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Towards a natural classification of Dothideomycetes 2: The genera *Cucurbidothis*, *Heterosphaeriopsis, Hyalosphaera*, *Navicella* and *Pleiostomellina* (Dothideomycetes *incertae sedis*)

HIRAN A. ARIYAWANSA[1,2,3], KASUN M. THAMBUGALA[2,3], JI-CHUAN KANG[1], SITI A. ALIAS[4], EKACHAI CHUKEATIROTE[2,3] & KEVIN D. HYDE[2,3]

[1] *The Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang 550025, Guizhou Province, China*
[2] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[4] *Institute of Biological Sciences, University of Malaya, 50603, Kuala Lumpur*
email: jichuank@yahoo.co.uk; kdhyde3@gmail.com

Abstract

By re-examining the generic types of Dothideomycetes genera, *incertae sedis* we can propose higher level positions according to the morphology based on modern taxonomic concepts but, more importantly, we illustrate the taxa so that they are better understood. In this way the taxa can be recollected and molecular data can be used to place them in a natural taxonomic framework of the Ascomycota. The generic types of *Cucurbidothis*, *Heterosphaeriopsis*, *Hyalosphaera*, *Navicella* and *Pleiostomellina* were re-examined in this study. A synopsis of the history and descriptions and illustrations of these genera are provided. *Cucurbidothis* is placed in *Cucurbitariaceae* as a distinct genus, while *Navicella* and *Pleiostomellina* are referred to *Melanommataceae* and *Parmulariaceae* respectively based on the morphological similarities. *Heterosphaeriopsis* and *Hyalosphaera* are retained in Dothideomycetes genera *incertae sedis* as they are not typical of any existing families of Dothideomycetes. Fresh collections of these genera are needed for further studies, so that they can be epitypified and molecular data can be analyzed to stabilize their natural classification.

Introduction

Dothideomycetes is the largest and possibly most phylogenetically diverse class within the phylum, Ascomycota (Hyde *et al*. 2013, Kirk *et al*. 2008, Schoch *et al*. 2009) and is characterized mainly by bitunicate asci with fissitunicate dehiscence (Hyde *et al*. 2013, Kirk *et al*. 2008). The class comprises a wide group of fungi that subsist in the majority of the niches where fungi can be found. Many species are saprobes, with many asexual states comprising important plant pathogens that cause serious problems to crop plants (Ariyawansa *et al*. 2013a,b, Liu *et al*. 2011, Manamgoda *et al*. 2012). Even though there is a large body of work comprising taxonomic and phylogenetic studies, most genera hypothesized to be members of Dothideomycetes remain under-studied and poorly understood within a systematic framework. Recent studies using multigene analysis and some coupled with morphology have provided the groundwork for classification in the Dothideomycetes (Liew *et al*. 2000, 2003, Lumbsch & Lindemuth 2001, Schoch *et al*. 2009, Spatafora *et al*. 2006, Zhang *et al*. 2012).

We have been studying the genera of Dothideomycetes(Ariyawansa *et al.* 2013a,b,c, Boonmee *et al*. 2011, Hyde *et al*. 2013, Liu *et al.* 2012, Wu *et al*. 2011, Zhang *et al*. 2012), especially Dothideomycetes *incertae sedis* to provide a natural classification of this large class (Ariyawansa *et al.* 2013b, Thambugala *et al*. 2014). The aim of the present study, the second paper in this series ((Ariyawansa *et al.* 2013b), is to re-visit the generic types of genera that have been categorized in Dothideomycetes *incertae sedis* and are poorly understood. In this paper we re-describe *Cucurbidothis*, *Heterosphaeriopsis, Hyalosphaera*, *Navicella* and *Pleiostomellina* from type material, provide illustrations and discuss suitable placements at the family and ordinal levels.

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Materials and methods

Specimen examination

The basic methodology used in this study was the same as Ariyawansa *et al.* (2013b). Type specimens were borrowed from BPI, FH, S, URM and W. Ascomata were rehydrated in water and 5% KOH prior to examination and sectioning. Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The fungi were examined under a Nikon ECLIPSE 80i compound microscope and photographed by a Cannon 450D 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). For each genus, the type species is listed together with a description of the genus, except in cases where there is only a single species in the genus. Notes on the type genus are provided along with full citations for the type species.

Results and discussion

Taxonomy

Cucurbitariaceae G. Winter

The family *Cucurbitariaceae* was introduced by Winter (1885) and typified with *Cucurbitaria berberidis* (Pers.) Gray. The family is characterized by aggregated, ostiolate ascomata on a basal stromatic structure, fissitunicate and cylindrical asci and pigmented, phragmosporous or muriform ascospores (Hyde *et al*. 2013). Currently *Cucurbitariaceae* consists of four sexual genera, *viz Cucurbitaria* Gray., *Curreya* Sacc., *Rhytidiella* Zalasky., and *Syncarpella* Theiss. & Syd., and two asexual genera *viz Pyrenochaeta* De Not., and *Pyrenochaetopsis* Gruyter *et al.* (Doilom *et al*. 2013, Hyde *et al*. 2013).

