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FULL PAPER

Richard T. Hanlin · María C. González · Omar Tortolero
Juan Renaud

A new species of *Ophiodothella* on *Casearia* from Venezuela

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Abstract *Ophiodothella casearіae* sp. nov. from leaves of *Casearia tremula* in Venezuela is described and illustrated.

Key words Ascomycota · Foliicolous ascomycete · Phyllachoraceae · Xylariales

Introduction

Foliicolous ascomycetes are a common inhabitant of the tropical phanerogamic flora, on which they often cause leafspot diseases. Collections of ascomycetes in Venezuela have yielded a foliicolous perithecial species on *Casearia tremula* (Griseb.) Wright (palo bonaire) (Flacourtiaceae), which was subsequently identified as belonging to the genus *Ophiodothella*. Comparison of the Venezuelan material with published descriptions of *Ophiodothella* species (Hanlin et al. 1992; Hsieh et al. 1997, 1998; Hyde 1999; Pearce and Hyde 1993) showed that it differs from the previously described species in several respects. Accordingly, it is described as a new species.

Materials and methods

Observations were made on living material collected in the field and the data were recorded. Small pieces of lesions were removed from living leaves to be killed and fixed for sectioning. Attempts to isolate the fungus in pure culture were unsuccessful. The host leaves were then air-dried in a standard botanical press for permanent preservation. Material to be sectioned was killed and fixed in formalin-propionic acid-alcohol (FPA) and embedded in paraplast, following procedures described previously (Hanlin and Tortolero 1989). For subsequent study, dried material was rehydrated in water before mounting on standard glass slides. Measurements of asci, ascospores, and conidia were made on material mounted in water; measurements of pycnidia and perithecia were made on sectioned material. Photographs were taken with a Nikon HFX camera on a Nikon SMZ stereo microscope (Nikon, Tokyo, Japan) or an Optiphot equipped with Nomarski differential interference contrast (DIC) (Nikon, Tokyo, Japan). Kodak Technical Pan film (Kodak, Rochester, USA) was used for photography.

R.T. Hanlin (✉)
Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274, USA
Tel. +1-706-542-1280; Fax +1-706-542-1262
e-mail: rhanlin@arches.uga.edu

M.C. González
Instituto de Biología, Universidad Nacional Autónoma de México, México City, DF, México

