
Neotropical pyrenomycetes: *Porosphaerella borinquensis* sp. nov. and its *Pseudobotrytis terrestris* anamorph

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Porosphaerella borinquensis is described and its connection to *Pseudobotrytis terrestris* is established *in vitro*. Its relationships at the generic and familial level are discussed. It is similar to *Porosphaerella cordanophora* in ascomatal and ascospore morphologies, although somewhat smaller. In both species the anamorphs have conidiogenous loci resembling spinules and pigmented, one-septate, ellipsoid to oblong conidia. *Pseudobotrytis terrestris* differs from *Cordana pauciseptata* by having conidiophores arranged in an umbellate fashion. Although the data are not shown here, nuclear large sub-unit ribosomal DNA from both *P. borinquensis* and *P. cordanophora* support their generic affinities. Other work has shown that *Porosphaerella* appears to have affinities to the *Coniochaetales*.

Key words: *Chaetosphaeriaceae*, *Cordana*, *Trichosphaeriaceae*.

Introduction

The genus *Porosphaerella* encompasses pyrenomycetes with small superficial ascomata, simple walls, one-septate ascospores and a *Cordana* anamorph (Müller and Samuels, 1982). It contains two species: *P. cordanophora* Müller & Samuels (Müller and Samuels, 1982) and *P. setosa* Romero & Samuels (Romero and Samuels, 1991). The genus was originally placed in the *Trichosphaeriaceae* (Müller and Samuels, 1982) and this placement was maintained by Barr (1990). The genus was transferred to the *Chaetosphaeriaceae* (Réblová *et al.*, 1999) although its placement in this family was not supported by DNA molecular analysis (Réblová and Winka, 2000). The true affinities of *Porosphaerella* appear to be close to the *Coniochaetales* (Huhndorf *et al.*, 2004).

Culture studies of a pyrenomycete collected in Puerto Rico produced an anamorph fitting the description of *Pseudobotrytis terrestris* (Timonin) Subram. (Subramanian, 1956). After comparing the teleomorph and anamorph to *P. cordanophora*, the pyrenomycete is described as a new species of *Porosphaerella*.

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Materials and methods

Ascomata contents were extracted and spread onto water agar plates. Germinating single and/or multiple ascospores were cut out from the medium and transferred to water agar, cornmeal agar and malt-extract agar in 6 cm diam. Petri-dishes, and incubated for 30 days at room temp. Colonies were observed for morphological characteristics and the presence of reproductive structures, particularly anamorphs. Ascomata were sectioned at 5 μm for light microscopy using the techniques of Huhndorf (1991) and images were captured using bright field (BF), phase contrast (PH) and differential interference microscopy (DIC). Images were captured and photographic plates were produced following the methods of Huhndorf and Fernández (1998).