Cucurbidothis Petr., Annls mycol. 19(3/4): 201 (1921) MycoBank: MB 1347

Saprobic on dead wood. *Sexual state*: *Ascomata* solitary or scattered, superficial, semi-immersed to erumpent, subglobose to broadly ellipsoid, coriaceous, ostiolate. *Ostiole* usually widely porate, with a well-developed neck. *Peridium* comprising 1–2 layers of thick-walled cells of *textura angularis*. *Hamathecium* of dense, long, cellular pseudoparaphyses, branching and anastomosing between and above the asci. *Asci* 8-spored, fissitunicate, clavate to broadly clavate. *Ascospores* oblong, reddish brown to dark yellowish brown, muriform. *Asexual state*: unknown.

Type species:—*Cucurbidothis pityophila* (J.C. Schmidt & Kunze) Petr., Annls mycol. 19(3/4): 201 (1921) MycoBank: MB 509319 (Fig. 1)

≡ *Sphaeria pityophila* J.C. Schmidt & Kunze [as 'pithyophila'], in Fries, Syst. mycol. (Lundae) 2(2): 425 (1823)

Saprobic on dead wood. *Sexual state*: *Ascomata* 380–450 × 360–420 µm ($\bar{x}$ = 430 × 400 µm, n = 10), solitary or scattered, superficial, semi-immersed to erumpent, subglobose to broadly ellipsoid, wall dark brown to black, coriaceous, ostiolate. *Ostiole* widely porate, with well-developed neck, ostiolar canal filled with a tissue of hyaline cells. *Peridium* 30–65 µm ($\bar{x}$ = 40 µm, n = 10) wide, comprising 1–2 layers of cells, outer layer composed of heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells. *Hamathecium* of dense, 2–4 µm ($\bar{x}$ = 3 µm, n = 10) diam., cellular pseudoparaphyses, branching, anastomosing between and above the asci, embedded in mucilage. *Asci* 100–140 × 7–10 µm ($\bar{x}$ = 120 × 8 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, with short, narrow pedicel, thickened and rounded at apex with an ocular chamber. *Ascospores* 15–19 × 3–6 µm ($\bar{x}$ = 16 × 5 µm, n = 40), uniseriate, partially overlapping, oblong, reddish brown to dark yellowishbrown, muriform, with 3 transverse septa and 1–2 vertical septa in the central cells when mature, constricted at the middle septum, smooth-walled, lacking a sheath. *Asexual state*: unknown.

Material examined:—CANADA. British Colombia: Victoria watershed, on dead wood of *Pinus monticola* Douglas ex D. Don (Pinaceae), 23 May 1950, *A.K Parker* (UBC-F3787!, paratype)

Notes:—*Cucurbidothis pityophila*, the type species of *Cucurbidothis* was initially introduced by Schmidt & Kunze (1823) as *Sphaeria pityophila* and placed in *Xylariaceae*. Petrak (1940) transferred *Cucurbidothis* as a

separate genus to *Cucurbitariaceae*, Pleosporales. Later, von Arx & Müller (1975) treated *Cucurbidothis* as a synonym of *Curreya*. After observing the paratype of *Cucurbidothis pityophila* from UBC, we proposed to maintain the species as a separate genus in *Cucurbitariaceae* based on its distinct characters. Both *Cucurbidothis* and *Curreya* have large muriform ascospores, cellular pseudoparaphyses and small ascomata, but *Cucurbidothis* differs from *Curreya* and other genera of *Cucurbitariaceae* in its superficial ascomata growth on conifer wood and strictly cylindrical asci (Barr 1990b, Checa 2004, Doilom *et al*. 2013).