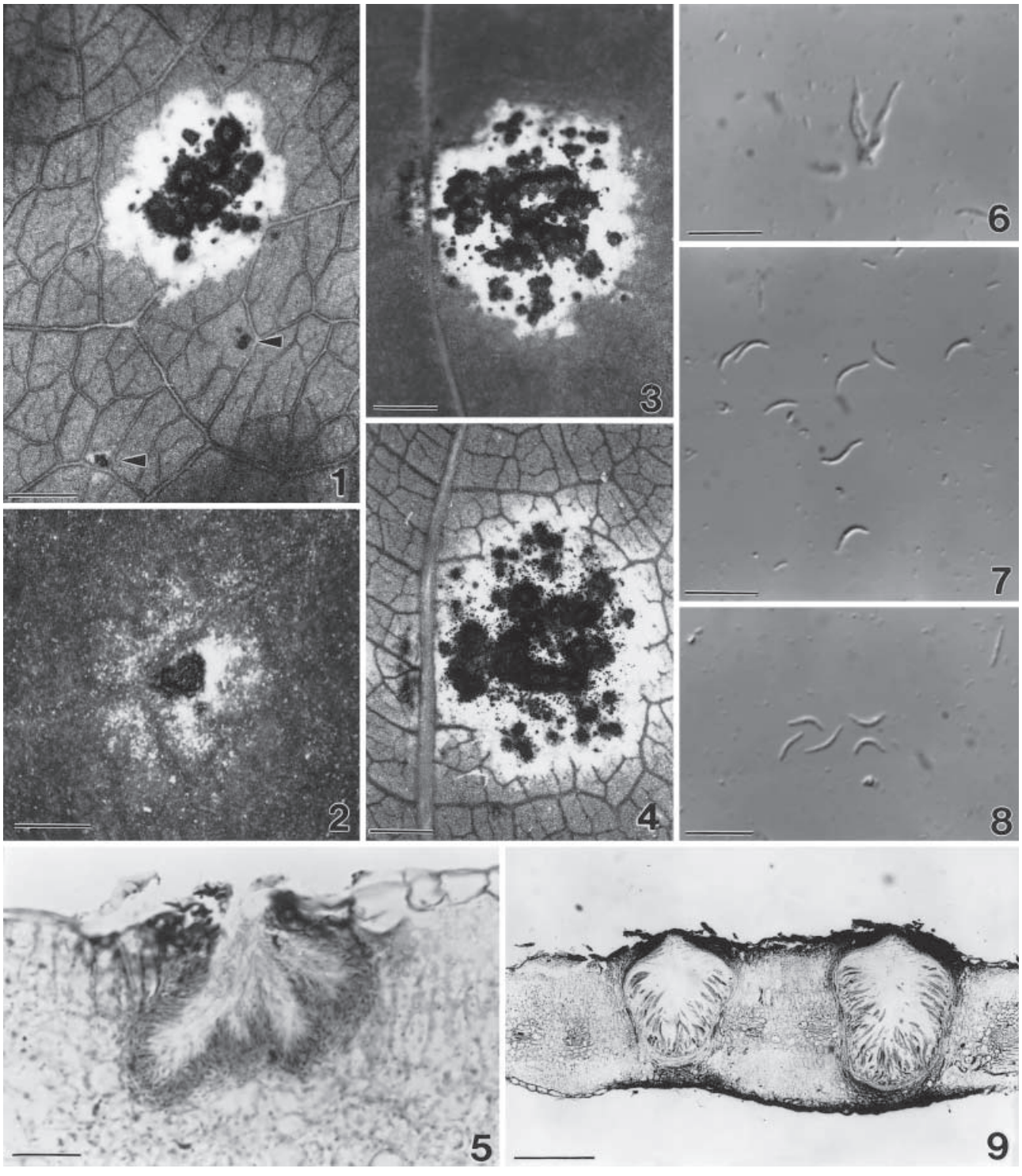
O. Tortolero · J. Renaud
Posgrado en Fitopatologia, Universidad Centro Occidental Lisandro Alvarado, Barquisimeto, Venezuela

Taxonomy

Ophiodothella casearіae Hanlin, M.C. González, Tortolero et Renaud, sp. nov. Figs. 1–19

Pycnidia ovoidea vel ellipsoidea, ostiolata, 92–148 × 82–158 μm, in contexto folii immersa, clypeo atro subjecta. Paries pycnidii 6–10 μm latus, ex hyphis hyalinis complanatis compositus. Cellulae conidiogenae 10.5–17.5 μm longae, basi 2–3 μm latae, apicem versus gradatim angustata. Conidia acrogena hyalina, aseptata, 8.6–11.4 × 1.75 μm, cylindrica, frequenter curvata vel sigmoidea.

Perithecia in contexto folii immersa, subglobosa, obpyriformia vel ellipsoidea, 304–364 × 265 μm, uni-vel biostiolata. Ostiolum erumpens, clypeo atro circumcinctum. Paries perithecii 16–24 μm latus, ex cellulis brunneis complanatis compositus. Paraphyses septatae, filiformes, numerosae. Asci unitunucati, ellipsoidei, apice late rotundati, 84.5–106.5 × 15–19 μm, octospori, annulo apicali iodo caerulescenti praediti. Ascosporae unicellulares, hyalinae, cylindricae vel apicem versus leviter attenuatae, curvatae, laeves, 53–67 × 3.5–4 μm.



Figs. 1–9. *Ophiodothella caseariae*. **1** Perithecial (*top*) and pycnidial (*arrowheads*) lesions on leaf of *Casearia*. **2** Close-up of pycnidial lesion on upper surface of leaf. **3** Perithecial lesion on upper surface of leaf. **4** Same lesion on underside of leaf. **5** Section through pycnidium. **6** Conidiogenous cells. Differential interference contrast (DIC). **7, 8** Conidia. DIC. **9** Two mature uniostiolate perithecia in leaf, with clypeus on upper and lower sides of leaf. *Bars* **1** 600 μm; **2** 300 μm; **3, 4** 700 μm; **5** 20 μm; **6–8** 10 μm; **9** 150 μm

Holotypus: GAM 12772, J. Renaud, in foliis vivis *Caseariae tremulae.*

Mycelium internal in host tissues, forming pale yellow, amphigenous lesions on leaves (Fig. 1). Pycnidial lesions scattered, rounded to irregular or elongate (Fig. 2), 98–240 × 91–141 μm, ($\bar{\chi}$ = 170 × 116 μm) discrete, with indistinct border, soon becoming black in center on upper surface of leaf, on lower leaf surface lesion evident only as a pale yellow area. Perithecial lesions rounded to irregular, 2.25–4.25 × 1.9–3.5 mm ($\bar{\chi}$ = 3 × 2.7 mm), with a raised, white

center that soon develops black areas (clypei) in center on both sides of leaf. Mature lesions with hemispherical to elongate, raised black clypei in center and a well-defined white border (Figs. 3, 4).

