

What is *Scirrhia*?

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Abstract: The ascomycetous genus *Scirrhia* is presently treated as a member of *Dothideomycetidae*, though uncertainty remains as to which family it belongs in *Capnodiales*, *Ascomycota*. Recent collections on stems of a fern, *Pteridium aquilinum* (*Dennstaedtiaceae*) in Brazil, led to the discovery of a new species of *Scirrhia*, described here as *S. brasiliensis*. Based on DNA sequence data of the nuclear ribosomal DNA (LSU), *Scirrhia* is revealed to represent a member of *Dothideomycetes*, *Capnodiales*, *Mycosphaerellaceae*. *Scirrhia* is the first confirmed genus in *Mycosphaerellaceae* to have well developed pseudoparaphyses and a prominent hypostroma in which ascospores are arranged in parallel rows. Given the extremely slow growth rate and difficulty in obtaining cultures of *S. brasiliensis* on various growth media, it appears that *Scirrhia* represents a genus of potentially obligate plant pathogens within *Mycosphaerellaceae*.

Key words:

Brazil
Capnodiales
Dothideomycetes
 ITS
 LSU
Mycosphaerellaceae
Pteridium
 systematics

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INTRODUCTION

The genus *Scirrhia* was originally known from four species (Fuckel 1870), and later lectotypified based on *S. rimosa*, a species known from stems of *Phragmites australis* (*Poaceae*) collected in Europe (Saccardo 1883). Based on its ascospores arranged in linear rows in a hypostroma of pseudoparenchymatal cells, bitunicate asci, hyaline, 1-septate ascospores, and the presence of pseudoparaphyses, Müller & von Arx (1962) placed the genus in *Dothiorales*, *Mycosphaerellaceae*, listing *Scirrhodothis* (Theissen & Sydow 1915), *Scirrhophragma* (Theissen & Sydow 1915) and *Metameris* (Theissen & Sydow 1915) as possible generic synonyms. All three genera have bitunicate asci, hyaline, 1-septate ascospores, pseudoparaphyses, and stromata with immersed, longitudinally arranged ascospores, appearing to justify this opinion.

In a later study, von Arx & Müller (1975) again placed *Scirrhia* in *Dothideaceae*, suborder *Dothideineae* in *Dothideales*. Barr (1972) questioned the synonymy of *Scirrhodothis* (lectotype species *S. confluens*), *Scirrhophragma* (type species *S. regalis*) and *Metameris* (type species *M. japonica*) under *Scirrhia*, and Sivanesan (1984) also chose not to list them as such, but included species that occurred on *Gymnospermae*, namely *S. acicola* and *S. pini*. Barr (1987) chose to reinstate these generic synonyms, and placed *Scirrhia* together with *Mycosphaerella* in *Dothideaceae*, *Dothideales*. Although little is known about

the three generic synonyms listed by Müller & von Arx (1962), the species on *Gymnospermae* are presently treated as separate genera, namely *Lecanosticta* (*L. acicola*) and *Dothistroma* (*D. pini*) (Crous *et al.* 2009a). Presently, the taxonomic position of *Scirrhia* remains obscure.

During a recent visit to Brazil, we collected fresh material of a species of *Scirrhia* on stems of a fern. The aims of the present study are, therefore, to identify the species of *Scirrhia*, and to see if the taxonomic position of the genus could be resolved.

MATERIALS AND METHODS

Isolates

Stems bearing ascospores were soaked in water for approximately 2 h, after which they were placed in the bottom of Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA), or potato-dextrose agar (PDA; Crous *et al.* 2009b). Ascospore germination patterns were examined after 24 h, and single ascospore cultures were established as described earlier (Crous *et al.* 1991, Crous 1998). Colonies were subcultured onto PDA and oatmeal agar (OA) (Crous *et al.* 2009b) and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Germinating ascospores died on MEA. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands.

