



Towards a natural classification of Dothideomycetes 3: The genera *Muellerites*, *Trematosphaeriopsis*, *Vizellopsis* and *Yoshinagella* (Dothideomycetes incertae sedis)

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Abstract

We re-examine the generic types of genera with unclear placement in Dothideomycetes. These genera were hitherto poorly illustrated or described. Following examination their appropriate familial or ordinal level placements based on modern morphology concepts may be better understood. The generic types of *Muellerites*, *Trematosphaeriopsis*, *Vizellopsis* and *Yoshinagella* were re-examined in this study. *Muellerites* is placed in *Dothidotthiaceae*, *Vizellopsis* in *Asterinaceae*, while *Trematosphaeriopsis* and *Yoshinagella* are retained in Dothideomycetes *incertae sedis*, as they are not typical of any existing families of Dothideomycetes. By illustrating and redescribing the genera we expect renewed interest for recollection and molecular study so that these dothideomycetous genera can be placed in a natural taxonomic framework.

Key words: Ascomycota, Dothideomycetes, taxonomy, types

Introduction

Dothideomycetes, the largest and most diverse class of Ascomycota (Hyde *et al.* 2013, Kirk *et al.* 2008, Schoch *et al.* 2006), is characterized by ascolocular ascoma development and bitunicate (or/and fissitunicate) asci (Hyde *et al.* 2013). The well known members of this class such as the species of *Capnodia*, *Botryosphaeria* and *Venturia* and the asexual genera *Diplodia*, *Fusicladium* and *Phyllosticta* are important plant pathogens (Chomnunti *et al.* 2011, Liu *et al.* 2012, Parisi *et al.* 1993). Most described species and genera are found as endophytes, saprobes or epiphytes growing on woody plant debris, decaying leaves or herbivore dung (Hyde *et al.* 2013, Malloch & Cain 1971). A smaller number are symbiotic as lichens (Lücking & Sérusiaux 2013, Lücking *et al.* 2013). Only one species, *Cenococcum geophilum* Fr. has been established as mycorrhizal with plant roots (LoBuglio *et al.* 1996). Lumbsch & Huhndorf (2010) included two subclasses and eleven orders in the class Dothideomycetes, whereas two orders, 34 families and around 184 genera were placed in Dothideomycetes *incertae sedis*.

We are in the process of studying genera of Dothideomycetes in order to provide a natural classification of this large class (Ariyawansa *et al.* 2013, Boonmee *et al.* 2011, Dai *et al.* 2012, Liu *et al.* 2011, 2012, Wu *et al.* 2011a, 2011b, Zhang *et al.* 2011, 2012, Hyde *et al.* 2013). These studies involved examination of type specimens of the genera, provision of modern descriptions and illustrations or photomicrographs and, wherever possible, linking to their asexual states, resulting with appropriate placement at the family and ordinal levels. In this study, we re-examined the type species of genera *Tirispora*, *Trematosphaeriopsis*, *Vizellopsis* and *Yoshinagella*.

Materials and methods

Examination of specimens

Type specimens were obtained from FH, S, URM and ZT (abbreviations according to Index Herbariorum 2013). The study methods followed are those reported in Chomnunti *et al.* (2011, 2014) and Liu *et al.* (2012). Fruiting bodies were rehydrated in water and/or 5% KOH prior to examination and sectioning. Hand sections of the ascocarps were mounted in water or lactic acid for microscopic studies and photomicrography. The materials were examined by a Nikon ECLIPSE 80i compound microscope and photographed by Canon 550D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3, Extended version 10.0 software (Adobe Systems, USA). Genus and species descriptions are given for all accepted genera.

Results and discussion

There is no sequence data for any members of these genera in GenBank and the data given below are based on morphology.

Taxonomy

Dothidotthiaceae Crous & A.J.L. Phillips, in Phillips *et al.*, Persoonia 21: 35 (2008) MycoBank: MB 511706

The family *Dothidotthiaceae* was introduced by Phillips *et al.* (2008) containing the single genus *Dothidotthia*. *Dothidotthia* was originally placed in *Botryosphaeriaceae* by Barr (1987). However, *Dothidotthia* formed a separate familial clade of the order Pleosporales in multi-gene phylogenetic analysis (Phillips *et al.* 2008, Zhang *et al.* 2012). *Dothidotthiaceae* is characterized by gregarious, erumpent, globose ascocarps, hyaline, septate pseudoparaphyses, 8-spored, bitunicate, clavate ascospores bearing ellipsoid, 1-septate ascospores (Hyde *et al.* 2013). The asexual state is *Thystroma* (Phillips *et al.* 2008).

