



Brazilian Semi-Arid Ascomycetes II: New and interesting records of Bertiaceae, Nitschkiaceae and Scortechiniaceae (Coronophorales, Sordariomycetes)

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With 2 figures and 1 table

Abstract: This is the second paper in a series of articles reporting the diversity of Dothideomycetes and Sordariomycetes in three enclaves of Atlantic Forest in the Caatinga Biome of the semi-arid region of Brazil. We provide illustrations, descriptions and notes of five species representing three families in the Coronophorales. *Bertia convolutispora* and *B. moriformis* are new records for South America, while *B. tropicalis* (Bertiaceae) and *Scortechinia diminutispora* (Scortechiniaceae) are new records for Brazil. *Fracchiaca broomeana* (Nitschkiaceae) has been previously reported from Brazil. Molecular analysis of partial sequences of the 28S nrDNA confirms the identifications of *B. tropicalis* and *S. diminutispora*.

Key words: Lignicolous Ascomycota, Hypocreomycetidae, taxonomy.

Introduction

Coronophorales is a monophyletic order that includes 26 genera distributed in five families (Huhndorf et al. 2004, Lumbsch & Huhndorf 2010, Mugambi & Huhndorf 2010, Vasilyeva et al. 2012). This order is characterized by superficial, stipitate, turbinate or subglobose to broadly ellipsoid, often collapsed ascomata, which may or may not be surrounded at the base by a subiculum. The cells of the peridium usually have pores called "Munk pores" and the members in one family (*Scortechiniaceae*) produce a quellkörper. A quellkörper is a cylindrical or cone-shaped structure orientated downward from inside the ascomatal apex, formed by a mass of thick-walled, hyaline

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cells that is probably involved in ascospore discharge (Mugambi & Huhndorf 2010). The asci are unitunicate, clavate to fusiform, stipitate and contain eight or more allantoid, subcylindrical, fusiform or ellipsoid, hyaline, unicellular or septate ascospores. Members of the order occur worldwide, predominantly as saprobes on bark and/or decorticated wood (Nannfeldt 1975a, Sivanesan 1978, Mugambi & Huhndorf 2010).

During a survey of ascomycetes in three enclaves of Atlantic Forest in the Caatinga biome of Brazil, five species in three genera were found representing three families. Descriptions and illustrations are presented below, including the phylogenetic placement for *Bertia tropicalis* Huhndorf, A.N.Mill. & F.A.Fern. and *Scortechinia diminutispora* Mugambi & Huhndorf.

Materials and methods

STUDY AREA AND MORPHOLOGICAL STUDY: Samples of dead twigs and decaying wood were collected from March 2011 to May 2013 in three enclaves of Atlantic Forest in the Caatinga biome of the semi-arid region of Brazil. Description of the study area and methods for collection and morphological examination of specimens have been previously described (Almeida et al. 2014b, Almeida et al. 2014a). Specimens are deposited in the Herbarium of the State University of Feira de Santana (HUEFS).

MOLECULAR STUDY: Ascomata were rehydrated in 50 μ L AP1 buffer for 3 h, followed by freezing at -80°C for 7 days before DNA extraction. DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California). PCR amplification and sequencing of 28S nrDNA were performed as described by Almeida et al. (2014b).

PHYLOGENETIC ANALYSES: The new sequences generated in this study were aligned with 36 sequences in three families of *Coronophorales* available from GenBank. Three taxa of *Hypocreales* were used as outgroup. The final dataset consisted of 41 sequences, which were aligned using the program MUSCLE[®] as implemented in Seaview 4.4 (Gouy et al. 2010). Manual corrections were performed using Mesquite 3 (Maddison & Maddison 2014). Ambiguous regions were excluded from the final alignment using Gblocks (Castresana 2000, Talavera & Castresana 2007). Alignments and trees are accessible through TreeBase under the reference number S18496 (<http://purl.org/phylo/treebase/phylows/study/TB2:S18496>). The General Time Reversible (GTR) model (Rodríguez et al. 1990) was determined as the best-fit model of evolution by jModeltest (Posada 2008). Independent maximum likelihood (ML) analyses were performed with RAxML 7.0.4 (Stamatakis et al. 2008) run on the CIPRES Portal v. 2.0 (Miller et al. 2010) with the default rapid hill-climbing algorithm and GTR model employing 1000 fast bootstrap searches. Bayesian analyses were performed using MrBayes v 3.12 (Huelsenbeck & Ronquist 2001, Huelsenbeck & Ronquist 2005) under the above model on the CIPRES Portal v. 2.0 (Miller et al. 2010). Constant characters were included and 100 million generations with trees sampled every 1000th generation were run resulting in 100,000 total trees. The first 10,000 trees were discarded as burn-in and Bayesian posterior probabilities (BPP) were determined from a consensus tree generated from the remaining 90,000 trees using PAUP* 4.0b10 (Swofford 2002).