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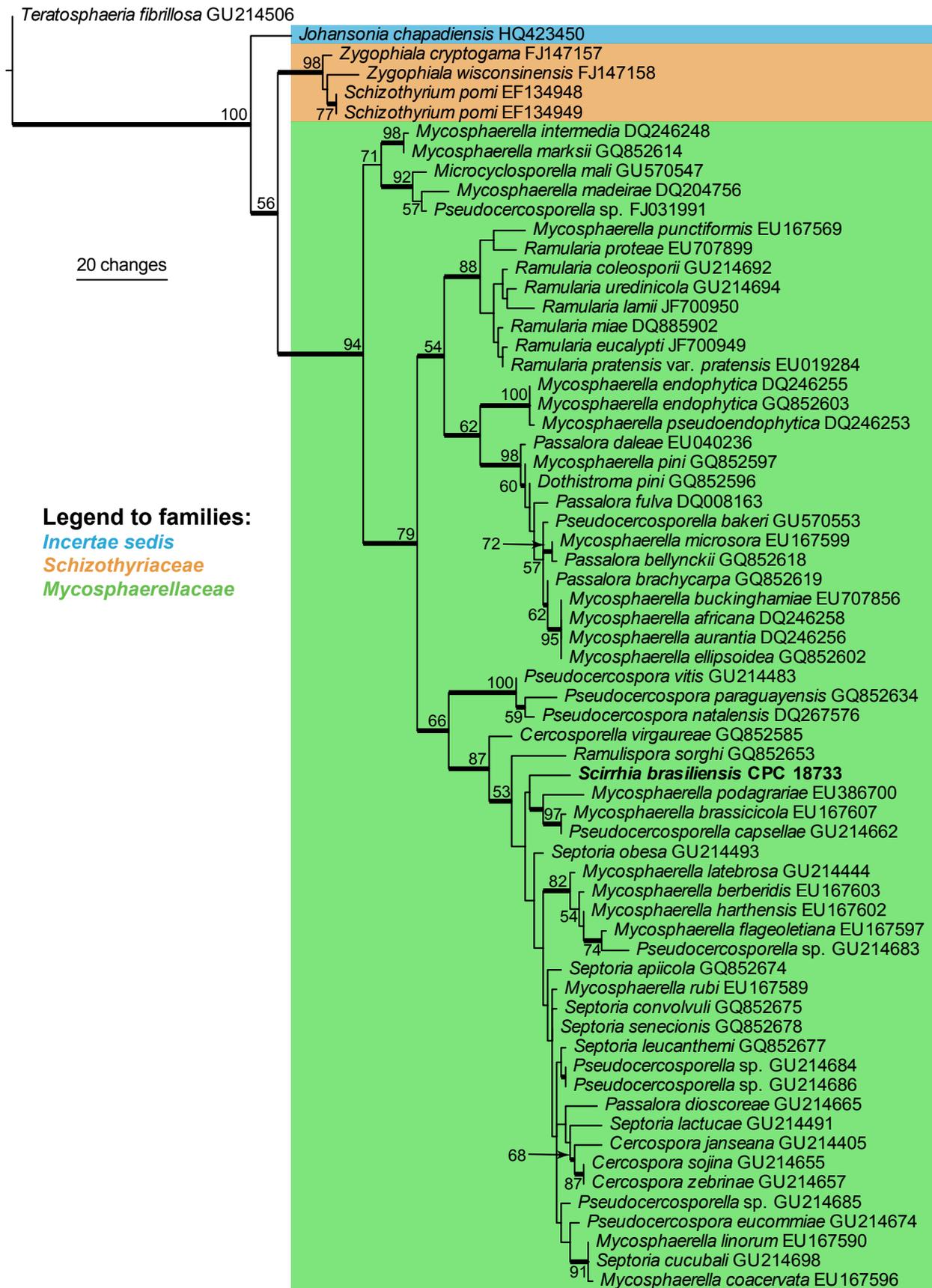


Fig. 1. The first of 1000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. The novel sequence generated for this study is shown in **bold**. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Teratosphaeria fibrillosa* (GenBank accession GU214506).