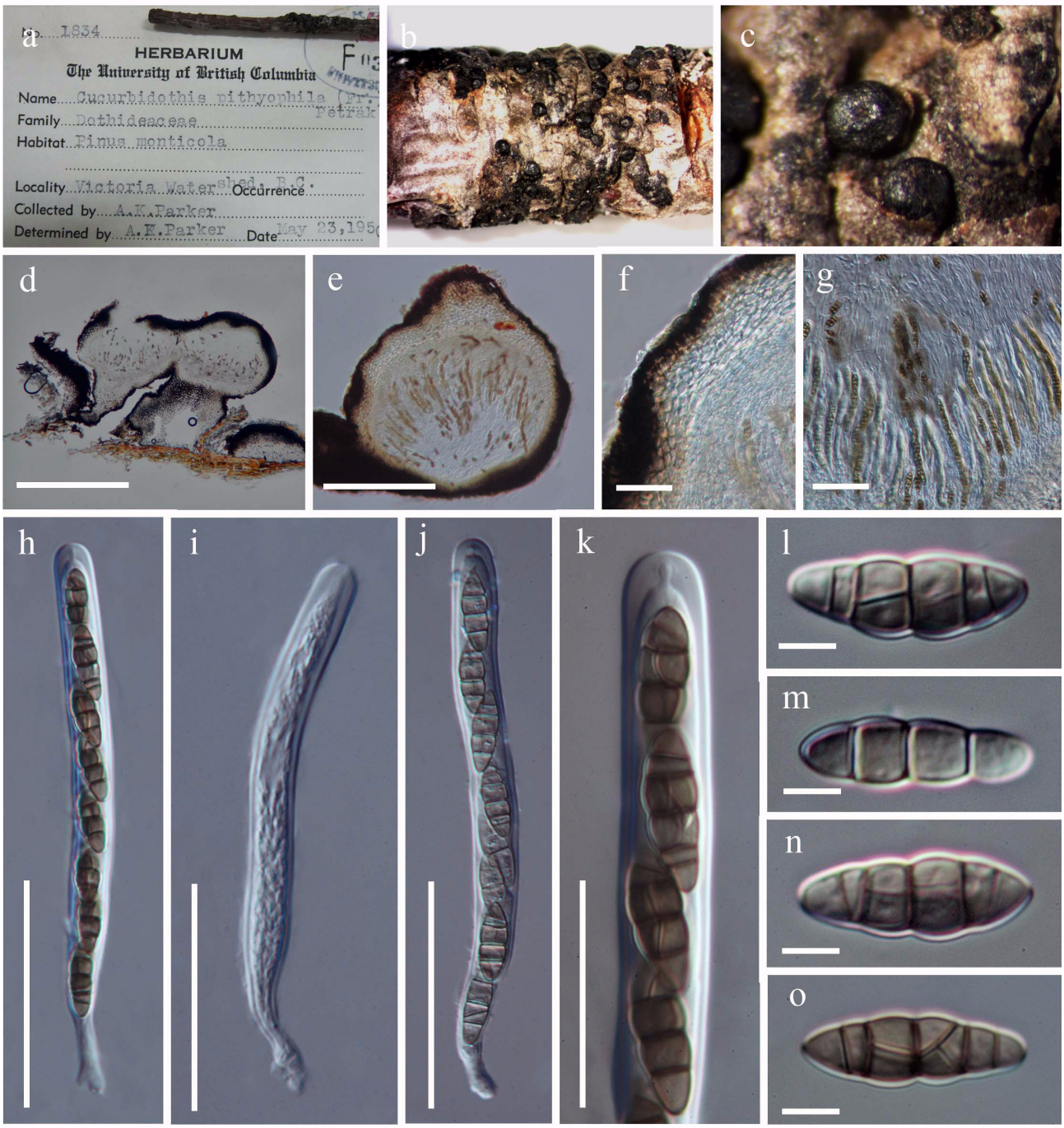

FIGURE 1. *Cucurbidothis pityophila* (paratype). a. Herbarium material. b. Ascomata on host substrate. c. Close-up of ascomata. d–e. Sections of ascomata. f. Close-up of peridium. g. Hamathecium of dense long cellular pseudoparaphyses, branching, anastomosing between and above. h–k. Asci with short, narrow pedicel. i–o. Reddish brown to dark yellowish brown, muriform ascospores. Scale bars: d–e = 100 µm, f–g = 10 µm, h–k = 15 µm, l–o = 5 µm.

Five *Cucurbidothis* species are listed in Index Fungorum (2013), but no molecular data is available for any of these. Therefore, fresh collections of the type species of the genus are needed so that molecular data can be obtained to confirm the natural taxonomic affinities of this genus. By giving a detailed description and illustrations we hope to provide impetus for future work.

Melanommataceae G. Winter

Based on globose or depressed perithecial ascomata, fissitunicate asci, pigmented phragmosporous ascospores and trabeculate pseudoparaphyses, Winter (1885) introduced *Melanommataceae* and typified the genus with *Melanomma* (Barr 1990a, Zhang *et al*. 2012). Barr (1983) treated *Melanommataceae* as a separate order, but recent molecular phylogenetic studies do not give any support to the division of Melanommatales from Pleosporales (Liew *et al.* 2000, Mugambi & Huhndorf 2009, Zhang *et al*. 2012). *Melanommataceae* asexual states are rarely hyphomycetous, with ontogenic structures (e.g. with annellidic conidiogenesis in *Exosporiella* and *Pseudospiropes*) or coelomycetous (e.g. "*Aposphaeria*"-like and *Pyrenochaeta*) (Zhang *et al*. 2012). Currently the family comprises 12 sexual genera and four asexual genera (Hyde *et al*. 2013).

Navicella Fabre, Annls Sci. Nat., Bot., sér. 6, 9: 96 (1879) [1878] MycoBank: MB 3429

Saprobic on dead wood. *Sexual state*: *Ascoma*ta solitary or scattered, erumpent through the outer layer of the host tissue to nearly superficial, coriaceous. *Ostiole* usually widely porate, with well developed neck. *Peridium* comprising 1–2 layers of thick-walled, *textura angularis*. *Hamathecium* of dense long trabeculate pseudoparaphyses, branching, anastomosing between and above the asci, embedded in mucilage. *Asci* bitunicate, fissitunicate, clavate to sub-cylindrical. *Ascospores* ellipsoidal with broadly rounded ends, multi-septate. *Asexual state:* unknown.

Type species*: Navicella julii* Fabre, Annls Sci. Nat., Bot., sér. 6, 9: 96 (1879) [1878] MycoBank: MB158755 (Fig. 2)

Saprobic on dead wood. *Sexual state*: *Ascomata* 750–890 × 460–600 µm ($\overline{x}$ = 810 × 520 µm, n = 10), solitary or scattered, erumpent through the outer layer of the host tissue to nearly superficial, coriaceous. *Ostiole* usually widely porate, with well developed neck, ostiolar canal filled with a tissue of hyaline cells, coriaceous. *Peridium* 33–45 µm ($\overline{x}$ = 38 µm, n = 10) wide, comprising a single layer of highly pigmented, dark brown to black, thick-walled cells of *textura angularis*. *Hamathecium* of dense 1–1.5 µm ($\overline{x}$ = 1.2 µm, n = 20) wide, long, trabeculate pseudoparaphyses, branching, anastomosing between and above the asci, embedded in mucilage. *Asci* 150–200 × 15–20 µm ($\overline{x}$ = 160 × 17 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate to sub-cylindrical, long pedicellate and thickened, rounded at apex, with a minute ocular chamber. *Ascospores* 55–75 × 8–12 µm ($\overline{x}$ = 60 × 10 µm, n = 40), biseriate, partially overlapping, ellipsoidal, 5–8-septate, primary septum euseptate, and others distoseptate, brown to chestnut-brown, verrucose, hyaline appendage at each end, lacking a sheath. *Asexual state:* unknown.