Results

Taxonomy

Bertia convolutispora K.D.Hyde, Nova Hedwigia 61(1–2): 142. 1995. Fig. 1A–E

ASCOMATA superficial, clustered, turbinate, apically or laterally collapsed when dry, with a sterile base, surface tuberculate, black, 460–850 μ m wide, 390–900 μ m high,

ostiole not seen. Munk pores present. Paraphyses not seen. ASCI unitunicate, clavate, long stipitate, apically truncate, with refractive apical ring, 8-spored, 147–213 × 10–15 µm. ASCOSPORES arranged in upper portion of the ascus, irregularly biseriolate, allantoid, curved at base, ends rounded, 1-septate, smooth, hyaline, 18–24 × 4.5–6 µm.

ANAMORPH: Unknown.

GEOGRAPHICAL DISTRIBUTION: Australia (Hyde 1995), Brazil (this paper).

MATERIAL EXAMINED: Brazil, Ceará, Ubajara, Ubajara National Park, on decorticated wood of unidentified plant, 4 May 2012, D.A.C.Almeida (HUEFS 131240).

Bertia moriformis (Tode) De Not., G. Bot. Ital. 1(1): 335. 1844 Fig. 1F–H

≡ *Sphaeria moriformis* Tode, Fung. Mecklenb. Sel. (Lüneburg) 2: 22. 1791

For additional synonyms see Index Fungorum (www.indexfungorum.org).

ASCOMATA superficial, clustered in small to large groups, turbinate, apex collapsed when dry and ascomata appearing cup-shaped, with a sterile base, surface tuberculate, black, 500–870 µm wide, 500–920 µm high, ostiole not seen. Munk pores present. Paraphyses not seen. ASCI unitunicate, clavate, long stipitate, thin-walled, 8-spored, 166–182 × 12–18 µm. ASCOSPORES irregularly arranged in upper portion of the ascus, fusiform, straight to curved, ends rounded, 1-septate, guttulate, smooth, hyaline, 24–40 × 3.5–6 µm.

ANAMORPH: Unknown.

GEOGRAPHICAL DISTRIBUTION: Brazil (this paper), Belgium, Canada, Denmark, England, Estonia, France, Germany, Italy, Romania, Sweden, Switzerland, Scotland (Corlett & Krug 1984), Poland (Farr et al. 2015), USA (Mugambi & Huhndorf 2010).

MATERIAL EXAMINED: Brazil, Bahia, Santa Teresina, Serra da Jibóia, on decorticated wood of unidentified plant, 12 Sep 2012, D.A.C.Almeida (HUEFS 192098); on decorticated wood of unidentified plant, 22 Jan 2013, D.A.C.Almeida (HUEFS 192164).

Bertia tropicalis Huhndorf, A.N.Mill. & F.A.Fern., Mycol. Res. 108(12): 1387. 2004. Fig. 1I–K

ASCOMATA superficial, isolated or clustered in small to large groups, turbinate, apex rounded, apically or laterally collapsed when dry, ascomata becoming cup-shaped, with a sterile base, surface tuberculate, black, 470–790 µm wide, 340–820 µm high, ostiole not seen. Munk pores present. PARAPHYSES hyaline, inflated, unbranched, 19–23 µm wide at base. ASCI unitunicate, clavate, long stipitate, thin walled, 8-spored, 149–247 × 14–21 µm. ASCOSPORES irregularly arranged in upper portion of the ascus, geniculate, curved at lower end, ends rounded, 1-septate, smooth, hyaline, 22–34 × 4.5–6 µm.

ANAMORPH: Unknown.

GEOGRAPHICAL DISTRIBUTION: Brazil (this paper), Costa Rica, French Guiana, Jamaica, Panama, Thailand, USA (Puerto Rico) (Huhndorf et al. 2004).

