ARTICLE

What is *Johansonia*?

Pedro W. Crous[1], Robert W. Barreto[2], Acelino C. Alfenas[2], Rafael F. Alfenas[2] and Johannes Z. Groenewald[1]

[1]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl

[2]Departemento de Fitopatologia, Universidade Federal de Viçosa, 36.570 Viçosa, MG, Brazil

Abstract: The bitunicate ascomycete genus *Johansonia* is presently treated as a member of *Saccardiaceae*, a family regarded as *incertae sedis* within the *Ascomycota*. Recent collections on leaves of a leguminous host, *Dimorphandra mollis,* in Mato Grosso, Brazil, led to the discovery of a new species of *Johansonia*, described here as *J. chapadiensis*. Based on DNA sequence data of the nuclear ribosomal DNA (LSU), *Johansonia* is revealed to represent a member of *Dothideomycetes*, *Capnodiales*. Although its family could not be resolved, it clustered basal to *Schizothyriaceae* and *Mycosphaerellaceae*, and could well represent a species of *Saccardiaceae*. DNA sequence data of other members of *Saccardiaceae* would be required, however, to confirm this classification.

Key words:
Dothideomycetes
Johansoniella
ITS
LSU
systematics

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INTRODUCTION

The genus *Johansonia* is based on *J. setosa* (Saccardo 1889), a species known from leaves of *Sapindaceae* collected in South America (Müller & von Arx 1962). Due on its superficial discoid ascomata, bitunicate asci and hyaline, 1-septate ascospores, Müller & von Arx (1962) were of the opinion that the genus belonged to *Schizothyriaceae*. In a later study, however, von Arx & Müller (1975) again placed it in *Saccardiaceae*, suborder *Dothideaceae* in *Dothideales*, based on the ascomata having an epithecium of branched hyphal elements. Barr (1993) again placed it in *Phillipsiellaceae* in *Loculoascomycetes*, while Lumbsch & Huhndorf (2007) concluded that it was a member of *Saccardiaceae*, a family they regarded as *incertae sedis* in *Ascomycota*. In recent studies on *Dothideomycetes* (Schoch *et al*. 2006, 2009), no mention is made of *Johansonia*. As there are presently no DNA sequence data represented for any species of *Johansonia* in GenBank, its taxonomic position remains obscure.

During a recent visit to Brazil, we collected fresh material of a species of *Johansonia* on leaves of a legume. The aims of the present study were, therefore, to identify the species of *Johansonia*, and at the same time to see if the taxonomic position of the genus could not be resolved.

MATERIALS AND METHODS

Isolates

Leaves bearing ascomata 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; Crous *et al*. 2009c). Ascospore germination patterns were examined after 24 h, and single ascospore and conidial cultures established as described earlier (Crous *et al*. 1991, Crous 1998). Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al*. 2009c), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Inc., 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), the internal transcribed spacer 1, the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et*

