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Cryptic but ubiquitous: Claviradulomyceae fam. nov.
with five novel species of the lenticel
fungus *Claviradulomyces* from Brazil

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Cryptic but ubiquitous: Claviradulomyceae fam. nov. with five novel species of the lenticel fungus *Claviradulomyces* from Brazil

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ABSTRACT

Claviradulomyces P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares was proposed to accommodate an apothecial fungus found growing on abnormal lenticels of *Erythroxylon mannii* Oliv. (Erythroxylaceae) in Africa (Ghana and Ivory Coast). After a second species of *Claviradulomyces* – *C. xylopiæ* R.W.Barreto, H.C.Evans & P.R.Johnst. – was found, also growing on abnormal lenticels of a plant belonging to a different family (Annonaceae) in South America (Brazil), it was conjectured that *Claviradulomyces* might be ubiquitous, but overlooked because of its cryptic nature. Here indications that this hypothesis may be correct were strengthened. Several collections of native Brazilian plants bearing seemingly abnormal (hypertrophied and spongy) lenticels belonging to five different plant families (Anacardiaceae, Asteraceae, Bignoniaceae, Fabaceae and Salicaceae) from several localities in schinii Brazil yielded typical structures of the asexual morph of *Claviradulomyces* – black pycnidia with rostrate ostiole containing fusoid-filiform hyaline conidia. Pure cultures were obtained for *Claviradulomyces* spp. collected from plants belonging to five of these host families. Five novel species of *Claviradulomyces* were recognized based on a combination of morphology and molecular features and are described herein as: *C. caseariae* sp. nov. (on *Casearia ulmifolia* Vahl ex. Vent.), *C. machaeriae* D.M.Macedo & R.W.Barreto, sp. nov. (on *Machaerium* sp.) *C. schini* sp. nov. (on *Schinus terebinthifolia* Raddi), *C. tabebuiae* sp. nov. (on *Tabebuia roseo-alba* (Ridl.) Sandwith) and *C. vermoniae* sp. nov. (on *Vernonia* sp.). A Bayesian inference analysis of the LSU and *rpb2* region of the known and novel species of *Claviradulomyces* supported the proposal of the new fungal family Claviradulomyceae fam. nov. (Ostropales). So far, *Claviradulomyces* is the only genus in this new family.

KEY WORDS
Asexual morph,
LSU,
Ostropales,
phylogeny,
new family,
new species.

RÉSUMÉ

Cryptique mais omniprésente : Claviradulomyceae fam. nov. avec cinq nouvelles espèces du Brésil du champignon des lenticelles Claviradulomyces.

Claviradulomyces P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares a été proposé pour héberger un champignon apothécial trouvé sur des lenticelles anormales d'*Erythroxylon mannii* Oliv. (Erythroxylaceae) en Afrique (Ghana et Côte d'Ivoire). Quand une deuxième espèce de *Claviradulomyces* – *C. xylopii* R.W.Barreto, H.C.Evans & P.R.Johnst. – a été trouvée, poussant également sur des lenticelles anormales d'une plante appartenant à une autre famille (Annonaceae) en Amérique du Sud (Brésil), il a été supposé que *Claviradulomyces* pourrait être omniprésent, mais ignoré en raison de sa nature cryptique. Ici, il a été constaté que cette hypothèse peut être correcte. Plusieurs collections de plantes brésiliennes indigènes portant des lenticelles apparemment anormales (hypertrophiées et spongieuses) appartenant à cinq familles de plantes différentes (Anacardiaceae, Asteraceae, Bignoniaceae, Fabaceae et Salicaceae) de plusieurs localités du Brésil ont donné des structures typiques de la forme asexuée de *Claviradulomyces* – pycnides noires à ostiole rostré contenant des conidies hyalines fusoid-filiformes. Des cultures pures de la plupart de ces champignons ont été obtenues à partir de plantes appartenant à cinq de ces familles d'hôtes. Cinq nouvelles espèces de *Claviradulomyces* ont été reconnues sur la base d'une combinaison de morphologie et de caractéristiques moléculaires et sont décrites ici comme suit: *C. caseariae* sp. nov. (sur *Casearia ulmifolia* Vahl ex. Vent.), *C. machaeriae* D.M.Macedo & R.W.Barreto, sp. nov. (sur *Machaerium* sp.), *C. schini* sp. nov. (sur *Schinus terebinthifolia* Raddi), *C. tabebuiae* sp. nov. (sur *Tabebuia roseo-alba* (Ridl.) Sandwith) et *C. vernoniae* sp. nov. (sur *Vernonia* sp.). Une analyse d'inférence bayésienne de la région LSU et *rp62* des espèces connues et nouvelles de *Claviradulomyces* a soutenu la proposition de la nouvelle famille fongique Claviradulomyceae fam. nov. (Ostropales). Jusqu'à présent, *Claviradulomyces* est le seul genre de cette nouvelle famille.