Pycnidia immersed in leaf tissue, epiphyllous, variable in shape, ovoid to transversely elliptical (Fig. 5), 92–148 × 82–158 μm ($\bar{\chi}$ = 120.5 × 120 μm), with single ostiolar papilla erumpent through host epidermis. Ostiole surrounded by blackened clypeus consisting of dark brown hyphae in host epidermis. Pycnidial wall 6–10 μm ($\bar{\chi}$ = 8.5 μm) wide, composed of flattened, hyaline hyphae. Cavity of pycnidium lined with crowded conidiogenous cells. Conidiogenous cells 10.5–17.5 μm long, 1.85–2.65 μm wide at base ($\bar{\chi}$ = 14 × 2.2 μm), tapering gradually to a slender tip on which conidia are formed (Fig. 6). Conidia one-celled, hyaline, 8.6–11.4 ($\bar{\chi}$ = 10) × 1.75 μm, cylindrical or widest at middle and tapering toward the ends, frequently curved or sigmoid (Figs. 7, 8).

Perithecia immersed in leaf tissue, filling interior of leaf, with erumpent ostiolar papilla (Fig. 9), 304–364 × 224–306 μm ($\bar{\chi}$ = 334 × 265 μm). Perithecia of two types, uniostiolate (Fig. 10) and biostiolate (Fig. 11). Uniostiolate perithecia subglobose to obpyriform, or widest at top with sides strongly constricted by veins or laterally flattened by compression of adjacent perithecia, ostiole epiphyllous. Biostiolate perithecia elliptical, with ostiolar papillae erumpent through both epidermal layers. Ostioles lined with slender periphyses. Area around ostiole and beneath uniostiolate perithecia surrounded by a blackened clypeus consisting of dark brown hyphae. Perithecial wall 16–24 ($\bar{\chi}$ = 19) μm wide, cells strongly flattened (Fig. 12), dark brown along top and bottom of perithecium, light brown to subhyaline along sides, with thickened walls on the exterior, thinner-walled and hyaline on the inside. Centrum containing numerous septate, filamentous paraphyses (Fig. 13). In uniostiolate perithecia, paraphyses and asci lining inside of perithecial wall; in biostiolate perithecia, paraphyses and asci limited to sides of perithecium and projecting inward. Asci thin-walled (unitunicate), ellipsoid, with broadly rounded apex (Fig. 14), 84.5–106.5 × 15–19 μm ($\bar{\chi}$ = 95 × 17 μm), 8-spored, arising from base of centrum and growing up among paraphyses. Ascus apex containing a small refractive ring (Fig. 15); ring in optical section appearing as two dots that stain blue in iodine (IKI) (Fig. 16). Ascospores hyaline, one-celled, 53–67 × 3.3–3.7 μm ($\bar{\chi}$ = 60 × 3.6 μm), cylindrical to slightly tapered toward the rounded ends, usually curved, smooth (Fig. 17).

Holotype: On living leaves of *Casearia tremula* (Griseb.) Wright (Flacourtiaceae). GAM 12772, via Agua Viva, Cabudare, Edo. Lara, Venezuela. Nov. 27, 1991, J. Renaud. Isotype deposited in VIA (Holmgren et al. 1990).

Etymology: From the Latin, *casearia*e, for the host genus.

Additional material examined: Venezuela: On *Casearia tremula*. via Agua Viva, Cabudare, Edo. Lara, Nov. 27, 1981, J. Renaud (GAM 12773); via Agua Viva, Cabudare, Edo. Lara. Nov. 6, 1986, J. Renaud (GAM 12770); via Agua Viva, Cabudare, Edo. Lara. Feb. 8, 1988, J. Renaud (GAM 12771).