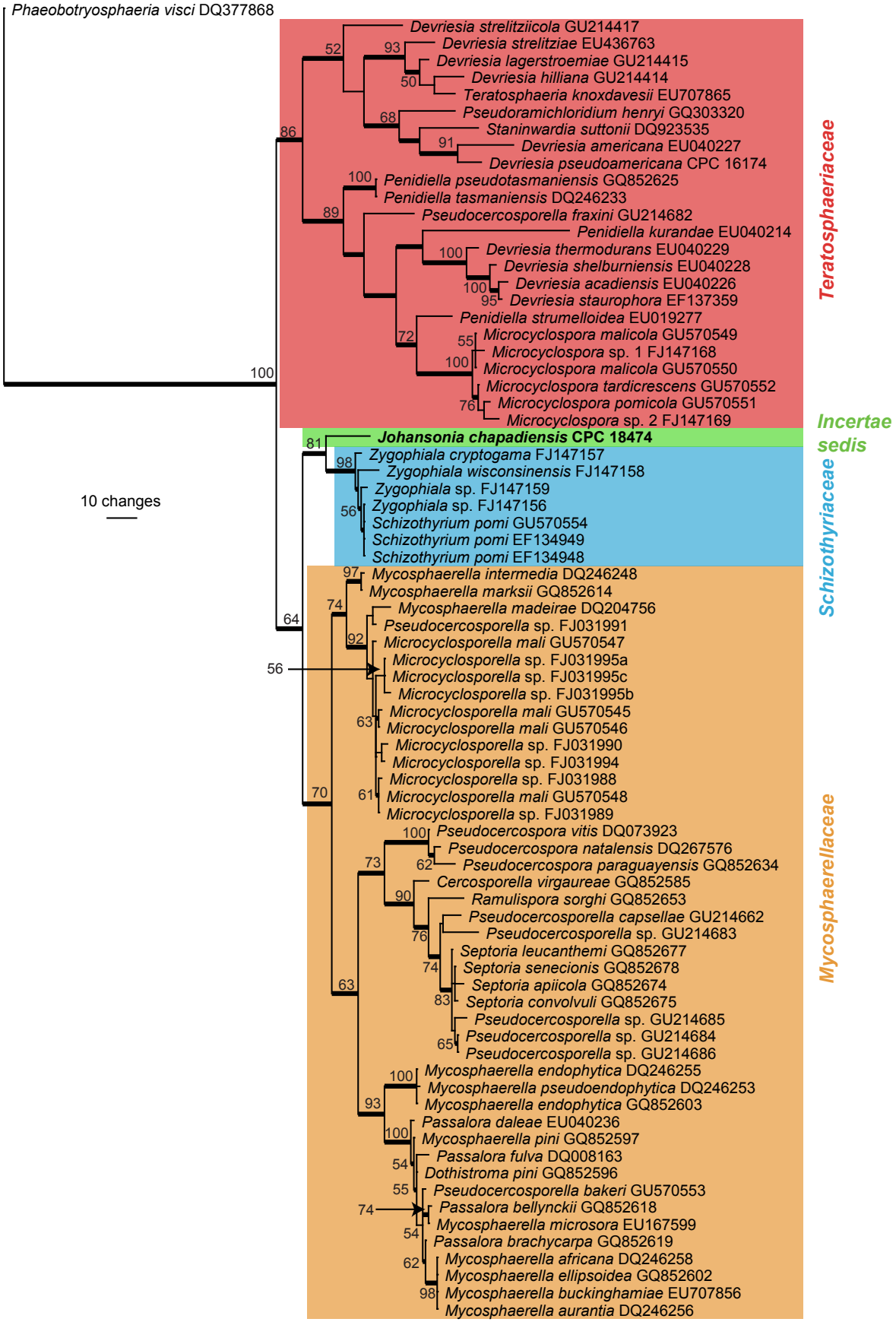

Fig. 1. The first of 1000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. The novel sequence generated for this study is shown in **bold**. Branches present in the strict consensus tree are thickened and important lineages are colour-coded. The tree was rooted to a sequence of *Phaeobotryosphaeria visci* (GenBank accession DQ377868).

al. 1990) and LSU1Fd (Crous *et al*. 2009b) 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, 2009a). 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 Frank *et al*. 2010 (TreeBASE study S10547). Alignment gaps were treated as new character states. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (<treebase.org/treebase/index.html>), and taxonomic novelties in MycoBank (<MycoBank.org>; Crous *et al*. 2004).

Morphology

The morphological description is 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 2 wk of growth on MEA, PDA and OA (Crous *et al*. 2009c) incubated at 25 ºC. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Growth characteristics were studied on MEA plates incubated for 2 wk in the dark at 25 °C.

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-level relationships (see notes under species descriptions). The manually adjusted LSU alignment contained 77 taxa (including the *Phaeobotryosphaeria visci* outgroup sequence) and, of the 731 characters used in the phylogenetic analysis, 171 were parsimony-informative, 96 were variable and parsimony-uninformative and 464 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 = 776, CI = 0.485, RI = 0.839, RC = 0.407). The phylogenetic tree of the LSU region (Fig. 1) show that the obtained sequence clusters basal to the *Schizothyriaceae*.

Taxonomy

Johansonia chapadiensis Crous, R.W. Barreto, Alfenas & R.F. Alfenas, **sp. nov.**
MycoBank MB517452
(Fig. 2)

Etymology: Named after the location where the holotype was collected, Chapada dos Guimarães, Mato Grosso, Brazil.

Johansoniae brasiliensis morphologice similis, sed ascosporis minoribus, (13–)15–19(–24) × (5–)6–7 μm, discernitur.

Typus: Brazil: Mato Grosso, Chapada dos Guimarães, on leaves of *Dimorphandra mollis* (*Leguminosae*; False Barbatimao), 18 Aug. 2010, *P.W. Crous, A.C. Alfenas & R. Alfenas*, (CBS H-20484 – holotypus, cultures ex-holotype CPC 18475, 18474 = CBS 128068). (GenBank accession numbers: ITS, HQ423449; LSU, HQ423450).