MOTS CLÉS
Formes asexuelles,
LSU,
Ostropales,
phylogénie,
famille nouvelle,
espèces nouvelles.

INTRODUCTION

The genus *Claviradulomyces* P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares was erected to accommodate a fungal species producing minute apothecia containing clavate/muricate paraphyses, unitunicate asci and hyaline, subcylindrical-filiform and unicellular ascospores. The asexual morph was described as being pycnidial, having a prominent neck and producing conidia much like the ascospores (Evans *et al.* 2010). This genus now comprises two species, *C. dabeicola* P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares and *C. xylopii* R.W.Barreto, H.C.Evans & P.R.Johnst., associated with two distantly related family hosts, Erythroxylaceae and Annonaceae, and reported from separate continents: *C. dabeicola* on *Erythroxylon mannii* Oliv. in Africa (Ghana and Ivory Coast) and *C. xylopii* on *Xylopia sericea* A.St.-Hil. in South America (Brazil). The habitat of both species is the abnormal (spongy and hypertrophied) lenticels on trunks or stems of woody hosts (Evans *et al.* 2010; Barreto *et al.* 2012). Although for *C. dabeicola* the suspicion was that the fungus caused a die-back on infected host-plants, this genus have not been properly investigated for the nature of its host association and it remains uncertain whether it is pathogenic or not or whether its colonization is systemic or localized and restricted to the lenticels. Phylogenetically it was found to belong to the order Ostropales, but its placement at the family level remained uncertain. It was firstly treated as a possible member of the Odontotremataceae (Evans *et al.* 2010) and later as *incertae sedis* by Barreto *et al.* (2012).

After the second species of *Claviradulomyces* – *C. xylopii* – was described it was then conjectured that this might

represent a large genus that remained unnoticed because of its small size and connection to a habitat entirely overlooked by mycologists. Therefore, *Claviradulomyces* might be ubiquitous, but obscure, because of its cryptic nature. Here, evidence was searched to test that hypothesis by randomly collecting and examining native plants in Brazil which appeared to be bearing hypertrophied lenticels. This search also aimed at generating a larger body of data which might produce a better resolution for the phylogenetic studies allowing for a clarification of the placement of *Claviradulomyces* within the Ostropales. Results of that investigation are presented herein.

MATERIAL AND METHODS

ISOLATES AND MORPHOLOGY

Since 2013, collections of various native Brazilian woody plants belonging to a range of plant families, bearing seemingly abnormal (hypertrophied and spongy) lenticels, were made. This followed no organized survey protocol, but only the chance observation of branches of plants bearing what appeared to be unusually large lenticels. Only samples of plants with which the collector (RWB) was familiar with or which were bearing flowers or fruits, allowing for easy identification by botanists, were included. Samples of branches were removed from plants with pruning scissors and left to dry naturally at well ventilated areas, in order to avoid mold overgrowth. The samples were examined under an Olympus SZX7 dissecting microscope and lenticels were scanned for the presence of typical *Claviradulomyces*

structures (sessile apothecia or rostrate pycnidia). Samples without any structures potentially belonging to *Claviradulomyces* were discarded.