Taxonomy

Porosphaerella borinquensis F.A. Fernández & S.M. Huhndorf, **sp. nov.**

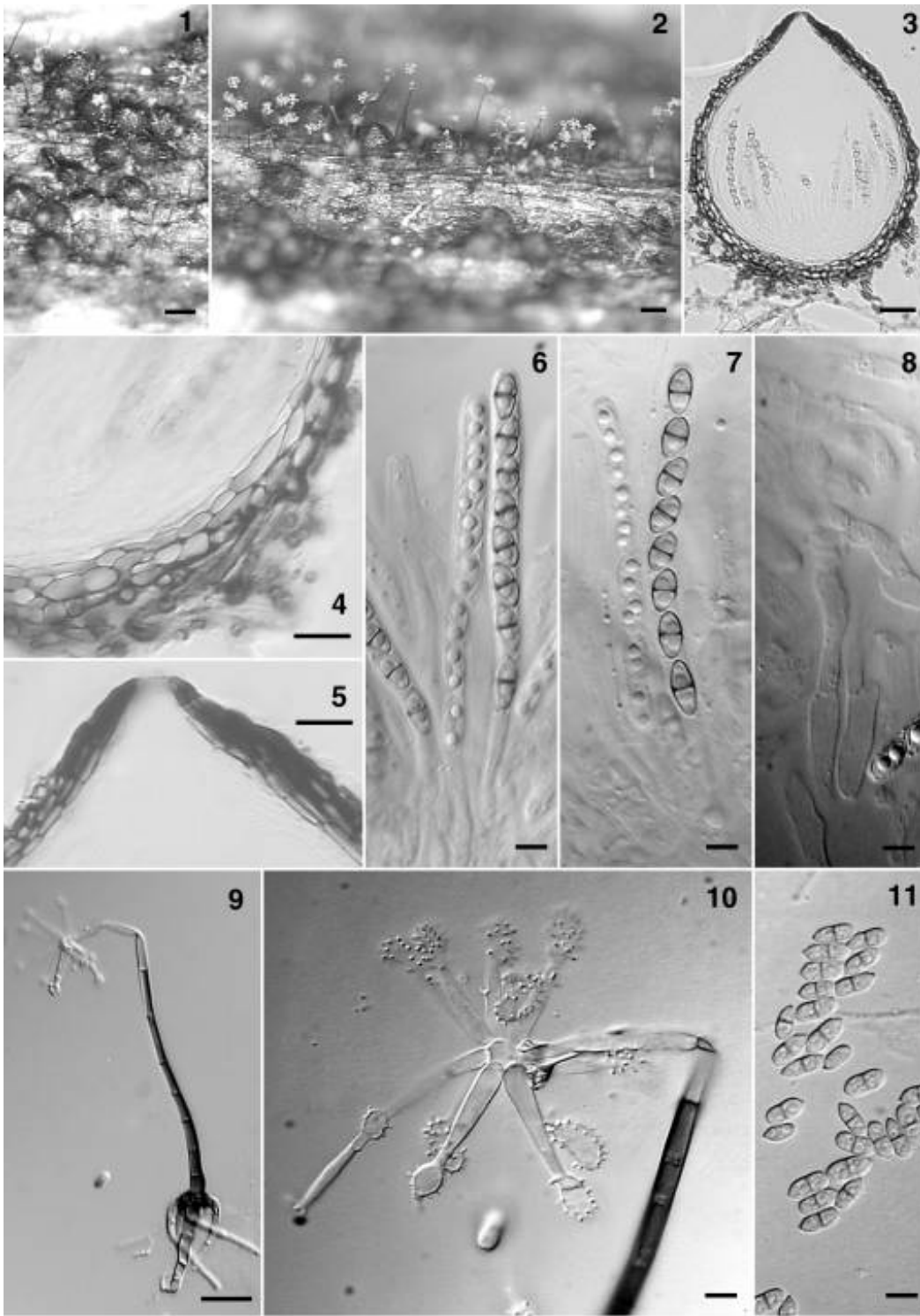
(Figs. 1-11)

Etymology: Refers to Borinquen, the indigenous name of Puerto Rico.

Ascomata globosa vel ovoidea, atrobrunnea, 0.12-0.13 mm diam., 0.12-0.15 mm alta, solitaria vel aggregata, superficialia; superficie glabrata. *Paries ascomatis* in sectione longitudinali 10-12 μm crassus. *Asci* cylindrici, brevi pedicellati, 60-74 \times 4-5 μm , unitunicatae, octospori, ascosporae uniseriatae. *Ascosporae* ellipsoideae, uniseptatae, ad septa constrictae, pallidae-brunneae, 7-9 \times 3-4 μm . *Anamorph*: *Pseudobotrytis terrestris* (Timonin) Subram. (Subramanian, 1956).

Ascomata globose to broadly ovoid, dark brown, 0.12-0.13 mm diam., 0.12-0.15 mm high, glabrous, scattered to gregarious, superficial. Ascomatal wall 10-12 μm thick in longitudinal section, composed of 3-5 layers of pseudoparenchymatic cells. *Ascomata apex* papillate. *Paraphyses* simple, unbranched, hyaline. *Asci* cylindrical, short-stalked, 60-74 \times 4-5 μm (\bar{x} = 65.4 \times 4.4 μm), unitunicate, apical ring inconspicuous, with 8 uniseriate ascospores. *Ascospores* ellipsoid, 1-septate, constricted, light brown, 7-9 \times 3-4 μm (\bar{x} = 7.3 \times 4.2 μm). *Conidiophores* 120-233 \times 4-6 μm , dark brown at the base and lighter towards the apex. *Conidiogenous cells* terminal, discrete, hyaline, in groups of 8 to 14 arranged in umbels, 15-26 \times 3-4 μm , swollen to 4-6 μm at the apex, with multiple conidiogenous loci appearing as spinules. *Conidia* mostly oblong, sometimes ellipsoid, 1-septate, light brown, 6.5-7.5 \times 2.5-3 μm . *Anamorph*: *Pseudobotrytis terrestris* (Timonin) Subram. (Subramanian, 1956).