Leaves with brown spots, but ascomata also occurring on dead and green leaf areas. *Mycelium* superficial, consisting of septate, branched, medium brown, verruculose to warty, 2–5 μm wide hyphae. *Ascomata* on lower leaf surface, superficial, situated on a hyphal stroma (occurring loosely on surface), discoid, dark brown, up to 300 μm diam, 200 μm high. *Exciple* 15–20 μm diam, consisting of 3–6 layers of brown *textura angularis* to *textura globulosa*. *Asci* in parallel layer, bitunicate with ocular chamber, sessile, narrowly ellipsoid to subcylindrical or clavate, 8-spored, 32–45 × 11–19 μm. *Paraphyses* intermingled among asci, hyaline, branched, septate, 1.5–2.5 μm wide, becoming somewhat darkened and branched towards the apical region, forming an epithecium. *Ascospores* hyaline, thick-walled, medianly 1-septate, thick-walled, constricted at the septum, prominently guttulate, (13–)15–19(–24) × (5–)6–7 μm. *Ascospores* after 24 h on MEA germinating from both ends, with germ tubes parallel to the long axis of the spore, developing lateral branches; ascospores remaining hyaline, prominently constricted, not distorting, 5–7 μm wide. *Setae* brown, erect, straight to curved, separate and surrounding ascomata, thick-walled, brown, smooth, with basal T-cell devoid of rhizoids, with slight taper towards apical cell, which is thin-walled, pale brown, and acutely to obtusely rounded, 5–10-septate, 130–260 × 4–5 μm; 2.5–3 μm wide at apical septum.

Culture characteristics: Colonies spreading, erumpent, with sparse aerial mycelium and diffuse, submerged margins. On PDA surface pale mouse-grey (centre), olivaceous-grey (middle) with smoke-grey to cream outer region; reverse olivaceous-grey; colonies reaching 5 mm diam. On OA smooth, somewhat slimy, surface umber to dark mouse-grey; margin diffuse, reaching 8 mm diam. On MEA, surface smoke-grey; reverse greyish-sepia, reaching 10 mm diam after 2 wk.

Additional specimen examined: Brazil: Pernambuco: Poço do Macaco, on *Inga* sp., 18 Sept. 1960, *Osvaldo Soares de Silva* (CBS H-5029 – isotype of *Johansonia brasiliensis*).

Notes: The generic name *Johansonia* is based on *J. setosa*, a species described from living leaves of *Sapindaceae* collected in South America. The genus is characterised by having loose, superficial, discoid ascomata situated on a hyphal stroma, and an exciple covering the bitunicate asci. *Paraphyses*, which are intermingled among asci, are hyaline,

Fig. 2. *Johansonia chapadiensis* (CBS H-20484 – holotype). **A.** leaves colonised with *J. chapadiensis*. **B–E.** Ascomata on leaf surface from above (B, C), below (D), and a vertical section though an ascoma (E). **F, L.** Germinating ascospores. **G.** Vertical section through ascoma. **H–J.** Asci. **K.** Ascospores. Bars: B, C = 300 µm; D, E = 150 µm; G, H = 20 µm; F, I–L = 10 µm.

branched, septate, and become somewhat darkened and branched towards the apical epithecium. Ascospores are hyaline and 1-septate. Ascomata are surrounded by brown, erect, straight to curved, septate setae (Müller & von Arx 1962). Based on these features, *J. chapadiensis* is a typical member of the genus *Johansonia*.

Morphologically, *J. chapadiensis* closely resembles *J. brasiliensis* (Fig. 3). The two species can be distinguished in that ascospores of *J. chapadiensis* are smaller, (13–) 15–19(–24) × (5–)6–7 µm, than those of *J. brasiliensis,* (18–24 × 6–7 µm). Furthermore, asci of *J. chapadiensis* are narrowly ellipsoid to subcylindrical or clavate, 32–45 × 11–19 µm, while those of *J. brasiliensis* are broadly ellipsoid, obovoid to subcylindrical, never clavate, and larger, 40–58 × 15–23 µm. Finally, setae in *J. chapadiensis* are more acutely rounded, 2.5–3 µm diam at the apical septum, while those of *J. brasiliensis* are bluntly rounded, and wider at the apical septum, 4–6 µm diam.

DISCUSSION

Although there are only 12 species of *Johansonia* listed in Index Fungorum, von Arx & Müller (1975) were of the opinion that *Johansoniella maranhensis* represented a further species of *Johansonia*. Batista *et al.* (1966) introduced the monotypic generic name *Johansoniella* (*Schizothyriaceae*), based on *J. maranhensis*, which they regarded as closely related to *Johansonia*. Morphologically, the description appears