Material examined:—FRANCE. Vaucluse, on dead wood, *H. Fabre* (S-F71928!, holotype)

Notes:—The genus is characterized by immersed to erumpent, globose ascomata with an elongated or rarely rounded apex and trabeculate pseudoparaphyses, branching, anastomosing between clavate or cylindrical asci and with multi-septate ascospores having hyaline appendages at each end (Barr 1990). Based on its saprobic nature on bark, Holm & Holm (1988) transferred *Navicella* to *Lophiostomataceae*. Barr (1990) referred *Navicella* to the *Massariaceae* based on the wide endotunica, thin apical ring and distoseptate ascospores, but this classification was not followed by Zhang *et al.* (2012) and Voglmayr & Jaklitsch (2011). *Navicella* shares similarities with *Melanommataceae* in processing superficial globose to subglobose, coriaceous ascomata with long, trabeculate pseudoparaphyses embedded in a gelatinous matrix and brown ascospores, but differs from the other genera of *Melanommataceae* in having, clavate or cylindrical asci with a long pedicel and ascospores with euseptate primary septum and others distoseptate with hyaline appendages at each end. Currently 116 *Navicella* species are listed in Index Fungorum (2013) but no molecular data is available for any of these species. Some of the lichenized species have been classified under *Navicella* (Aptroot 2001, 2003), thus most of these may need accommodating in other genera. We assign *Navicella* to *Melanommataceae* pending molecular investigation.

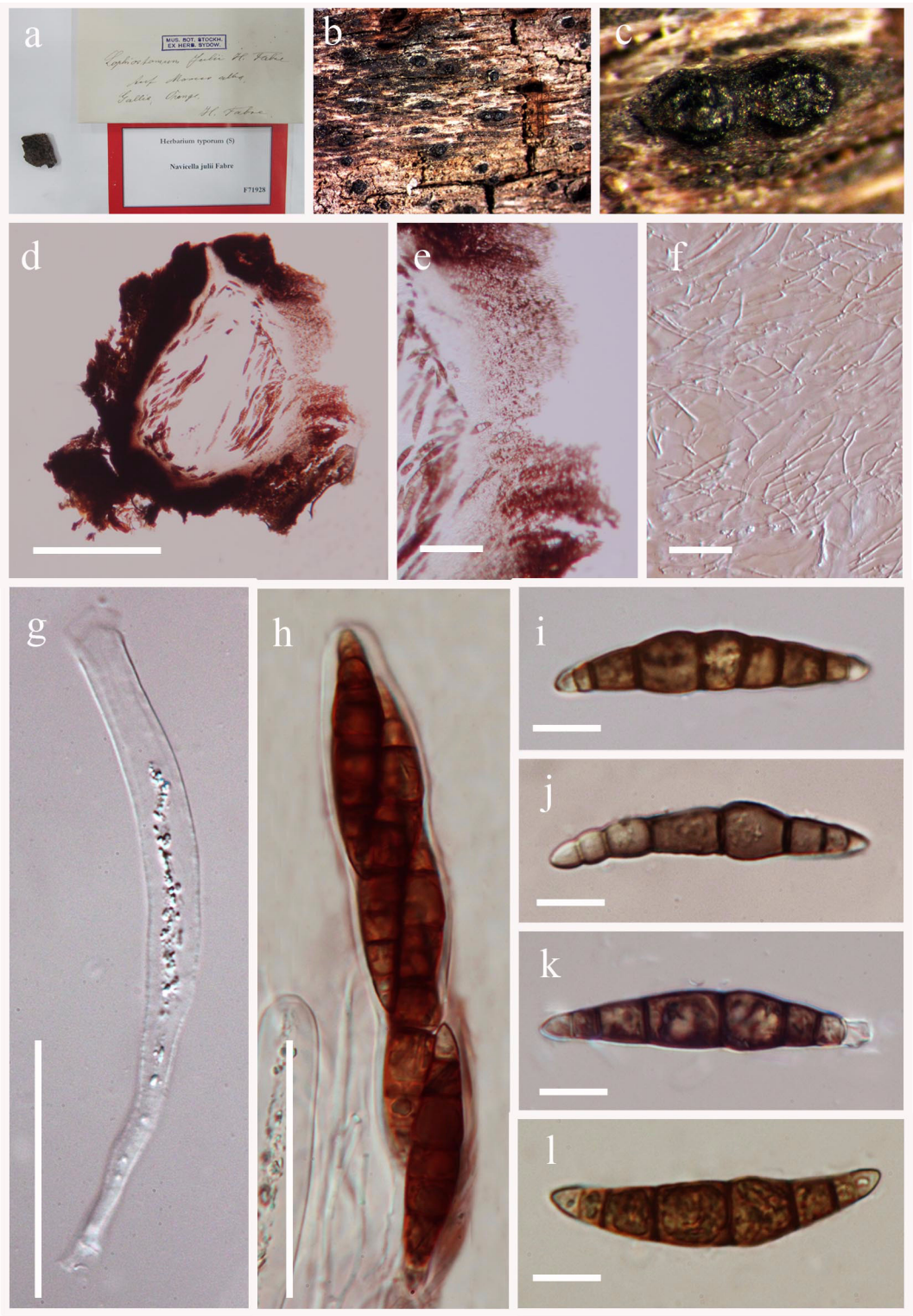

FIGURE 2. *Navicella julii* (holotype). a. Herbarium material. b. Ascomata on host substrate. c. Close up of ascomata. d. Section of ascoma. e. Section of the peridium. f. Long trabeculate pseudoparaphyses, branching and anastomosing between and above the asci. g–h. Immature and mature asci with long pedicels. i–l. Brown to chestnut-brown ascospores. Scale bars: d–e = 100 µm, e–f = 10 µm, g–h = 15 µm, i–l = 5 µm.