The fungus is known only from the type locality. Attempts to find it on the host in other locations have been unsuccessful.

Areas of the leaf containing perithecial lesions become greatly thickened, attaining about twice the thickness of uninfected tissue (Fig. 18).

Perithecial initials develop deep inside the leaf tissue and are provided with slender trichogynes that extend beyond the surface of the epidermis (Fig. 19).

The mode of conidiogenesis could not be determined with certainty because of the small size of the conidiogenous cells.

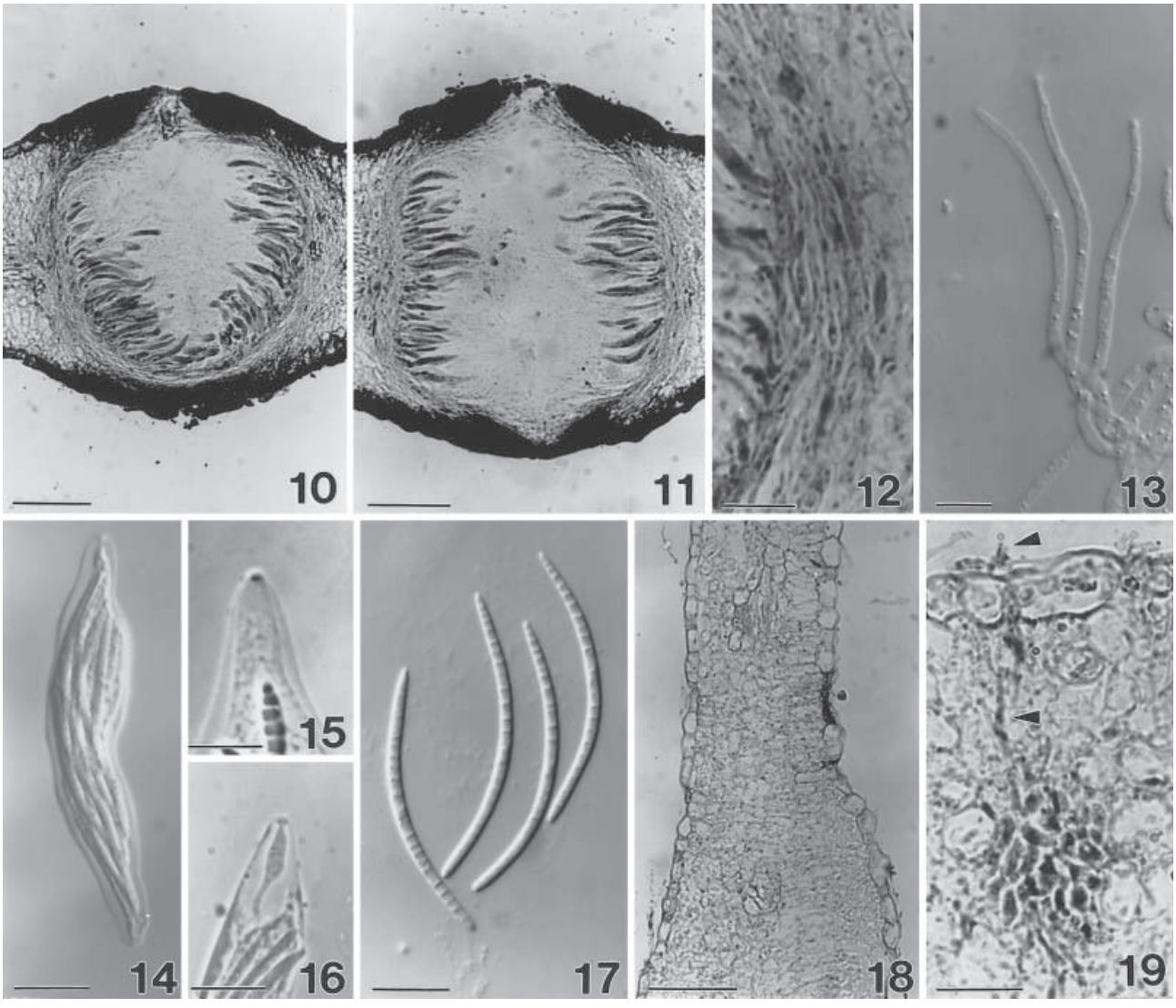
Discussion

Ophiodothella is a genus of perithecial ascomycetes that is characterized by the formation of ostiolate perithecia immersed in host leaves in which a blackened clypeus typically occurs around the ostiole and beneath the perithecium opposite the ostiole. The unitunicate asci possess an amyloid apical ring and contain hyaline, scolecosporous ascospores. *Ophiodothella casearia*e is most similar in ascus and ascospore size to *O. circularis* (Bres.) Petr. and *O. floridana* Chardón, and less so to *O. edax* (Berk. & Broome) Höhn. and *O. neurophila* Syd. (Hanlin et al. 1992; Hsieh et al. 1997, 1998; Hyde 1999; Pearce and Hyde 1993). Of these four species, *O. edax* has spermogonia but lacks paraphyses. The other three species have paraphyses but lack spermogonia. No species of *Ophiodothella* has been reported on *Casearia* or on any member of the Flacourtiaceae. Because none of the described species combines the characteristics of the *Casearia* pathogen, we are therefore designating it as a new species.

An unusual feature of *O. casearia*e is the occurrence of ostioles on both the top and bottom of the same perithecium. Boyd (1934) reported that the perithecia of *O. vaccinii* E.S. Boyd were biostiolate; this was confirmed by Hanlin (1990a), who pointed out however, that uniostiolate perithecia are also common in this species. The occurrence of biostiolate perithecia of the type described for *O. casearia*e and *O. vaccinii* is unusual among the perithecial ascomycetes. Theissen and Sydow (1915) described a similar phenomenon in *Catacauma biguttulatum* (Theiss.) Theiss. (≡*Phyllachora biguttulata* Theiss.), and in *Diachora onobrychidis* (DC.) J. Müll. (Arx and Müller 1954) the asci are arranged in an equatorial band around the perithecium and are lacking along the bottom. Biostiolate perithecia also are occasionally observed in *Melanospora zamiae* Corda grown in culture.