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), ITS 1, the 5.8S rRNA gene, ITS 2 and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al.* 1990) and LSU1Fd (Crous *et al.* 2009c) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment, and subsequent phylogenetic analysis followed the methods of Crous *et al.* (2006, 2009d). Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search and results are discussed in the relevant species notes where applicable. Based on the Blast results, the novel sequence was added to the alignment of Crous *et al.* 2010 (TreeBASE study S10980). Alignment gaps were treated as new character states. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.Mycobank.org; Crous *et al.* 2004).

Morphology

Morphological descriptions are based on preparations made from host material in clear lactic acid, with 30 measurements determined per structure, and observations made with a Nikon SMZ1500 dissecting microscope, and with a Zeiss Axioscope 2 microscope using differential interference contrast (DIC) illumination. Colony characters and pigment production were noted after 3 mo of growth on PDA and OA (Crous *et al.* 2009b) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

RESULTS

Phylogeny

Approximately 1700 bases, spanning the ITS and LSU regions, were obtained from the sequenced culture. The LSU region was used in the phylogenetic analysis for the generic placement (Fig. 1) and ITS to determine species-rank relationships (see notes under the species description). The manually adjusted LSU alignment contained 66 taxa including the outgroup sequence and, of the 749 characters used in the phylogenetic analysis, 104 were parsimony-informative, 51 were variable and parsimony-uninformative, and 594 were constant. Only the first 1000 equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 1 (TL = 415, CI = 0.494, RI = 0.851, RC = 0.421). The phylogenetic tree of the LSU region (Fig. 1) show that the obtained sequence clusters together with species belonging to *Cercospora*, *Pseudocercospora*, *Pseudocercospora* and *Septoria* in *Mycosphaerellaceae*.

Taxonomy

Scirrhia rimosa (Alb. & Schwein.) Fuckel, *Jb. nassau. Ver. Naturk.* **23–24**: 221 (1870).

Basionym: *Sphaeria rimosa* Alb. & Schwein., *Consp. Fung.*: 13 (1805).
(Fig. 2)

Specimen examined: **Czech Republic**: Lipnik, on culms (stems) of *Phragmites australis*, June 1942 (Reliquiae Petrakianae Institut für Systematische Botanik Graz, exsiccati no. 1871, BPI 1111233).

Hypostroma on upper leaf surfaces, dark brown to black, immersed beneath epidermis, becoming raised, irregularly ellipsoid, to 20 mm long, 5 mm wide. *Ascomata* immersed in brown stroma of *textura angularis*; arranged in parallel rows, opening by a 10–15 µm diam central ostiole; ascomata to 250 µm diam, wall 10–20 µm wide. *Asci* 80–130 × 13–18 µm, hyaline, smooth, cylindrical, sessile in fascicles, short-stipitate, bitunicate with ocular chamber, 3–4 µm diam. *Pseudoparaphyses* hyaline, cellular, smooth, distributed among asci, septate, branched, prominently constricted at septa, 5–8 µm diam, extending above asci. *Ascospores* hyaline, smooth, biseriate in asci, guttulate, fusoid-ellipsoidal, widest just above septum, tapering towards both ends, but more prominently towards lower end, prominently constricted at the median septum, thin-walled, (20–)21–23(–26) × 6–7(–7.5) µm; ascospores germinating on host with germ tubes at right angles to the long axis of spore, but remaining hyaline, not distorting.

Notes: Despite several attempts, we have thus far been unable to recollect fresh material of this species on *Phragmites*, and thus no DNA sequence data are available. The nature of the hypostroma, ascomatal arrangement, asci, ascospores, and pseudoparaphyses, closely match that of the species described below. The specimen described and illustrated here agrees with the original description as well as locality and host of this species, occurring on stems. A later Schweinitz collection on *Zizania* (*Poaceae*) from the USA proved to be a misidentified *Leptosphaeria zizaniicola* (Ellis & Everhart 1892). We are unaware of any confirmed reports of *Scirrhia rimosa* from North America.