Muellerites L. Holm, Svensk bot. Tidskr. 62: 231 (1968) MycoBank: MB 3285

Type species: *Muellerites juniperi* (E. Müll. & Arx) L. Holm, Svensk bot. Tidskr. 62: 231 (1968) MycoBank: MB 297818 (Fig. 1)
Basionym: *Gibberidea juniperi* E. Müll. & Arx, Phytopath. Z. 24: 359 (1955)

Saprobic on needle-like leaves of cypress. Sexual state: *Ascomata* forming at the base of the leaves, 400–650 mm diam., solitary to gregarious, superficial, coriaceous, globose, black, with indistinct ostiole, easily removed. *Peridium* 50–70 µm thick, comprising a single layer composed of thick-walled, brown cells of *textura angularis*. *Hamathecium* of ca. 1 µm wide dense, septate, branch pseudoparaphyses. *Ascii* 170–218 × 25–29 µm ($\bar{x} = 197.8 \times 27.1$ µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, with a short pedicel, apically rounded with an ocular chamber. *Ascospores* 40–46 × 10.5–12 µm ($\bar{x} = 43.2 \times 11.6$ µm, n = 20), 2-seriate, ellipsoid to fusiform, dark brown, 3–4-septate, straight to curved, rounded or with acute ends, smooth-walled, surrounded by a mucilaginous sheath. Asexual state: Unknown.

Notes:—The type species of the monotype genus *Muellerites* was originally described by Holm (1968) from the collection of *Gibberidea juniperi* E. Müll. & Arx (1955). This genus is characterized by globose, small and black ascocarps forming at the base of the cypress leaves, and cylindrical ascospores containing eight, dark brown ascospores with 3–4 septa. The peridium is composed of one layer composed of thick-walled, brown, cells of *textura angularis*. Based on these characters, we place *Muellerites* in the family *Dothidotthiaceae*. *Dothidotthia* grows on wood, has clustered, erumpent, ascocarps with central ostioles, and ellipsoid, 1-septate, brown to chestnut or pale brown ascospores (Hyde *et al.* 2013). *Muellerites* differs in having solitary, somewhat gregarious ascocarps with indistinct ostioles, forming on pine leaves and fusiform, 3–4-septate, dark brown ascospores.

Material examined:—SWITZERLAND. Airolo: Raetia, Ramosch, Lunga, 1900 m, on leaves of *Juniperus nana* Willd. (Cupressaceae), 16 July 1949, E. Müller (ZT Myc 49038!, holotype).



FIGURE 1. *Muellerites juniperi* (holotype). a, b. Herbarium specimens. c–d. Ascomata on host. e, f. Sections of ascomata. g, h. Peridium. i–m. Ascii with pseudoparaphyses. n–t. Ascospores. Scale bars: c, d = 500 µm, e–g = 50 µm, i–m = 10 µm, n–t = 5 µm.

Asterinaceae Hansf., Mycol. Pap. 15: 188 (1946) MycoBank: MB 80492

The family *Asterinaceae* was introduced by Hansford (1946) and is typified by *Asterina* Lév. The important features of *Asterinaceae* are the superficial, web-like, black colonies that form on the upper and lower surface of living leaves and are typical of *Asterinaceae* (Hofmann 2010, Hofmann *et al.* 2010, Hosagoudar *et al.* 2013, Hyde *et al.* 2013). The superficial, small, black thyrothecia open in the centre by radial or star-like splitting (Hosagoudar 2012), and grow below the radial mycelium which frequently produce hyphopodia (Hyde *et al.* 2013). Thyrothecia of this family are closely attached to the host plant cuticle and have an upper dark wall (scutellum) which is one cell layer thick and composed of isodiametrical or cylindrical cells (Hofmann *et al.* 2010), however the lower