Only the asexual stage of the fungus was found on all specimens bearing lenticels with *Claviradulomyces*-like colonies. Isolations from such structures were made by direct transfer of pycnidia onto tap water-agar (TWA) plates with a sterile fine-point needle. These structures were left for 5–10 minutes on the medium surface, in order to allow them to absorb water and release conidia. After this period, the pycnidia were gently rolled over the surface of the medium with the needle. A “trail” of conidia was hence spread over the TWA surface. Then, with the tip of the sterile needle, individual conidia were collected and transferred to plates containing potato dextrose-agar (PDA) and left in an incubator at 25°C. Plates were observed daily for colony development and individual colonies were transferred onto new plates in order to avoid contaminant overgrowth. Colonies suspected to belong to *Claviradulomyces* were transferred to potato carrot-agar (PCA) slants and left in a refrigerator at 4°C for later analysis.

Slides containing fungal structures were mounted on lactophenol or lactofuchsin. Measurements and photographs were taken with an Olympus BX 51 light microscope fitted with an Olympus E330 camera. Morphology of colonies was described after 40 days of growth on PDA and malt extract-agar (MEA) incubated at 25°C under 12 hours daily light regime. Colony color terminology followed Rayner (1970). Representative herbarium samples and pure cultures were deposited in the local herbarium (VIC) and culture collection (COAD) belonging to the Universidade Federal de Viçosa.

DNA ISOLATION, AMPLIFICATION AND ANALYSIS

Fungal DNA was extracted from 15 days old colonies formed on PD (potato-dextrose) using Wizard® Genomic DNA Purification Kit (Promega, WI) according to the manufacturer's instructions. Part of the large ribosomal subunit (LSU) was amplified with the primers LR0R and LR5 (Vilgalys & Hester 1990) and RNA polymerase II second largest subunit (*rpb2*) with 5F2 and 7CR (Reeb *et al.* 2004) using DreamTaq™ Master Mix (Fermentas) by following the manufacturer's instructions. The PCR reaction and conditions were the same described by Fernandes *et al.* (2013). The amplicons were sequenced on both directions (Macrogen, Korea) employing the same primers used for PCR amplification. A consensus sequence was computed from the forward and reverse sequences using DNA dragon software (<http://www.dna-dragon.com/index.php>). Additional sequences used in the analysis were retrieved from GenBank, including the outgroup *Pleopsidium chlorophanum* (Wahlenb.) Zopf and *Acarospora laqueata* Stizenb. ex Flagey (Table 1). Sequences were aligned with the software Muscle v.3.6 (Edgar 2004) on MEGA 5.0 program (Tamura *et al.* 2011). Bayesian analysis was conducted with MrBayes v.3.2.3 (Huelsenbeck & Ronquist 2001) to reconstruct the phylogeny of the order Ostropales. MrModeltest v.2.3 (Posada & Buckley 2004) were used to select the most suitable nucleotide substitution model. The Markov Chain Monte Carmelo (MCMC) analysis used four chains and was

run for 10 million generations or until the average standard deviation of split sequences dropped below 0.01. Trees were saved at each 1000 generations. The analysis was hosted by CIPRES science gateway portal at San Diego supercomputer center (Miller *et al.* 2010). The program Tracer v1.5 (Rambaut & Drummond 2003) was used to ensure the convergence of the chains and the consensus tree was calculated in MrBayes v.3.2.3 (Huelsenbeck & Ronquist 2001) where the first 25% of sampled trees were discarded as “burnin”. Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

RESULTS

PHYLOGENETIC ANALYSES

The LSU consensus sequences obtained for the *Claviradulomyces* isolates showed a high level of identity to *C. dabeicola* and *C. xylopii* isolates available in GenBank. The combined data presented by Papong *et al.* (2009), Baloch *et al.* (2010) and Barreto *et al.* (2012) were used to construct the phylogenetic tree (Fig. 1). The alignment data matrix consisted of 53 taxa (including the outgroup sequence) and 936 characters in which 436 sites were variable and 337 sites were parsimony informative. Sequences of the five new species of *Claviradulomyces* formed, together with the two previously described species of *Claviradulomyces*, an isolated and well-supported clade (posterior probability of 1.0) within the Ostropales.

Division ASCOMYCOTA (Berk.) Caval.-Sm.
Class PEZIZOMYCETES O.E.Eriksson & Winka
Order OSTROPALES Nannf.

Family CLAVIRADULOMYCEAE
L.L.Duarte, D.M.Macedo & R.W.Barreto, fam. nov.