Scirrhia brasiliensis Crous, O.L. Pereira, Alfenas & R.F. Alfenas, **sp. nov.**

Mycobank MB560601
(Fig. 3)

Etymology: Named after the country where the holotype was collected, Brazil.

Scirrhiae rimosae morphologicis similis, sed asco saepe minus quam octosporo et ascosporis dissimiliter contractis.

Typus: **Brazil**: Minas Gerais: Viçosa, Serra do Brigadeiro, S20°41.487, W42°29.875, on stems of *Pteridium aquilinum* (*Dennstaedtiaceae*), 22 Aug. 2010, P.W. Crous, O.L. Pereira, A.C. Alfenas & R.F. Alfenas,

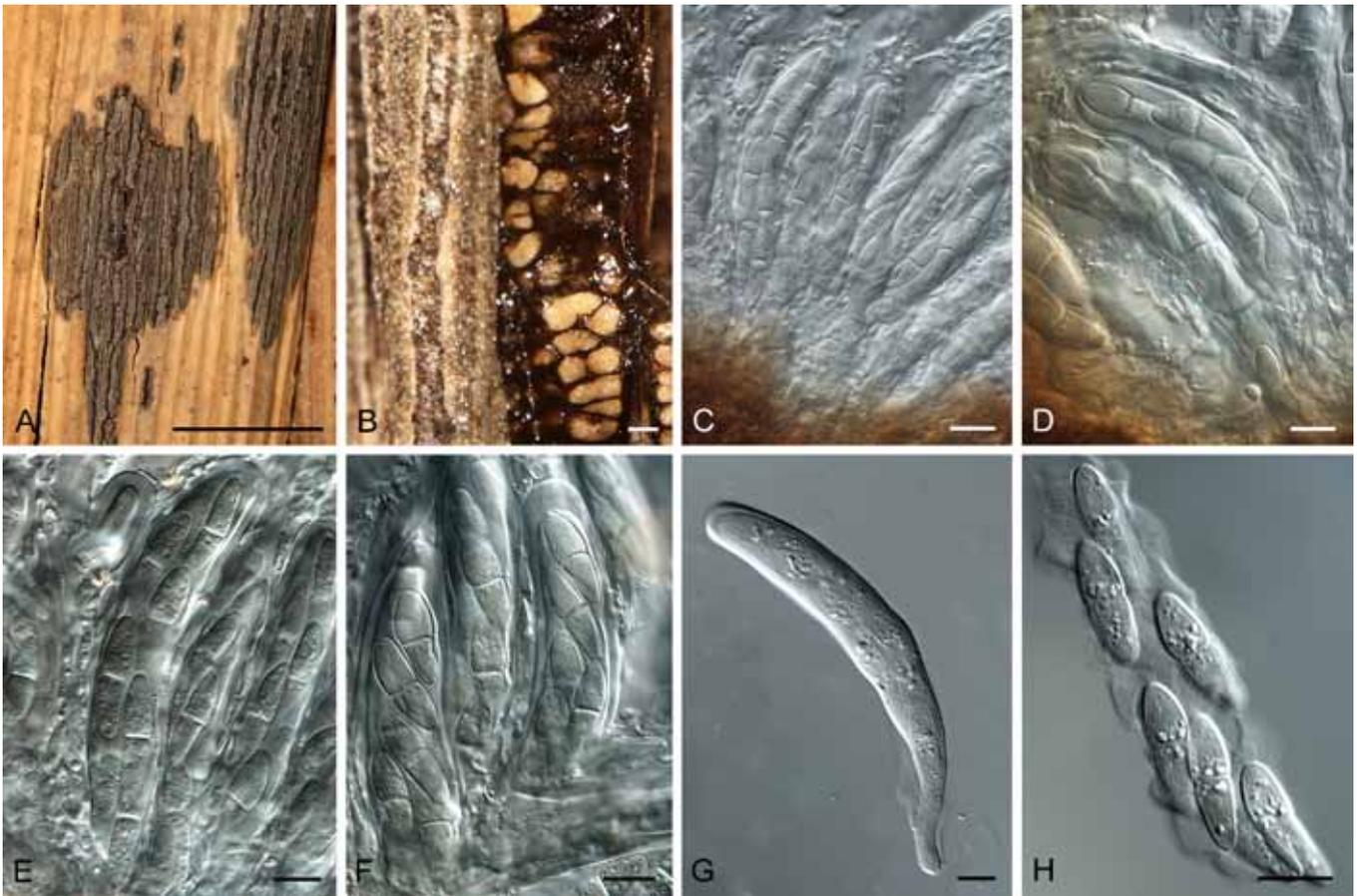


Fig. 2. *Scirrhia rimosa* (BPI 1111233). **A.** Hypostroma on leaf of *Phragmites australis*. **B.** Horizontal section through ascomata. **C–F.** Asci intermingled among pseudoparaphyses. **G.** Ascus with basal stalk. **H.** Ascospores inside disintegrated ascus. Bars: A = 5 mm; B = 250 μ m, all others = 10 μ m.

(CBS H-20538 – holotypus; cultures ex-holotype CPC 18734, 18735, 18733 = CBS 128762). (GenBank accession numbers: CPC 18733, ITS JN197292, LSU JN197293).

Stem cankers narrowly fusoid, brown, at times with red-purple margin, visible on shoots and stems of host, up to 5 mm wide, 3 cm long; raised areas forming longitudinal cracks in the host epidermis through which erumpent ascomata protrude. *Ascomata* to 160 μ m diam, 200 μ m high, subepidermal, situated in rows in a brown hypostroma of *textura angularis*; wall consisting of to 6 layers of *textura angularis*, to 60 μ m diam; cells brown, becoming flattened between ascomata, to 20 μ m diam; ostioles ellipsoid, single, central, to 