ascoma wall is poorly developed. Pseudoparaphyses are frequently inconspicuous or missing (Hofmann 2010). Ascii are globose to clavate, bitunicate, which produce brown, mostly two-celled ascospores (von Arx & Müller 1975, Hofmann 2010, Hyde *et al.* 2013). Asexual states of *Asterinaceae* have been reported in *Acarella*, *Asterostomella*, *Asterostomula*, *Asterostromina*, *Clasterosporium*, *Elachopeltis*, *Eriothyrium*, *Leprieurina*, *Mahanteshamomyces*, *Peltasterella*, *Pirozynskia*, *Septothyrella*, *Sirothyriella*, *Thyrinula* and *Triposporium*, but this need to be confirmed (Hyde *et al.* 2013).

Vizellopsis Bat., J.L. Bezerra & T.T. Barros, Publções Inst. Micol. Recife 637: 5 (1969)
MycoBank: MB 5748

Type species:—*Vizellopsis grevilleae* Bat., J.L. Bezerra & T.T. Barros, Publções Inst. Micol. Recife 637: 5 (1969) MycoBank: MB 325517 (Fig. 2)

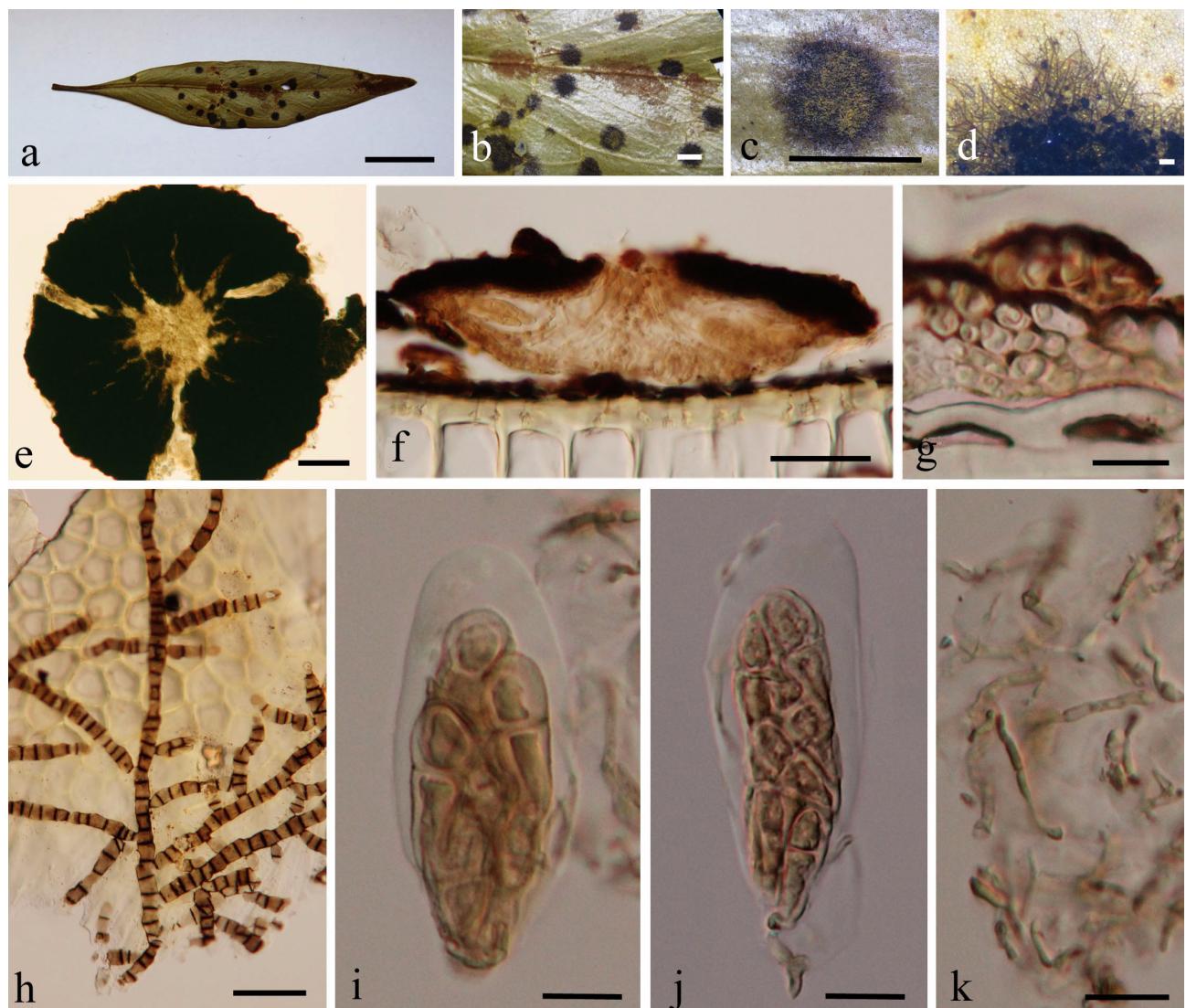


FIGURE 2. *Vizellopsis grevilleae* (holotype). a. Herbarium material. c, d. Colonies on the host. e, f. Superficial mycelium on host. g. Thyrothecia with slit-like opening ostiole. h. Upper wall of thyrothecia. i. Section of thyrothecia. k. Section of immature thyrothecia. l. Branched mycelium with strongly thick septa. m. Pseudoparaphyses. n, o. Ascii with ascospores. Scale bars: b = 20 mm, c–f = 2 mm, g–k = 50 µm, l–o = 10 µm.

Epiphytic on upper surface of living leaves. *Colonies* 1–2 mm diam., superficial, thin, circular, dark brown to black, without hyphopodia. *Hyphae* long, 7–13 µm wide, flexuous, reticulately branched, septate, dark brown, strongly constricted and dark at the septa, straight or curved. *Sexual state*: *Thyrothecia* forming below the mycelium, 250–350 µm diam., scattered to gregarious, superficial, aggregated in the center of colonies, orbicular,