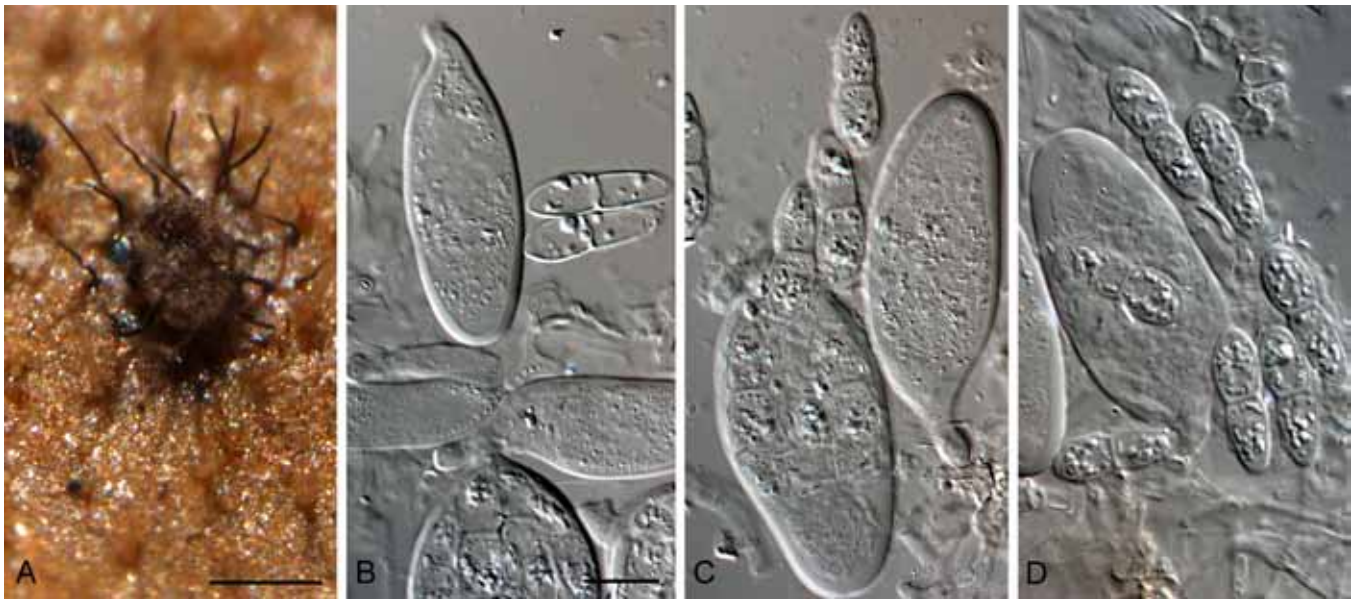

Fig. 3. *Johansonia brasiliensis* (CBS H-5029 – isotype). **A.** Ascoma on leaf. **B–D.** Asci and ascospores. Bars: A = 300 µm; B–D = 10 µm.

somewhat different, as the ascomata are described as having an upper wall layer (though this may be an epithecium), and setae around the ascomata, as well as on top of the ascomata. Regardless of these supposed differences, von Arx & Müller (1975) treated *Johansoniella* in synonym with *Johansonia*. A re-examination of the holotype specimen (URM 47621) found it to be depauperate, and hence the status of *Johansoniella* could not be resolved in the present study.

An attempt to make a key to the species described to date based on published descriptions has not proven feasible, as too many species either have similar ascospore dimensions, or are insufficiently known. Based on published descriptions, most taxa only seem distinct if aspects such as dimenions of the ascospores, asci and setae are combined with host and distribution. However, as most taxa have been recorded once only, the value of these characters seems unreliable, and hence a key would only be feasible once the specimens of all described taxa have been re-examined to help resolve possible species synonymies.

Recent studies focused on elucidating the higher order phylogeny of *Dothideomycetes* (Schoch *et al*. 2009) and *Capnodiales* (Crous *et al*. 2009b) did not treat *Johansonia*, as the present collection represents the first known cultures of this genus. Von Arx & Müller (1975) were of the opinion that *Johansonia* belonged to *Saccardiaceae*, a treatment accepted by Lumbsch & Huhndorf (2007), though they regarded it as a family *incertae sedis* within *Ascomycota*. Based on the DNA phylogeny generated in the present study (Fig. 1), we can reveal that *Johansonia* belongs to the *Dothideomycetes* (*Capnodiales*), and is closely related to *Schizothyriaceae* and *Mycosphaerellaceae*. However, whether it is a member of the *Saccardiaceae* (von Arx & Müller 1975, Lumbsch & Huhndorf 2007), could not be confirmed, as presently there are no known cultures of this family available for comparison. Parts of *Saccardiaceae* have been transferred to *Schizothyriaceae* von Arx & Müller (1975), and thus its close relationship to the *Schizothyriaceae* suggests *Saccardiaceae* a likely family for this genus, pending further collections and study.

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