MATERIAL EXAMINED: Brazil, Ceará, Ubajara, Ubajara National Park, on decorticated wood of unidentified plant, 3 May 2012, D.A.C.Almeida (HUEFS 131251); 4 May 2012, D.A.C.Almeida

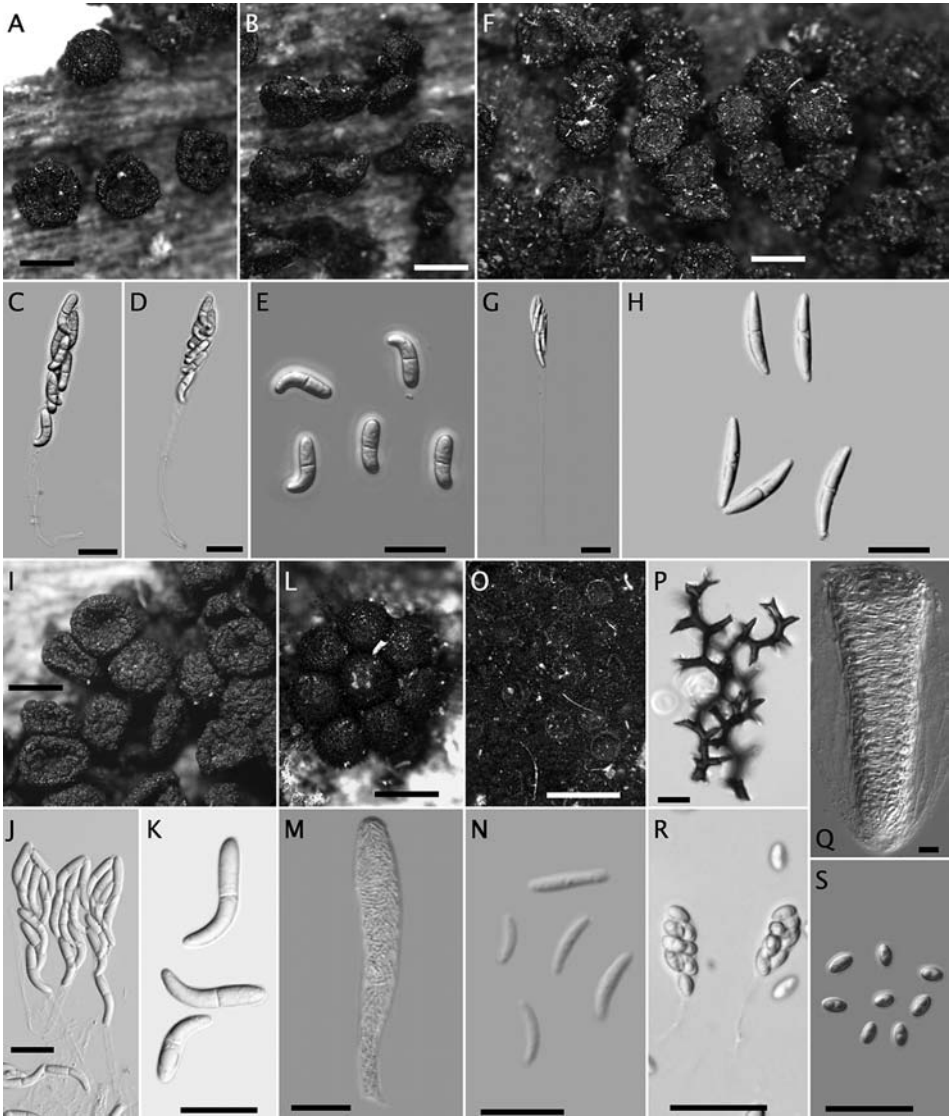


Fig. 1. A–E. *Bertia convolutispora*. A. Ascomata turbinate and apically collapsed. B. Ascomata laterally collapsed. C–D. Asci. E. Ascospores. F–H. *Bertia moriformis*. F. Ascomata turbinate and apically collapsed. G. Ascus with ascospores. H. Ascospores. I–K. *Bertia tropicalis*. I. Ascomata turbinate, apically and laterally collapsed. J. Asci with ascospores. K. Ascospores. L–N. *Fracchiaria broomeana*. L. Ascomata. M. Polysporous asci. N. Ascospores. O–S. *Scortechinia diminutispora*. O. Ascomata apically collapsed and surrounded by a subiculum. P. Dichotomously branched hyphae of the subiculum. Q. Quellkörper. R. Asci with ascospores. S. Ascospores. Bars: A–B, F, I, L, O = 500 µm; C–E, G–H, J–K, M, P–S = 20 µm; N = 10 µm.