flattened, black, ostiolate in the center, opening by irregular splitting when mature, membranaceous to slightly coriaceous, easily removed from host surface. *Upper wall* comprising cells of *textura prismatica*, radiating outwardly in parallel rows from the central ostiole, basal wall layer poorly developed. *Hamathecium* composed of dense, septate, branched pseudoparaphyses and asci inclined from the base and rim towards the central ostiole. *Asci* 55–70 × 22–24 µm ($\bar{x} = 64.7 \times 24.3$ µm, n = 10), 8-spored, bitunicate, clavate, pedicel poorly developed, apically rounded with an ocular chamber. *Ascospores* 20–25 × 5–8 µm ($\bar{x} = 23.1 \times 7.6$ µm, n = 10), 2–3-seriate, irregularly arranged, broad fusiform to long obovoid, brown, 1-septate, smooth-walled. Asexual state: Unknown.

Notes:—*Vizellopsis* was introduced by Batista *et al.* (1969) and is typified by *V. grevilleae* Bat. This monotypic genus is characterized by black colonies on the leaf surface formed by a brown mycelium with flexuous hyphae which have strongly constricted and dark septa. Batista *et al.* (1969) placed *Vizellopsis* in the family *Microthyriaceae*, according to the black, circular, flattened thyrothecia. Lumbsch & Huhndorf (2010) included this genus as Dothideomycetes *incertae sedis*, as the morphological characters are not similar to *Microthyriaceae*. Based on the small, black thyrothecia, forming below the dark brown mycelium, comprising radiating cells and having concentrically ridged surface, we place *Vizellopsis* in the family *Asterinaceae*. *Vizellopsis* has strongly thickened and septate mycelium which is different from other genera of *Asterinaceae*.

Material examined:—NEW CALEDONIA. South Province: Yate, on leaves of *Grevillea exul* Lindl., 11 September 1966, B. Huguenin 76.296 (URM 655060!, holotype).

Dothideomycetes *incertae sedis*

Lumbsch & Huhndorf (2010) listed 184 genera in Dothideomycetes genera *incertae sedis* because familial placements are unclear. These doubtful genera lack molecular data in GenBank and need phylogenetic analyses to establish which families they belong to. We have examined the type materials of *Trematosphaeriopsis* and *Yoshinagella* and although we can make suggestions as to their familial relationships, we leave them in this group as it is not obviously apparent where the genera can be placed.

