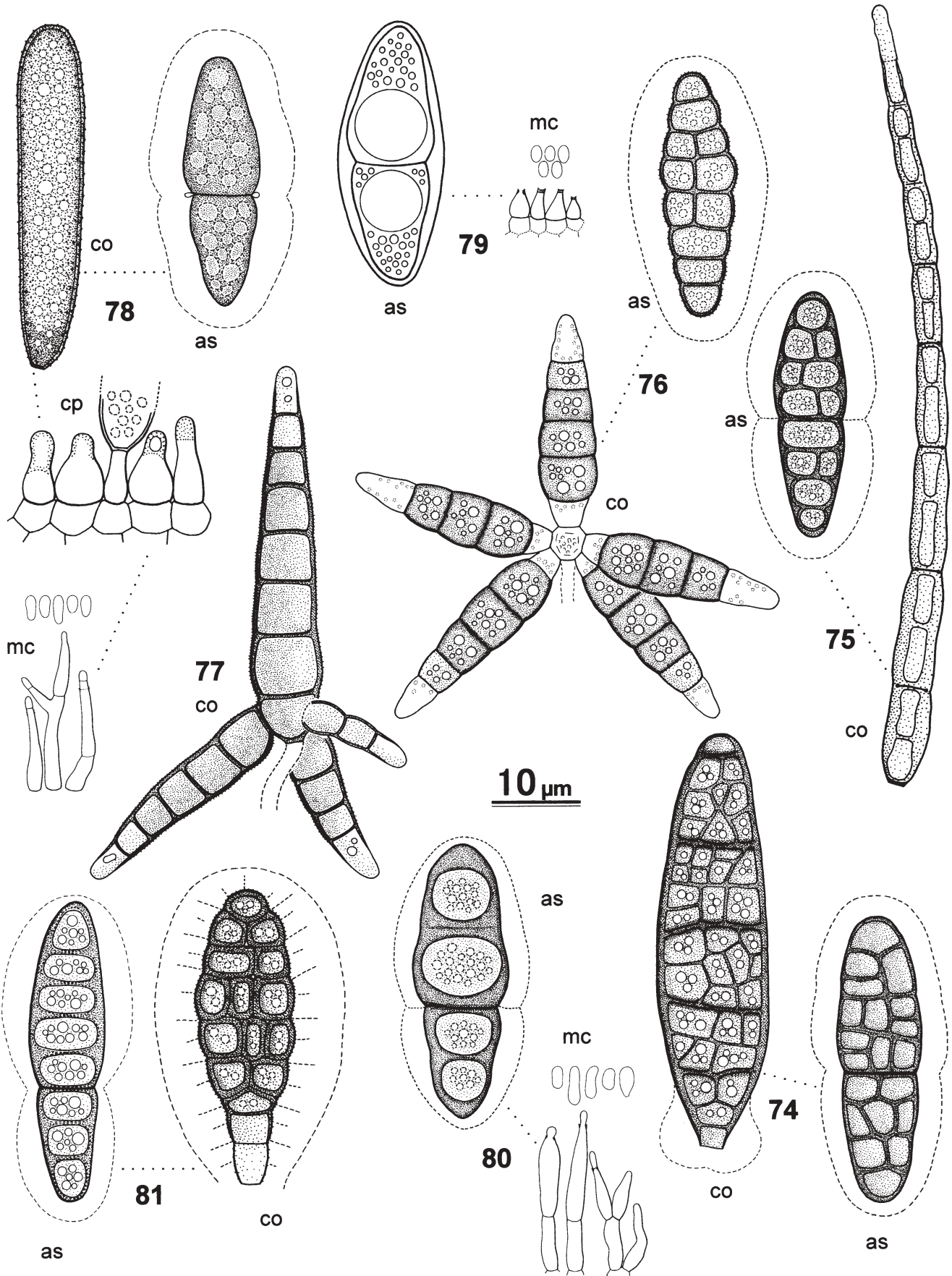
Figs. 58–64. *Splanchnonema mori*. 58, 59 Ascospores. 60 Ascus. 61 Ascoma in longitudinal section. 62 Microconidioma in longitudinal section. 63 Microconidioma with exuding mass of microconidia. 64 Microconidia. (58 from HHUF 28555; 59–61 from HHUF 28554; 62–64 from culture MAFF 239514 = JCM 12846). Bars 58–60, 64 20µm; 61–63 200µm

24µm (mean = $58.8 \times 20.9\mu\text{m}$, $n = 50$), 3-septate with a submedian primary septum (0.52–0.60; mean = 0.56, $n = 50$).

Specimens examined: On twigs of *Morus* sp.: Hokkaido University Botanical Garden, Sapporo, Hokkaido, 141°20.4'E, 43°03.4'N, June 28, 1928, M. Terui (HHUF 790), June 30, 1931, S. Ito (SAPA); Chitose-farm of Hirosaki University, Hirosaki, Aomori, 140°28.1'E, 40°34.1'N, May 24, 2002, Y. Takahashi (HHUF 28554), Hattaribetsu, Hokkaido, June 3, 1928, K. Sasaki (SAPA). On twigs of *Morus australis* Poir.: Hokkaido University Botanical Garden, Sapporo, Hokkaido, 141°20.4'E, 43°03.4'N, June 30, 2000, KT. 339 (HHUF 28551); July 7, 2000, KT. 354 (HHUF 28552); June 6, 2004, KT. 1724



Figs. 65–73. *Splanchnonema argus*. 65, 66 Ascospores. 67 Ascus. 68 Ascoma in longitudinal section. 69 Ascomal wall. 70, 71 Conidia. 72 Conidia with sheath (in India ink). 73 Conidioma in longitudinal section. (65, 68, 69 from HHUF 28659; 66, 67 from HHUF 28656; 70, 72, 73 from culture MAFF 239515 = JCM 12847; 71 from culture MAFF 239662 = JCM 13152). Bars 65–67, 69–72 20µm; 68, 73 200µm