Parmulariaceae E. Müll. & Arx ex M.E. Barr

The family *Parmulariaceae* was invalidly introduced by Müller & von Arx (1962) and later validated by Barr (1979). The family is clearly polyphyletic and contains a range of ascostromata types, ascomata and even thyriothecia, ascus and ascospores forms (Inácio & Cannon 2008). Lumbsch & Huhndorf (2010) included 34

genera in the family, while Hyde *et al*. (2013) accepted 30 genera. *Parmulariaceae* is characterized by shield-like to star-shaped or elliptical to boat-shaped ascostromata with or lacking appressoria and pseudoparaphyses along with fissitunicate, short pedicellate asci and 1-septate ascospores. Asexual states of the family are coelomycetous or hyphomycetous (Hyde *et al*. 2013).

Pleiostomellina Bat., J.L. Bezerra & H. Maia, in Batista & Bezerra, Portug. acta biol., Sér. B 7(4): 373 (1964) MycoBank: MB 4204

Type species:—*Pleiostomellina pernambucensis* Bat., J.L. Bezerra & Cavalc., in Batista & Bezerra, Portug. acta biol., Sér. B 7(4): 374 (1964) MycoBank: MB 337168 (Fig. 3)

Saprobic on leaves. *Sexual state*: *Ascostromata* solitary to gregarious, superficial to semi-immersed, dark brown to black, carbonaceous, flattened, with numerous locules in a circle, cells of ascostromata comprising brown-walled cells of *textura angularis*. *Locules* 80–120 × 400–450 µm ($\overline{x}$ = 100 × 410 µm, n = 10), globose to subglobose, with individual central ostioles. *Peridium* of locules 16–25 µm diam ($\overline{x}$ = 20 µm, n = 10), comprising one layer of thin-walled, heavily pigmented, small, dark brown to black cells of *textura angularis*. *Pseudoparaphyses* absent. *Asci* 65–80 × 35–50 µm ($\overline{x}$ = 70 × 40 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, thick-walled, without a pedicel and with a distinct ocular chamber. *Ascospores* 38–44 × 15–18 µm ($\overline{x}$ = 42 × 16 µm, n = 40), 2–3-seriate, ellipsoidal, with rounded ends, hyaline when immature and dark brown to reddish brown at maturity, 1-septate, constricted at the septum, verrucose. *Asexual state*: unknown.