Trematosphaeriopsis Elenkin, Izv. Imp. St.-Peterburgsk. Bot. Sada 1(4): 146 (1901)
MycoBank: MB 5523

Type species: *Trematosphaeriopsis parmeliana* Jacz. & Elenkin, Izv. Imp. St.-Peterburgsk. Bot. Sada 1(4): 146 (1901)
MycoBank: MB 221591 (Fig. 3)

Probable synonymy: *Homostegia parmeliana* (Jacz. & Elenkin) Vouaux, Bull. Soc. mycol. Fr. 28: 198 (1912)
Trematosphaeris parmeliana (Jacz. & Elenkin) Clem., Gen. fung. (Minneapolis): 35 (1909)

Lichenicolous on thallus. Sexual state: *Ascostromata* 1–1.5 mm high, 1–2.5 mm diam., solitary, scattered, superficial, subglobose, depressed in the center, reddish-yellow, coriaceous, lacking an ostiole. Wall of ascostromata, thick, three layered, with an outer thin layer composed of fragmented or disintegrated tissue, with middle 40–120 µm wide layer composed of orange to reddish-yellow cells of *textura angularis*, and thick inner layer composed of reddish-yellow to hyaline cells of *textura oblita* and becoming black when dry; peridium surrounding the locules, 20–30 µm thick, composed of hyaline small cells intermixed with ascogenous hyphae and pseudoparaphyses. *Locules* 100–200 µm high, 80–150 µm diam., immersed in the upper part of ascostromata, globose to subglobose, some with narrow necks. *Peridium* of locules 10–25 µm thick, composed of small pale reddish yellow cells of *textura oblita*. *Hamathecium* of dense, 1–2 µm wide, hyaline, non-septate, unbranched, pseudoparaphyses. *Asci* 60–80 × 10–12 µm ($\bar{x} = 70.8 \times 11.8$ µm, n = 20), 8-spored, bitunicate, cylindrical, with a short pedicel; apical chamber not observed. *Ascospores* 18–26 × 4–5.5 µm ($\bar{x} = 21.9 \times 5.1$ µm, n = 20), 2-seriate, ellipsoid to fusiform, sometimes slightly curved, hyaline to pale yellow, 3-septate when mature, smooth to verrucose, with rounded ends. Asexual state: Unknown.

Notes:—*Trematosphaeriopsis*, introduced by Elenkin (1901), is monotypic and represented by *T. parmeliana*. This fungus is characterized by large ascostromata, growing superficially on lichens and producing 10–25 locules formed only under the periphery of upper wall. *Trematosphaeriopsis* is similar with the genera in *Trypetheliaceae* by having the globose, orange ascostromata forming on lichens (Hafellner 2001, Hyde *et al.* 2013, Lücking *et al.* 2007). However, *Trematosphaeriopsis* differs by having ascostromata with the cupped-socket area in centre and

producing fusiform ascospores with 1 to 4 septa (Hafellner 2001). Genera in *Trypetheliaceae* have the ascostromata with central ostiole, and with fusiform-ellipsoid to oblong ascospores with distosepta and sometimes additional eusepta (Aptroot 1991, Hyde *et al.* 2013). The morphologic characters of this genus do not fit well in any existing Dothideomycete families and we therefore retain it in Dothideomycetes, genera *incertae sedis*.