20 μ m diam, situated on a dark brown, depressed neck, to 50 μ m wide, 15 μ m high; ostiole lined with hyaline, cylindrical periphyses with rounded ends, to 25 μ m long, 3 μ m wide. *Asci* 90–130 \times 8–11 μ m, fasciculate, intermingled between pseudoparaphyses, subcylindrical, with thick wall, 2–4 μ m diam, inconspicuous apical chamber, 2–3 μ m diam; wall appearing multi-layered with fissitunicate dehiscence, widest in middle of ascus, tapering to a long, thin, basal part and lobed base, to 9 μ m wide; asci initially with 8 immature ascospores, of which some dissolve, leaving 4–6 mature ascospores per ascus. *Pseudoparaphyses* hyaline, cellular, smooth, septate, anastomosing, extending above asci, 3–5 μ m wide. *Ascospores* fusoid-ellipsoidal, hyaline, smooth,

guttulate, obliquely uniseriate in asci, medianly 1-septate, at times constricted at the septum, widest in the middle of apical cell, tapering towards both ends, (17–)20–23(–27) \times (5–)6–7(–8) μ m; germinating from both ends, with germ tubes generally parallel to the long axis; spores neither darkening nor distorting.

Culture characteristics: Colonies after 3 mo on PDA and OA with sparse aerial mycelium and even margins, olivaceous-grey (surface and reverse), reaching to 6 mm diam, extremely slow growing, with brown, diffuse pigment visible in agar surrounding colony; colonies sterile. Most ascospores died upon germination, and only a few of the germinated spores on PDA survived to form extremely slow-growing colonies, suggesting this pathogen to be highly obligate in nature.