Whether the spores designated herein as conidia represent an anamorph or andromorph of the fungus is uncertain. Attempts to germinate them were unsuccessful. The presence of perithecial initials with trichogynes that extend beyond the surface of the epidermis suggests that an andromorph is present in the life cycle of the fungus.

Ophiodothella has traditionally been classified in the Phyllachoraceae (Eriksson and Hawksworth 1993) of the



Figs. 10–19. *Ophiodothella casearia*e. **10** Section through uniostiolate perithecium. **11** Section through biostiolate perithecium with laterally attached asci. **12** Close-up of perithecial wall. **13** Filamentous paraphyses. DIC. **14** Mature ascus with ascospores. DIC. **15** Ascus apex. DIC. **16** Dark ascus ring stained blue in iodine. **17** Mature ascospores. DIC. **18** Section through *Casearia* leaf showing uninfected (*top*) and infected (*bottom*) portions. **19** Section through leaf with perithecial initial and trichogyne (*arrowheads*) extending to leaf surface. *Bars* **10, 11** 50 μm; **12, 13, 15, 16** 10 μm; **14** 20 μm; **17, 19** 15 μm; **18** 40 μm

Phyllachorales, primarily because of its general similarity to species of *Phyllachora*, also a foliicolous pathogen with immersed, ostiolate perithecia and hyaline, one-celled ascospores. One difference between the two genera, however, that is regarded as taxonomically significant is the apical ring in the ascus; in *Phyllachora* it is nonamyloid, whereas in *Ophiodothella* it stains blue in iodine (Hanlin 1990b). Additionally, the conidia (or spermatia) described for *O. casearia*e are very similar to those described for species of *Diatrype*, *Diatrypella*, *Eutypa*, and *Eutypella* of the Diatrypaceae (Xylariales) (Glawe and Rogers 1982a,b), although they tend to be somewhat smaller. These characteristics suggest a relationship between *Ophiodothella* and the Xylariales, and such a placement is supported by molecular data. In an analysis of 18S rDNA sequences of *O. vaccinii*, this species grouped with *Pestalosphaeria* of the Xylariales rather than with *Phyllachora* (Silva 1996). *Pestalosphaeria* also forms nonstromatic, ostiolate perithecia that are immersed in the substrate, often leaves, and the unitunicate asci have an amyloid apex. The ascospores, however, are septate and brown in *Pestalosphaeria* (Hanlin 1990b). Thus it appears that *Ophiodothella* is best accommodated in the Xylariales.

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