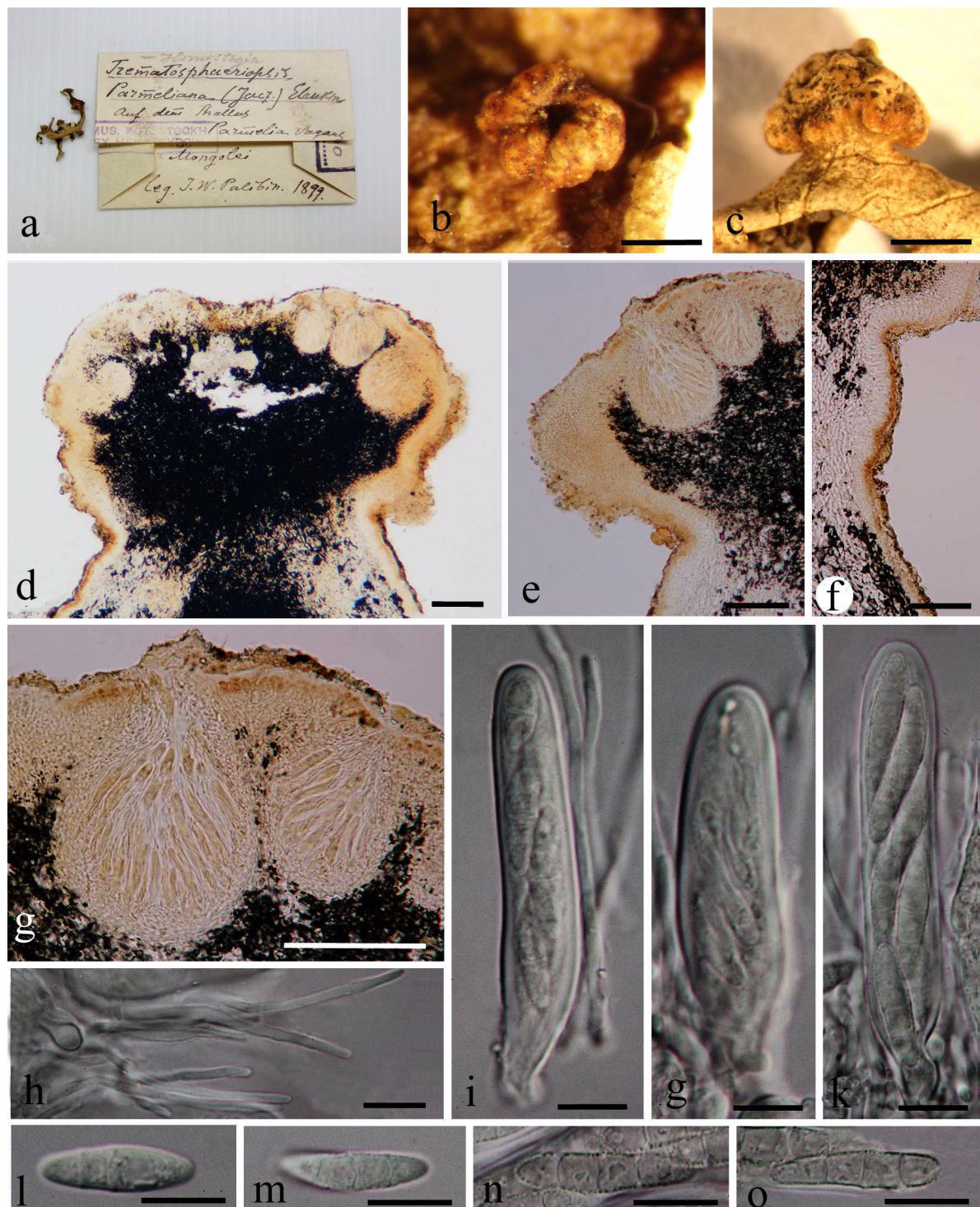


FIGURE 3. *Trematosphaeriopsis parmeliana* (isotype). a. Herbarium material. b, c. Reddish yellow ascostromata on the host. d, e. Section of ascostroma. f. Wall of ascostroma. g. Ascostroma with locules. h. Pseudoparaphyses. i–k. Asci with ascospores. l–o. Ascospores. Scale bars: b, c = 1 mm, d–g = 100 µm, h–o = 10 µm.

Material examined:—MONGOLIA. Hentiy: Kerulen River, on the thallus of *Parmelia molliuscula* var. *vagans* (Nyl.) Nyl., 1899, J.W. Palibin (S F45300!, isotype).

Parasitic on upper surface of living leaves. Sexual state: *Ascostromata* solitary, scattered to gregarious, superficial, cushion-like, flat, rounded, irregular at the margin, black, coriaceous, flattened at the centre. *Locules* immersed in the ascostromata, arranged in a circular margin, globose to irregular. Wall of ascostromata thick, two layered, with outer layer composed of black and thin tissue; inner layer composed of light brown to hyaline, large cells of *textura angularis*; peridium that surrounds the locules, composed of small hyaline cells intermixed with ascogenous hyphae and pseudoparaphyses. *Hamathecium* composed of dense, septate, branched, pseudoparaphyses, branching above the asci. *Asci* 8-spored, bitunicate, cylindrical, with a short furcate pedicel, apically rounded with an apical ocular chamber. *Ascospores* 1-seriate, fusiform, pointed at the ends, hyaline, 3-septate, smooth-walled. Asexual state: Unknown.