Notes: The only culture of a species of *Scirrhia* presently available in the CBS culture collection, *S. aspidiorum* (CBS 204.66, on *Pteridium aquilinum*, UK; GenBank accession number: ITS, JN197294) turned out to be related to *Phoma herbarum* (*Didymellaceae*, *Pleosporales*; de Gruyter et al. 2010), and thus unrelated to *S. brasiliensis*. Although the specimen from which this culture was derived (ETH 2662) was not examined, examination of another collection under this name (The Netherlands, Baarn, Groenevelt, *Pteridium*



Fig. 3. *Scirrhia brasiliensis* (ex-type CPC 18733). **A.** Fronds of *Pteridium aquilinum*. **B, C.** Stems with cankers extending into inner tissue. **D.** Hypostroma viewed from above. **E–G, K.** Sections through ascomata. **H–J.** Ostiolar area of ascomata, showing depressed neck. **L–N.** Bitunicate asci (arrow denotes apical chamber). **O, P.** Germinating ascospores. **Q.** Ascospores. Bars: B, C = 5 mm; D = 1 mm; E–H = 160 μ m, all others = 10 μ m.

aquilinum, May 1962, J.A. von Arx, CBS H-17938), revealed a typical species of *Scirrhia* to be present on this fern, but no culture is presently available to confirm that it is the same taxon represented by CBS 204.66. Müller & von Arx (1962) treat *S. aspidiorum* in detail, citing ascospores as being 10–

15 \times 3–4 μ m, thus being significantly smaller than those of *S. brasiliensis*. The ITS sequence of *S. brasiliensis* did not retrieve any highly identical hits with a megablast search of the NCBI's GenBank nucleotide database; the closest hits represent species of *Septoria*, e.g. *Septoria digitalis* GenBank

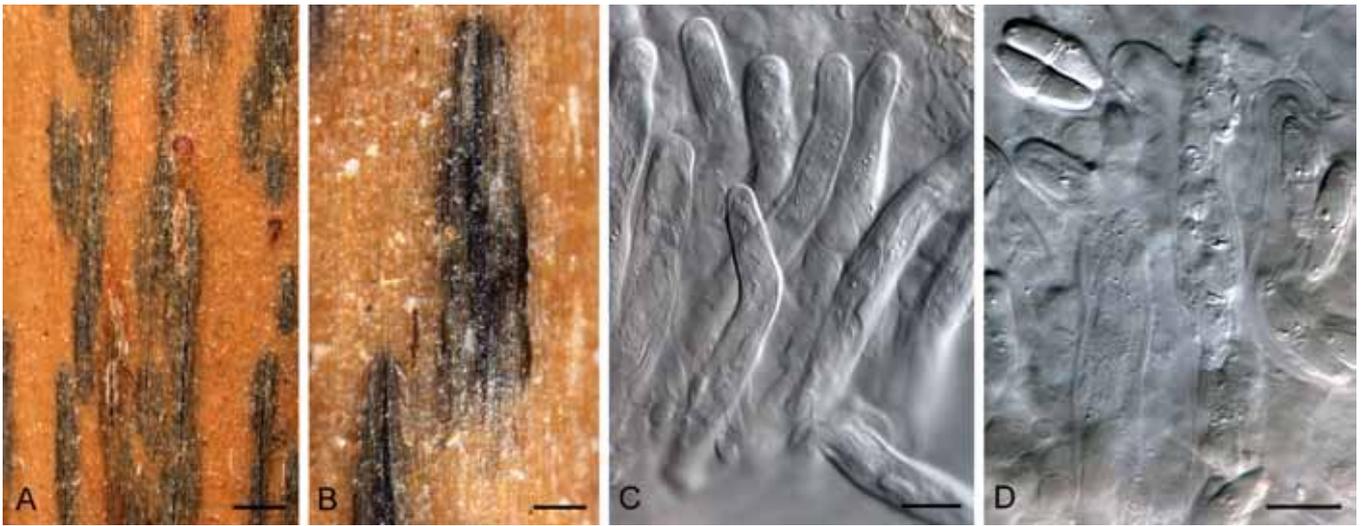


Fig. 4. *Scirrhodothis confluens* (isotype specimen CBS H-4806). **A, B.** Superficial view of stromata with imbedded ascomata. **C.** Asci. **D.** Broken asci with *Didymella*-like ascospores. Bars: A, B = 2 mm; C, D = 10 μ m.