(HUEFS 137810); 16 Nov 2012, D.A.C.Almeida (HUEFS 192133); on twigs of unidentified plant, 15 Nov 2012, D.A.C.Almeida (HUEFS 192152); Bahia, Santa Teresina, Serra da Jibóia, on decorticated wood of unidentified plant, 12 Sep 2012, D.A.C.Almeida (HUEFS 192100); 13 Sep 2012, D.A.C.Almeida (HUEFS 192096); Piauí, Caracol, Serra das Confusões National Park, on decorticated wood of unidentified plant, 29 Mar 2011, D.A.C.Almeida (HUEFS 42850).

Fracchiaea broomeana (Berk.) Petch [as 'broomeiana'], Ann. R. Bot. Gdns Peradeniya 6(4): 333. 1917. Fig. 1L–N

≡ *Sphaeria broomeana* Berk. [as 'broomeiana'], Hooker's J. Bot. Kew Gard. Misc. 6: 231. 1854.

For additional synonyms see Index Fungorum (www.indexfungorum.org).

ASCOMATA superficial, clustered on a stroma, hemispherical to globose, surface echinulate, black, spines short, conical, usually bifurcate at apex, 300–500 µm diameter, up to 300 µm high, ostiole inconspicuous. Munk pores present. PARAPHYSES not seen. ASCI unitunicate, clavate, short stipitate, thin-walled, apical ring absent, polysporous, 91–122 × 9.5–16 µm, spore-bearing part 60–80 µm. ASCOSPORES irregularly arranged in upper portion of the ascus, cylindrical, straight or curved, aseptate, smooth, hyaline, 5–11 × 1–2 µm.

ANAMORPH: Unknown.

GEOGRAPHICAL DISTRIBUTION: Argentina, Australia, Brazil, China, Czechoslovakia, France, Gambia, Ghana, India, Italy, Japan, New Zealand, Nicaragua, Pakistan, Sierra Leone, Sri Lanka, USA, Venezuela, Yugoslavia, Zimbabwe (as *Nitschkia broomeiana*, Nannfeldt 1975b).

MATERIAL EXAMINED: Brazil, Ceará, Ubajara, Ubajara National Park, on wood of unidentified plant, 19 May 2013, D.A.C.Almeida & A.N.Miller (HUEFS 192220); 4 May 2012, D.A.C.Almeida (HUEFS 137812).

Scortechinia diminutispora Mugambi & Huhndorf, Mycologia 102(1): 202. 2010. Fig. 1O–S

ASCOMATA superficial, surrounded by or embedded in a thick subiculum, scattered, subglobose, apex collapsed when dry and ascomata becoming cup-shaped, surface smooth, black, 155–280 µm wide, 160–190 µm high, ostiole not seen; subiculum well developed, sometimes growing on stromata of *Annulohypoxylon* sp., composed of dichotomously branched, brown, septate, thick-walled hyphae, with pointed ends, 4–7.5 µm wide, extending from ascomata 20–44 × 15–32 mm. Munk pores present. QUELLKÖRPER present, subconical, 217–300 µm long, 90–109 µm wide at the base. PARAPHYSES not seen. ASCI unitunicate, clavate, long stipitate, thin-walled, apical ring absent, 8-spored, 26–44 × 7–9.5 µm, spore-bearing part 15.5–22 µm. ASCOSPORES irregularly arranged in upper portion of the ascus, ellipsoid, aseptate, with two guttules, smooth, hyaline, 4.5–7(–8) × 2.5–4 µm.

ANAMORPH: Unknown.

GEOGRAPHICAL DISTRIBUTION: Brazil (this paper), Ecuador (Mugambi and Huhndorf, 2010).

Table 1. New sequences obtained in this study.