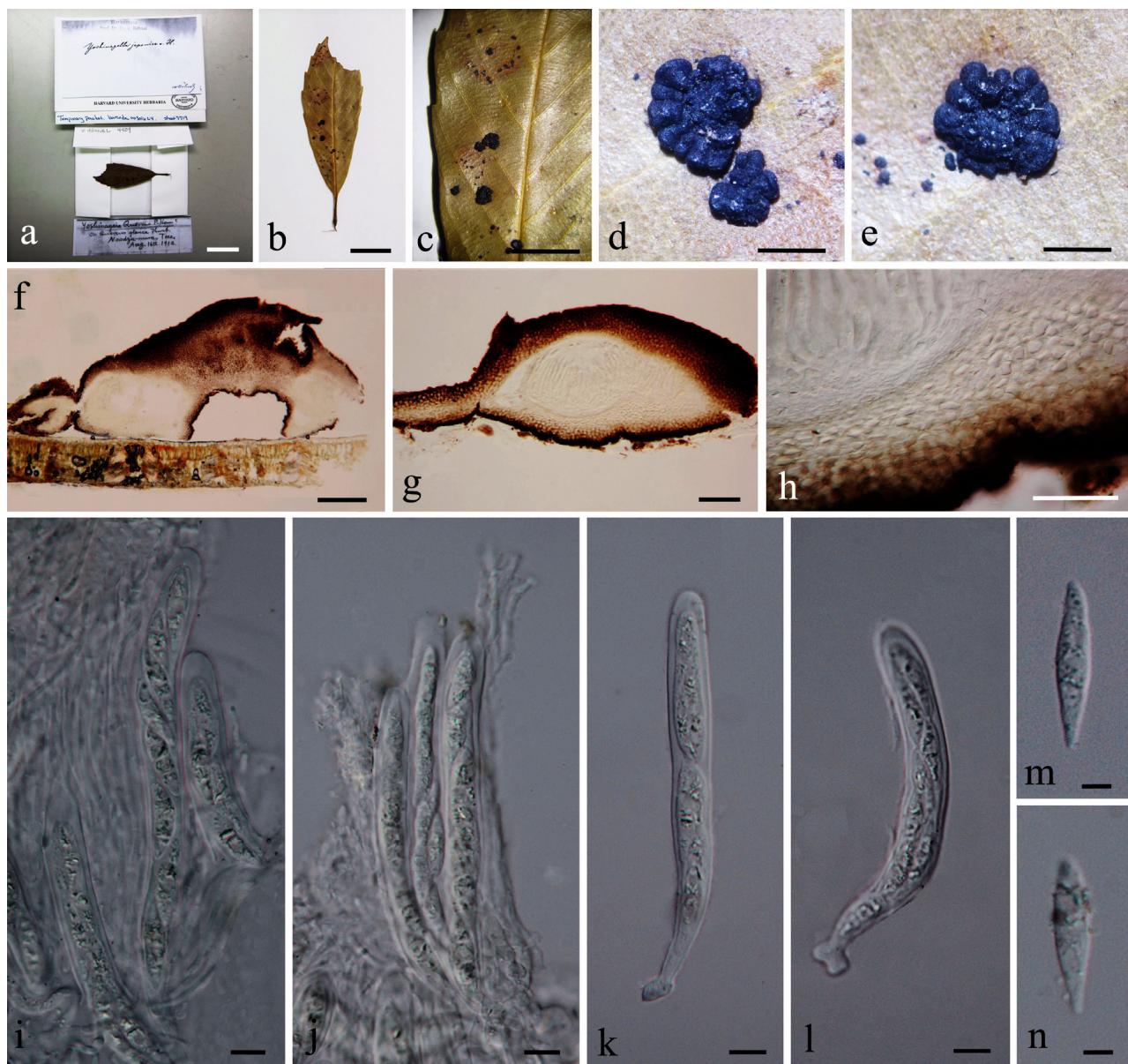


FIGURE 4. *Yoshinagella japonica* (lectotype). a, b. Herbarium material. c–e. Black ascostromata on host. f, g. Section of ascostroma. h. Wall of ascostromata and peridium surrounding the locules. i–l. Ascii with pseudoparaphyses. m, n. Ascospores. Scale bars: a = 50 mm, b = 25 mm, c = 5 mm, d = 1mm, e = 250 µm, g = 100 µm, h = 50 µm, i–l = 10 µm, m, n = 5 µm.