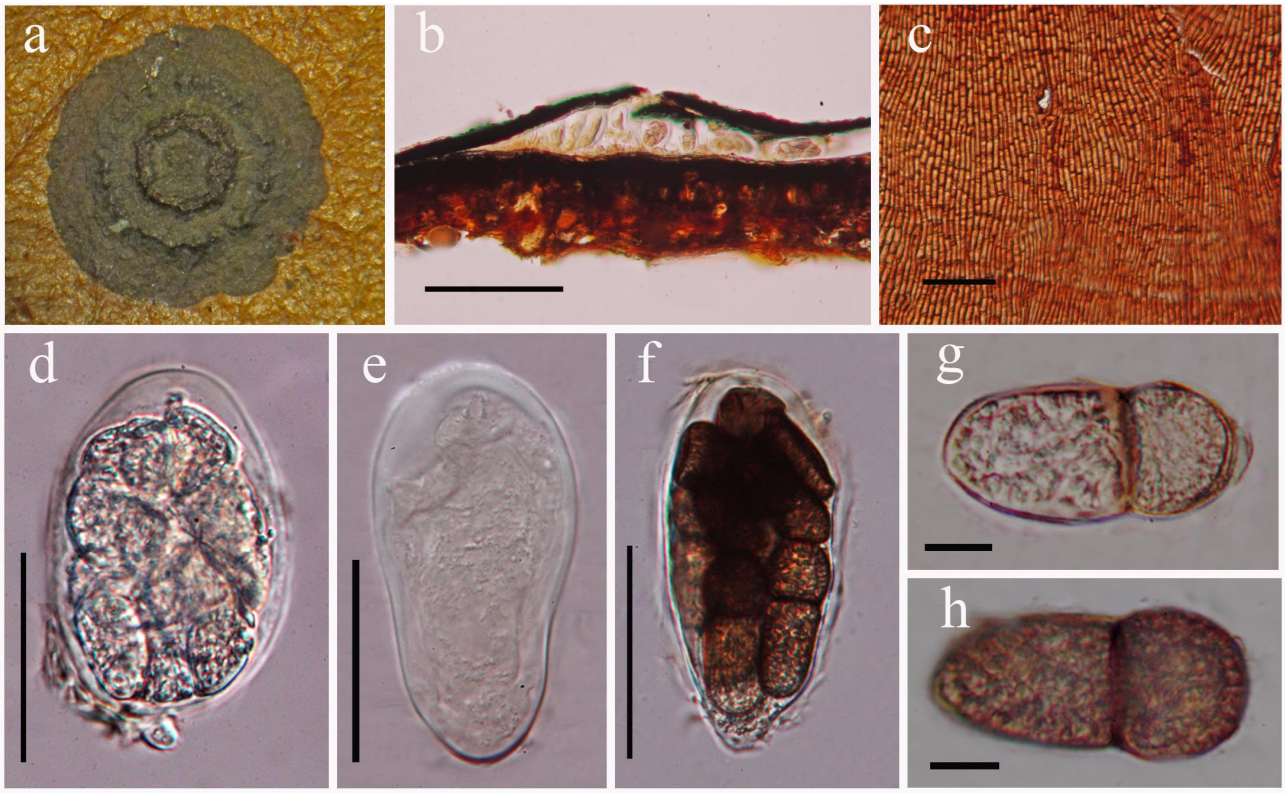

FIGURE 3. *Pleiostomellina pernambucensis* (holotype). a. Close-up of ascostroma. b. Section of ascostroma. c. Close-up of the peridium. d–f. Mature and immature asci without a pedicel. g–h. Dark brown to chestnut-brown ascospores. Scale bars: b = 100 µm, c = 10 µm, d–f = 10 µm, g–h = 5 µm.

Material examined:—BRAZIL. João Pessoa: 11 August 1968, *R. Garnier* (URM!, holotype).

Notes:—Based on superficial, flattened carbonaceous ascomata with numerous locules in a circle, cylindrical asci without a pedicel and 2–3-seriate, ellipsoidal, 1-septate, verrucose ascospores, Batista (1964) introduced *Pleiostomellina* as a monotypic genus typified by *P. pernambucensis*. *Pleiostomellina* shows similarities with Parmulariaceae in having solitary to gregarious, carbonaceous ascomata with multi-locules, fissitunicate, cylindrical asci and dark brown to reddish brown, 1-septate, verrucose ascospores. *Pleiostomellina* differs from the

other genera of *Parmulariaceae* in having thick-walled, bitunicate asci without a pedicel, lack of pseudoparaphyses and 2–3-seriate ascospores having verrucose spore walls (Hyde *et al*. 2013). We refer *Pleiostomellina* to Parmulariaceae based on its similarities with *Parmularia*. The generic type needs recollecting, epitypifying and sequencing so that phylogenetic analysis can be used to confirm family relationships.

Dothideomycetes genera *incertae cedis*

Huhndorf & Lumbsch (2010) included two orders, 34 families and over 100 genera in Dothideomycetes *incertae sedis* because the exact placement of the species within this order is uncertain.

Heterosphaeriopsis Hafellner, Nova Hedwigia, Beih. 62: 175 (1979)

Type species: ***Heterosphaeriopsis fulvodisca*** (Pat.) Hafellner, Beih. Nova Hedwigia 62: 176 (1979) MycoBank: MB 315200 (Fig. 4)
≡ *Karschia fulvodisca* Pat., Bull. Soc. mycol. Fr. 11(4): 218 (1895)