Species	Voucher info	Provenance	GenBank accession numbers
<i>Bertia tropicalis</i>	HUEFS 192152	Ubajara National Park – Brazil	KT003701
<i>Scortechinia diminutispora</i>	HUEFS 194245	Ubajara National Park – Brazil	KT003703

MATERIAL EXAMINED: Brazil, Paraíba, Areia, on decorticated wood of unidentified plant and on stromata of *Annulohyphoxylon* sp., 4 Jul 2012, D.A.C.Almeida (HUEFS 192050); Ceará, Ubajara, Ubajara National Park, on decorticated wood of unidentified plant and on stromata of *Annulohyphoxylon* sp., 20 May 2013, D.A.C.Almeida & A.N.Miller (HUEFS 194245).

Phylogenetic analyses

DNA samples of only two species, *Bertia tropicalis* and *Scortechinia diminutispora*, were successfully sequenced and are listed in Table 1. The original LSU nrDNA alignment comprised 41 taxa and 1,192 characters. After ambiguous regions were excluded with Gblocks, the final dataset consisted of 1,029 characters. Maximum likelihood analysis produced a single most likely tree (Fig. 2). The Brazilian specimen of *Scortechinia diminutispora* grouped with the holotype from Ecuador and possessed an identical LSU nrDNA sequence. Both formed a strongly supported clade (84% RAxML BV; $\geq 95\%$ PP) along with another species of *Scortechinia* and *Neofracchiaea callista* (Berk. & M.A.Curtis) Teng in the Scortechiniaceae. *Bertia tropicalis* from this study formed a strongly supported clade (100% RAxML BV; $\geq 95\%$ PP) along with four other sequences of *B. tropicalis*, including the sequence from the holotype (AY695262). They grouped with other species of *Bertia* and *Gaillardiaella pezizoides* Pat. in the Bertiaceae with strong support (100% RAxML BV; $\geq 95\%$ PP).

Discussion

Bertia convolutispora was originally described by Hyde (1995), who collected it on submerged wood in a freshwater stream. He suggested that the semi-helical (J-shape) ascospores could be an adaptation to fix to substrates for dispersal in the aquatic environment. Our specimen (Fig. 1A–E), however, was found in a terrestrial environment, suggesting the holotype may have been accidentally introduced into the aquatic habitat. The ascomata of the Brazilian specimen collapse laterally or apically when dry (Fig. 1A–B). This characteristic was not seen by Hyde (1995), probably because his material was not dry enough since it was collected from submerged wood. *Bertia biseptata* Sivan. & W.H.Hsieh, a species so far known only from terrestrial environments (Hsieh et al. 1995), has ascospores similar to *B. convolutispora*, but differs by having longer (25–35 μm), biseptate ascospores with the lower end strongly curved upwards. *Bertia latisporea* (Corlett & J.C.Krug) Lar.N.Vassiljeva also has geniculate

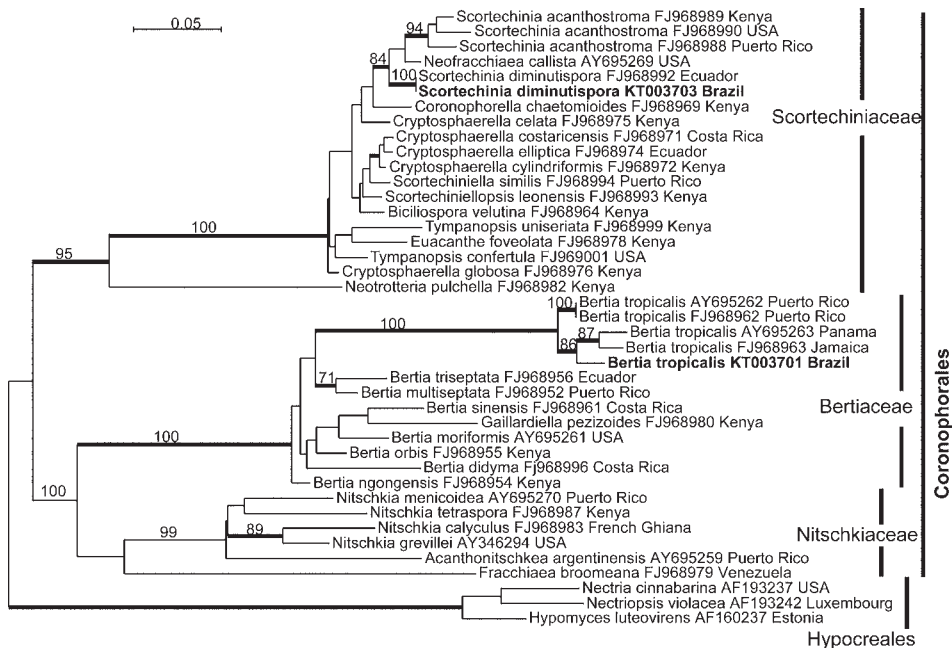


Fig. 2. Phylogram of the most likely tree generated from RAXML maximum likelihood analyses of LSU nrDNA partial sequences. Bootstrap values $\geq 70\%$ are shown above branches. Thickened branches indicate Bayesian posterior probabilities $\geq 95\%$. Brazilian specimens are indicated in bold.