Notes:—*Yoshinagella*, introduced by Höhn (1913), is typified by *Yoshinagella japonica* Höhn. This genus is defined by large, black ascostromata with irregular margins containing locules which are globose to irregular. *Yoshinagella* contains three species: *Y. japonica* Höhn. and *Y. nuda* F. Stevens growing on the leaves of *Quercus glauca* Thunb. and *Cibotium chamissoi* Kaulf., respectively (Höhn 1913, Stevens 1925), and *Y. phyllostachydis* I.

Hino & Katum. on dead stems of *Phyllostachys bambusoides* Siebold & Zucc. (Hino & Katumoto 1955). *Yoshinagella* can be compared with *Coccoidea* and *Cookella*, two genera that have superficial, circular to subcircular, pulvinate to effuse discoid, and cushion-like, pigmented ascostromata. *Coccoidea* differs as the locules grow upwards from the basal portion linearly, and opening with an apical ostiole (Eriksson 1981, Inácio & Cannon, 2002). In *Cookella*, locules are small, and scattered in the ascostromata (Hyde *et al.* 2013). In *Yoshinagella* locules are only arranged in a circle at the periphery of the ascostromata. *Yoshinagella* may be similar with *Parmularia* in having large, black ascostromata with locules lined at the surface. The ascostromata in *Parmularia* have radially raised areas from the centre (Hyde *et al.* 2013, Inácio & Cannon 2008). However, in *Yoshinagella*, ascostromata are only raised at the margins, and are thicker. Species in *Yoshinagella* can also be compared with *Yoshinagaia quercus* which has ascomata directly formed on leaves and produces elliptical ascospores without septa (Sivanesan & Hsieh 1995). *Yoshinagella* might be placed in *Botryosphaeriaceae* or Dothideales. However, fresh collections and molecular analyses are needed to confirm the relationships. Thus we remain *Yoshinagella* in Dothideomycetes genera *incertae sedis*. We examined good material of one specimen, herein designated as lectotype.

Type species: *Yoshinagella japonica* Höhn., Fragm. Mykol. 122: 293 (1913) MycoBank: MB 248507 (Fig. 4)

Parasitic on upper surface of living leaves. Sexual state: *Ascostromata* 0.5–0.7 mm high, 1–2.5 mm diam., solitary, scattered to gregarious, superficial, cushion-like, flat, rounded, irregular at the margin, black, coriaceous, with flattened centre. *Locules* 200–250 µm high, 400–500 µm diam., immersed in the ascostromata, arranged in a circle at the margin, globose to irregular. Wall of ascostromata thick, two layered, with outer layer composed of black and thin tissue; inner layer 80–150 µm thick, composed of light brown to hyaline, large cells of *textura angularis*; peridium surrounding the locules, 15–20 µm thick, composed of hyaline small cells intermixed with ascogenous hyphae and pseudoparaphyses. *Hamathecium* composed of 1 µm wide, dense, septate, pseudoparaphyses, branching above the asci. *Asci* 90–120 × 9–15 µm ($\bar{x} = 109.1 \times 12.7$ µm, n = 20), 8-spored, bitunicate, cylindrical, with a short furcate pedicel, apically rounded with an apical ocular chamber. *Ascospores* 18–24 × 4.5–6 µm ($\bar{x} = 22.3 \times 5.3$ µm, n = 20), 1-seriate, fusiform, pointed at the ends, hyaline, 3-septate, smooth-walled. Asexual state: Unknown.

Material examined:—JAPAN. Kochi: Tosa, Noodzu-mura, on leaves of *Quercus glauca* Thunb. (*Fagaceae*), 16 August 1912, (FH 00301624!, **lectotype, here designated**).

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