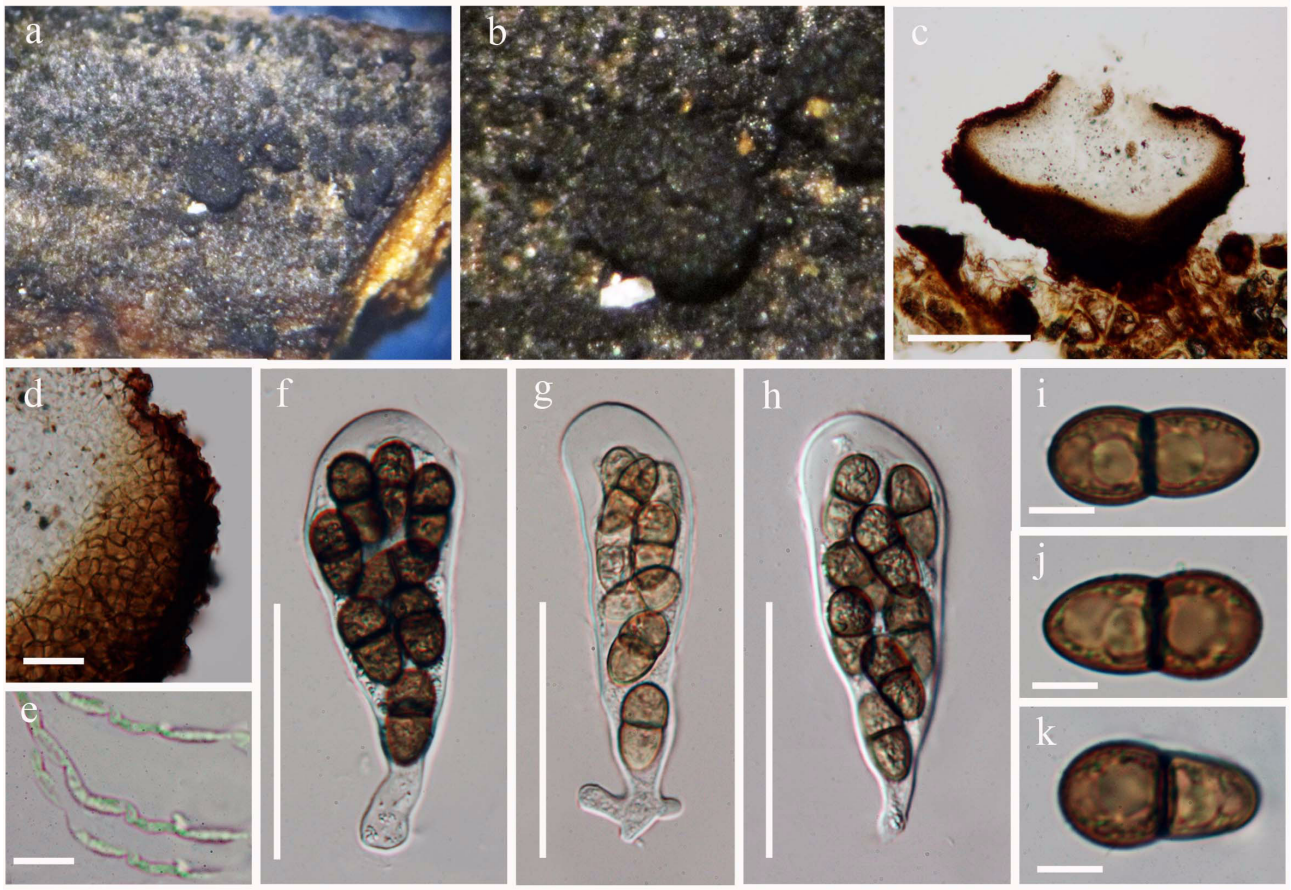

FIGURE 4. *Heterosphaeriopsis fulvodisca* (holotype). a. Ascomata on host substrate. b. Close-up of ascoma. c. Section of ascoma. d. Close-up of peridium. e. Cellular pseudoparaphyses. f–h. Mature and immature asci with short, broad, furcate pedicels. i–k. Light brown to dark brown ascospores. Scale bars: c = 100 µm, d–e =10 µm, f–h = 10 µm, i–k = 5 µm.

Saprobic on dead wood. *Sexual state*: *Ascomata* 340–380 × 420–480 µm ($\overline{x}$ = 350 × 440 µm, n = 10), semi-immersed to superficial, solitary or scattered, dark brown to black, with flattened base, globose or subglobose, carbonaceous, discharging ascospores via a longitudinal slit-like opening. *Peridium* 40–80 µm ($\overline{x}$ = 55 µm, n = 10) thick, comprising 2-layers, outer layer composed of heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells. *Pseudoparaphyses* 2–3 µm ($\overline{x}$ = 3 µm, n = 20) wide, cellular, numerous. *Asci* 75–95 × 20–30 µm ($\overline{x}$ = 80 × 24 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate to broadly-clavate, with short, broad, furcate pedicel, thickened and rounded at apex. *Ascospores* 18–22 × 8–12 µm ($\overline{x}$ = 20 ×

10 µm, n = 40), biseriate to partially overlapping uniseriate near the base, obovoid, 1-septate, and deeply constricted at the septum, light brown to dark brown, walls smooth to verrucose. *Asexual state*: unknown.

Material examined:—SWEDEN. on dead bark (FH, holotype).

Notes:—*Heterosphaeriopsis* is presently monotypic. The genus is placed in Dothideomycetes, genera *incertae sedis* in Index Fungorum (2013) and Lumbsch & Huhndorf (2010). *Heterosphaeriopsis* is characterized by superficial, carbonaceous ascomata with a flattened base, cellular pseudoparaphyses and asci with an unusual furcate pedicel and 1-septate brown ascospores. It resembles some species in *Hysteriaceae* and *Patellariaceae* in possessing hysterothecia opening via a longitudinal slit (Hyde *et al.* 2013). This combination of characters does not fit well in any Dothideomycete families and therefore we retain this genus in Dothideomycetes, genera *incertae sedis*. Morphology coupled with molecular data is essential to establish the correct placement of this unusual genus.

Hyalosphaera F. Stevens, Trans. Ill. St. Acad. Sci. 10: 172 (1917) MycoBank: MB 2393

Pathogenic or *saprobic* on leaves. *Sexual state*: *Ascomata* superficial, solitary or scattered, globose to subglobose, smooth. *Peridium* composed of smooth-walled, hyaline cells. *Pseudoparaphyses* absent. *Asci* 8-spored, bitunicate, narrowly obovoid, with thickened, short, furcate pedicel. *Ascospores* filiform to subcylindrical, multi-septate with narrow rounded ends, yellow to chestnut-brown. *Asexual state:* unknown.

Type species:—*Hyalosphaera miconiae* F. Stevens, Trans. Ill. St. Acad. Sci. 10: 172 (1917) MycoBank: MB 215013 (Fig. 5)