Figs. 74–81. Ascospores (*as*), conidia (*co*), conidiophores (*cp*), and microconidia (*mc*) of Pleomassariaceae. **74** *Pleomassaria maxima*. **75** *Pl. swidae*. **76** *Pl. siparia* “type A”. **77** *Prosthemia canba*. **78**

Asteromassaria macroconidica. **79** *A. pulchra*. **80** *Splanchnonema mori*. **81** *S. argus*. All same magnification

(HHUF 28555); Campus of Hirosaki University, Hirosaki, Aomori, 140°28'E, 40°35'N, Apr. 14, 2001, KT. 484 (HHUF 28553), Sapporo, Hokkaido, July 3, 1931, Y. Imai (SAPA). On twigs of *Morus alba* L.: Maruyama, Sapporo, Hokkaido, July 12, 1931, S. Ito (SAPA); Sapporo, Hokkaido, June 1925, S. Ito (SAPA). Single ascospore culture: isolated from HHUF 28555 (MAFF 239514 = JCM 12846).

Notes: Obviously, this species is close to *S. phorcioides*. Both species were described by Miyake (1916) at the same time as species of *Massaria* De Not., and they were distinguished by the ascospore septation; 3-septate (1+1+1) in the present fungus, and (3-)4(-5)-septate (usually 2+1+1) in *S. phorcioides*. Because of similarities of ascospore dimensions, Teng (1934) suggested that both are identical species. However, we considered that they are different as the ascospores formed in culture were consistently 3-septate in *S. mori*.

8. *Splanchnonema argus* (Berk. & Broome) Kuntze, Rev. Gen. Pl. 3: 531, 1898.

Anamorph: *Myxocyclus polycistis* (Berk. & Broome) Sacc., Ann. Mycol. 6: 559, 1908. Figs. 65–73, 81

Ascomata 350–450 µm high, 850–1000 µm in diameter, immersed, scattered to 2–3-crowded, depressed globose. Ascomal wall 80–135 µm thick at sides, composed of angular brown cells 5–15 µm in diameter. Pseudoparaphyses 1.5–3 µm thick. Asci 195–286 × 28.5–38 µm (mean = 235.5 × 33.8 µm, *n* = 35), clavate, 8-spored. Ascospores 51–68 × 12.5–18 µm (mean = 60.4 × 15.8 µm, *n* = 57), L/W 3.5–4.3 (mean = 3.8, *n* = 57), cylindrical to clavate, 6–7-septate (mostly 3+1+2), with a primary septum submedian (0.56–0.65; mean = 0.61, *n* = 57), brown, with a sheath constricted at the primary septum.

Cultural characteristics: The growth rate and color are slightly different depending on the isolates. For example, in culture MAFF 239515 (=JCM 12847) and MAFF 239663 (=JCM 13153), colonies on PDA attain 45–60 mm in diameter after 4 weeks at 20°C in the dark, White (1A1) to Pale Grey (1B1), lanose, with White (1A1) entire margin; reverse Olive Grey (1D2); no pigment produced. In culture MAFF 239662 (=JCM 13152), colonies grow slowly, 18–20 mm in diameter after 4 weeks, Yellowish Grey (4E3); reverse Blond (4B4); no pigment produced. On RSA, *Myxocyclus* state formed within 2 months. Conidiomata 450–680 µm high, 800–1200 µm wide at the top, 500–800 µm wide at the base, acervular, immersed to erumpent, scattered, cupulate to trapezoid in longitudinal section. Wall mainly composed of hyaline to pale brown prosenchymatic cells, partly with angular cells 3–15 µm in diameter, with dark brown margin. Conidiophores up to 200 µm long, 2–4 µm thick, filiform, hyaline, septate, with slime coating. Conidiogenous cells 7–15 × 3.5–6 µm, holoblastic, cylindrical, yellow to pale brown. Conidia 49–60(–64) × 17–20.5 µm (mean = 55.7 × 18.3 µm, *n* = 50), L/W 2.7–3.4 (mean = 3.1, *n* = 50), cylindrical to clavate, muriform, with (6-)7–8(-9) transverse septa, thick-walled, yellow to brown (paler at the basal cells), echinulate, with a mucilaginous sheath 5 µm thick (later enlarges up to 20 µm thick).

Specimens examined: On twigs of *Betula* sp.: Arishima, Niseko, Abuta, Hokkaido, 140°42.27'E, 40°48.27'N, Sept. 22, 2004, YO. and SH. 1753a (HHUF 28659), 1753b (HHUF 28660). On twigs of *Betula ermanii*: Hakkoda, Aomori, 140°52.4'E, 40°42.2'N, Oct. 19, 2004, SH. 1756–1758 (HHUF 28656–28658). Single ascospore cultures: isolated from HHUF 28659 (MAFF 239662 = JCM 13152); isolated from HHUF 28656 (MAFF 239663 = JCM 13153); Tissue isolation culture: isolated from dead twigs of *Betula platyphylla* Sukaczew var. *japonica* (Miq.) H. Hara (at Biratori, Hokkaido), YO. 03-36 (MAFF 239515 = JCM 12847).

Notes: Tonolo (1956) observed only a “*Phoma* type” conidial state in culture from the ascospore of *S. argus*. However, the connection between *S. argus* and *M. polycistis* was suggested by several authors (Barr 1982; Saccardo 1908) based on their occurrences on same substratum. In this study, we obtained evidence of the congenetic relation of these morphs in culture for the first time.

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