FJ557246; Identities = 507/534 (95 %), Gaps = 8/534 (1 %), and *Cercospora*, e.g. *Cercospora piaropi* GenBank HQ902254; Identities = 514/547 (94 %), Gaps = 9/547 (2 %) (data not shown).

DISCUSSION

Persoon (1796) was the first to use the term “stroma” to define structures on or in which ascomata of the genus *Sphaeria* were borne. Fruiting (1867) introduced the terms “epistroma” for the hyaline pseudoparenchymatous crust formed in the outer layers of the host cortex, in which conidia were produced, and under which a “hypostroma” was formed in which ascomata developed. Based on the concepts of Fruiting, Ruhland (1900) introduced the terms “ectostroma” and “entostroma”. Blain (1927) subsequently defined *Scirrhia rimosa* as having a dothideoid epistroma and a hypostroma of compressed pseudoprosenchyma. The same hypostroma anatomy observed in *S. rimosa* was also found in *S. aspidiorum* and *S. brasiliensis*, and appears to be typical for the genus.

Although the type species of *Hadrotrichum*, *H. phragmitis*, has been linked to *S. rimosa* as a possible anamorph of the genus *Scirrhia* (Fuckel 1870), this relationship has never been proven and has been disputed (von Arx & Müller 1975). The phylogenetic position of *Hadrotrichum* presently remains unknown, and this genus will have to be recollected to resolve this issue. Presently, no anamorph connections have been confirmed for *Scirrhia*, and none formed in culture for *S. brasiliensis*.

Von Arx & Müller (1975) considered *Scirrhophragma*, *Metameris* to be synonyms of *Scirrhia*. Holm & Holm (1978) mentioned that although the taxa were closely related, the synonymy was controversial given different types of centrum development in some of the species. Barr (1992) regarded *Scirrhia* to represent a genus

in *Dothideales*, while members of *Metameris* were found to have pseudoparaphyses and 1–2-septate, *Didymella*-like ascospores (*Pleosporales*). Furthermore, based on its small ascomata, broadly cylindrical to slightly obclavate asci with a short, thick, knob-like pedicels, as well as its monocotyledonous host preference, Zhang *et al.* (2012) suggested *Metameris* to belong to *Phaeosphaeriaceae*, though molecular data are still lacking to resolve its final placement.

Lumbsch & Huhndorf (2010) list *Scirrhia* under *Dothideaceae* and *Metameris* under *Phaeosphaeriaceae*. The lectotype species of the genus *Scirrhophragma*, *S. confluens* (isotype CBS H-4806; Fig. 4), was examined in the present study and found to differ from *Scirrhia* in that the ascomata were locules formed in a stroma, not in linear rows in a hypostroma as in *Scirrhia*, and ascospores were *Didymella*-like. Although *Scirrhophragma* is clearly not a synonym of *Scirrhia*, the synonymy of these genera cannot be resolved in the absence of cultures, and they are best retained as separate entities until more data become available.

Kirschner & Chen (2010) stated that the systematic position of *Scirrhia* was also in need of re-examination, and referred to a paper by Suetrong *et al.* (2009), who placed *S. annulata* (*Juncaceae*) within *Mycosphaerellaceae*. The clustering of *S. brasiliensis* in this family in the present study supports this finding and confirms *Scirrhia* as the first genus within *Mycosphaerellaceae* (*Capnodiales*, *Dothideomycetes*) with well developed pseudoparaphyses and an epi- and hypostroma. Other ascomycetous genera that have recently been shown to cluster in *Mycosphaerellaceae* include *Phaeocryptopus* and *Rosenscheldiella* (Winton *et al.* 2007, Sultan *et al.* 2011). Further collections are now required to resolve the questions: does *S. rimosa* cluster with *S. brasiliensis*, and what is the status of its reported synonyms, *Scirrhophragma*, *Scirrhophragma* and *Metameris*?

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