ascospores, but can be separated by its longer ascospores (30–49 μm). Unfortunately, molecular data of *B. convolutispora* are lacking to assess its phylogenetic relationship with other species of *Bertia*. This is the first record of *B. convolutispora* for South America and only the second known collection of this species.

The characteristics of the Brazilian material of *Bertia moriformis* (Fig. 1F–H) agree with the description presented by Corlett & Krug (1984), except it has longer asci (166–182 vs. 92–140 μm) and smaller ascospores (24–40 \times 3.5–6 vs. 34.5–52.5 \times 4.5–6.5 μm). In addition to the type variety, two other varieties were proposed: *B. moriformis* var. *latispora* Corlett & J.C.Krug and *B. moriformis* var. *multiseptata* Sivan. (Corlett & Krug 1984). The former differs by its broader (6–8.5 μm) and geniculate ascospores, whereas the latter is distinct in having 3–8-septate ascospores (Corlett & Krug 1984). These two varieties were elevated to species rank as *B. latispora* (Corlett & J.C.Krug) Lar.N.Vassiljeva (Vasilyeva 1998) and *B. multiseptata* (Sivan.) Huhndorf, A.N.Mill. & F.A.Fern. (Huhndorf et al. 2004), respectively. Phylogenetic analysis based on LSU nrDNA partial sequences provided additional evidence to separate *B. moriformis* and *B. multiseptata* (Mugambi & Huhndorf 2010). Our phylogenetic analysis based on LSU nrDNA of *B. moriformis* and *B. multiseptata* from GenBank also supports previous results (Fig. 2). Molecular data for *B. latispora*, however, is presently lacking. This is the first record of *B. moriformis* for South America.

Huhndorf et al. (2004) proposed *B. tropicalis* for several specimens collected in tropical regions (see geographical distribution above). Our material (Fig. 1I–K) agrees well with the original description (Huhndorf et al. 2004), except the ascomata are shorter (340–820 vs. 892–1135 µm) and paraphyses are wider (19–23 vs. 13–16.5 µm). *Bertia convolutispora* and *B. latispora* also have similar ascospores, but can be distinguished by having shorter and longer ascospores (16–21 and 30–49 µm, respectively). Although there are no sequences available for *Bertia convolutispora* and *B. latispora*, the molecular analysis confirms our identification (Fig. 2). This is the first record of *B. tropicalis* for Brazil.

The Brazilian specimens of *Fracchiaea broomeana* (Fig. 1L–N) have characters in accordance with the descriptions presented by Berlese (1900), Petch (1917) and Nannfeldt (1975b). This species was previously reported for Brazil by Nannfeldt (1975b).

Scortechinia diminutispora is morphologically similar and phylogenetically close to *S. acanthostroma* (Mont.) Sacc. & Berl., both having collabent ascomata embedded in a subiculum and ellipsoid, hyaline, aseptate ascospores (Subramanian & Sekar 1990, Mugambi & Huhndorf 2010). *Scortechinia acanthostroma* can be separated by having asci with a larger spore-bearing part (18–30 × 8–11 vs. 15–18 × 6–7 µm) and larger ascospores (6–10 × 3.5–5 vs. 5–6 × 2–3 µm). Our material has guttulate ascospores as in *S. acanthostroma*. Although Mugambi & Huhndorf (2010) did not mention it in their original description of *S. diminutispora*, this characteristic is present in their images of the species (see Fig. 7v). The Brazilian specimens (Fig. 1O–S) agree with the original description with exception of the subconical shape of the quellkörper and apparent fungicolous growth on stromata of an unidentified species of *Annulohyphoxylon*. These differences must be considered intraspecific variations as both isolates have identical LSU nrDNA sequences (Fig. 2). This is the first record of *S. diminutispora* for Brazil.

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