Figs. 1-11. *Porosphaerella borinquensis* (from holotype). **1.** Ascomata on the substrate. **2.** Conidiophores on the substrate. **3.** Longitudinal section through ascoma. **4.** Longitudinal section showing the ascomal walls. **5.** Longitudinal section showing ostiole. **6, 7.** Asci. and ascospores. **8.** Paraphyses. **9.** Conidiophore on CMA. **10.** Apex of conidiophore showing umbel of polyblastic conidiogenous cells with denticles. **11.** Conidia from Bars: 1, 2 = 100 μm ; 3, 9 = 20 μm ; 4, 5 = 10 μm ; 6-8, 10-11 = 5 μm .



Habitat: On *Cecropia* leaf.

Known distribution: Brazil, Canada, Cuba, Democratic Republic of Congo (Zaire), Jamaica, Japan, India, New Zealand, Panama, Poland, Puerto Rico, Thailand, USA (Georgia, Iowa).

Material examined: USA, Puerto Rico, Luquillo Mountains, Bisley watershed 3, on *Cecropia* leaf, 27 January 1997, F.A. Fernández smh 3138 (**holotype:** F).

Notes: Geographical distribution is almost entirely based on reports of occurrence of the anamorph (Krzemieniewska and Badura, 1954; Morris, 1955; Subramanian, 1956; Ellis, 1971; Tokumasu and Aoiki, 2002; Castañeda-Ruiz *et al.*, 2003; Photita *et al.*, 2003).

Discussion

Porosphaerella borinquensis is similar to *P. cordanophora* in ascomatal, ascospore and conidium morphologies, differing only in being somewhat smaller than in *P. cordanophora*. There are also similarities between *Pseudobotrytis* and *Cordana pauciseptata* Preuss, the anamorph of *P. cordanophora*. The complex branching of the conidiogenous cells in *Pseudobotrytis*, for example, can be viewed as multiple repeats of a *Cordana* conidiophore in an umbellate arrangement. Also, conidiogenous loci resembling spinules and pigmented, one-septate, ellipsoid to oblong conidia are present in both species. Similarities between *Pseudobotrytis* and *C. pauciseptata* were also recognized by Krzemieniewska and Badura (1954) who placed them in the Cordanea-group. These morphological similarities and a comparison of partial sequences of the nuclear large sub-unit ribosomal DNA from both *P. borinquensis* and *P. cordanophora* (data not shown) support their generic affinities. *Porosphaerella cordanophora* was found to have affinities to the *Coniochaetales* (Huhndorf *et al.*, 2004).

Porosphaerella setosa Romero & Samuels resembles both *P. cordanophora* and *P. borinquensis* in the one-septate ascospores with terminal pores (Romero and Samuels, 1991). Presence of stiff setae on the ascomata distinguishes it from the other two species.

The biological connection between *Porosphaerella borinquensis* and *Pseudobotrytis terrestris* is proven by culture of ascospores *in vitro*. The anamorph produced *in vitro* fits the characteristics of those found on the natural substrate. Two species of *Pseudobotrytis* have been described: *P. terrestris*, and *P. bisbyi* Timotin (Timotin, 1961). *Pseudobotrytis terrestris* was first isolated from the rhizosphere of alfalfa and described as *Spicularia terrestris* Timotin (Timotin, 1940). Apparently, the name *Spicularia* was improperly emended and misapplied to *S. terrestris*; the genus *Umbellula* E.F. Morris was erected, and the new combination *Umbellula terrestris* (Timonin)

E.F. Morris created (Morris, 1955). It was later determined that *U. terrestris* and *Pseudobotrytis fusca* Krzemieniewska & Badura were congeneric, giving rise to *Pseudobotrytis terrestris* (Subramanian, 1956). The anamorph is cosmopolitan in distribution and has been isolated from soil and plant substrates in temperate and tropical areas (Krzemieniewska and Badura, 1954; Morris, 1955; Subramanian, 1956; Castañeda-Ruiz *et al.*, 2003; Photita *et al.*, 2003). Similarly, the anamorph of *P. cordanophora* is cosmopolitan (Müller and Samuels, 1982). It appears that both species of *Porosphaerella* are widely distributed as indicated by the distribution of their anamorphs.

Eleven species of *Cordana* have been reported, some of which have already been transferred into other anamorphic genera (Castañeda-Ruiz *et al.*, 1999). Except for *C. pauciseptata*, their relationships to the genus *Porosphaerella* are unknown.

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