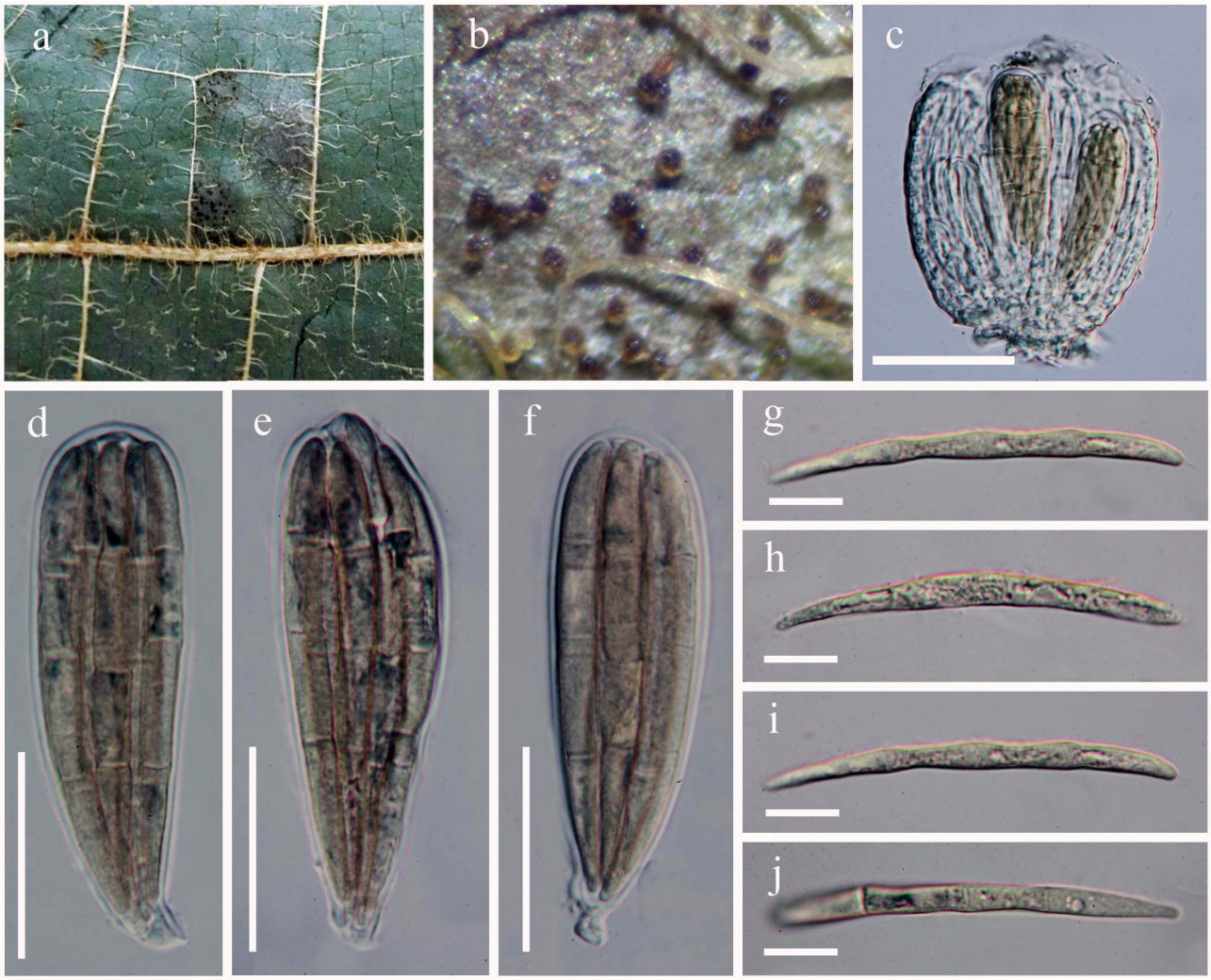

FIGURE 5. *Hyalosphaera miconiae* (holotype). a. Herbarium material. b. Ascomata on host substrate. c. Section of ascoma. d–f. Mature asci with short pedicel. g–j. Brown to chestnut-brown ascospores. Scale bars: c = 100 µm, d–f = 15, g–i = 5 µm.

Pathogenic or *saprobic* on leaves. *Sexual state*: *Ascomata* 90–190 × 80–130 µm ($\bar{x}$ = 130 × 110 µm, n=10), superficial, solitary or scattered, globose to subglobose, yellowish brown to dark brown, base flattened on the host surface, smooth, dehiscence via a irregular slit appearing on upper part of the wall. *Peridium* 2.5–5 µm ($\bar{x}$ = 4 µm, n=10) thick, composed of hyaline cells of *textura angularis*. *Pseudoparaphyses* absent. *Asci* 50–65 × 12–15 µm ($\bar{x}$ = 58 × 14 µm, n=20), 8-spored, bitunicate, narrowly obovoid, with short furcate pedicel, thickened and rounded at apex. *Ascospores* 47–54 × 4–5 µm ($\bar{x}$ = 50 × 5 µm, n=40), uniseriate, filiform, 3-septate with narrow rounded ends, guttulate, yellow to chestnut-brown, verruculose or smooth-walled, lacking a sheath. *Asexual state*: unknown.

Material examined:—COSTA RICA. El Limon: Valle de Puerto La Cruz, Federal District, on leaf of Melastomataceae, 17 January1928, *H. Sydow* (286A), (BPI 635922!, holotype).

Notes:—Currently, four *Hyalosphaera* species are listed in Index Fungorum (2013) and no DNA sequence data is available for any of these species. *Hyalosphaera* consists of fleshy to gelatinous ascomata whose asci lack an amyloid reaction. Ascomata are possibly apothecioid and ascospores are possibly released by deliquescence of the entire ascomata. *Hyalosphaera* resembles the unusual genus *Corynelia* (Benny *et al*.1985) by apothecioid ascomata and ascospores released by deliquescence of the entire ascomata. Therefore the genus might be better accommodated in Coryneliales. We however, retain this genus in Dothideomycetes, genera *incertae sedis* until morphology coupled with molecular data can resolve the correct placement of this unusual genus.

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