MYCOBANK NUMBER. — MB 811908.

DIAGNOSIS. — Member of the order Ostropales, according to the phylogenetic analysis of LSU sequences. Sporulating on hosts with abnormal, hypertrophied and spongy lenticels. Sexual morph (when present) apothecial. Apothecia sessile, containing clavate-muricate paraphyses and unitunicate asci, containing eight subcylindrical-filiform ascospores. Asexual morph pycnidial. Pycnidia spherical to subspherical ostiolate, rostrate, producing filiform to narrow acerose conidia, commonly aseptate and often bearing a subtle heel next to the base.

Genus *Claviradulomyces*
P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto &
D.J.Souares

Fungal Biology 114 (1): 43 (2010).

TYPE SPECIES. — *Claviradulomyces dabeicola* P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares (Evans *et al.* 2010: 43).

TABLE 1. — GenBank accession number of sequences belonging to species treated in this study. Newly generated sequences in **bold**.

ISOLATE	<i>rpb2</i>	LSU
<i>Absconditella sphagnorum</i> Vězda & Poelt	HM244777	AY300824
<i>Acarospora laqueata</i> Stizenb. ex Flagey	AY641024	AY58464
<i>Acarosporina microspora</i> (R.W. Davidson & R.C. Lorenz) Sherwood	AY584682	AY584643
<i>Astrochapsa astroidea</i> (Berk. & Broome) Parnmen, Lücking & Lumbsch	JX420945	JX421443
<i>Belonia russula</i> Körb. ex Nyl.	HM244780	HM244759
<i>Carbacanthographis stictica</i> Staiger & Kalb	JX420875	JF828974
<i>Chapsa alborosella</i> (Nyl.) Frisch	JX465320	JX421440
<i>Chapsa indica</i> A. Massal.	JX465322	JX465295
<i>Chapsa leprocarpa</i> (Nyl.) Frisch	JX420942	JX421455
<i>Chapsa niveocarpa</i> Mangold	JX420828	FJ708487
<i>Chapsa patens</i> (Nyl.) Frisch	JX420941	JX421458
<i>Chapsa phlyctidioides</i> (Müll. Arg.) Mangold	HM244783	KC020260
<i>Chapsa pulchra</i> (Müll. Arg.) Mangold	HM244784	KC020261
<i>Chapsa sublilacina</i> (Ellis & Everh.) M. Cáceres & Lücking	JX420842	JX421466
<i>Chromatochlamys muscorum</i> (Th. Fr.) H. Mayrhofer & Poelt	—	AY607731
<i>Claviradulomyces caseariae</i> sp. nov.	MW191755	MK931434
<i>Claviradulomyces dabeicola</i> P.R.Johnst., D.C.Park, H.C.Evans, R.W.Barreto & D.J.Souares	—	GQ337897
<i>Claviradulomyces macheriae</i> D.M.Macedo & R.W.Barreto, sp. nov.	—	MW148819
<i>Claviradulomyces schini</i> sp. nov.	—	KP975456
<i>Claviradulomyces tabebuiae</i> sp. nov.	MW191754	KP975454
<i>Claviradulomyces vernoniae</i> sp. nov.	MW191753	KP975455
<i>Claviradulomyces xylopii</i> R.W.Barreto, H.C.Evans & P.R.Johnst.	—	NG042732
<i>Coenogonium leprieurii</i> (Mont.) Nyl.	AY641032	AF465442
<i>Coenogonium luteum</i> (Dicks.) Kalb & Lücking	AY641038	AF279387
<i>Coenogonium pineti</i> (Ach.) Lücking & Lumbsch	HM244786	AY300834
<i>Crutarndina petractoides</i> (P.M. Jörg. & Brodo) Parnmen, Lücking & Lumbsch	JX420891	JX421664
<i>Cryptodiscus gloeocapsa</i> (Nitschke ex Arnold) Baloch, Gilenstam & Wedin	HM244788	AF465440
<i>Cyanodermella viridula</i> (Berk. & M.A. Curtis) O.E. Erikss	HM244792	HM244763
<i>Diploschistes actinostomus</i> (Ach.) Zahlbr.	KF688546	KF688496
<i>Diploschistes cinereocaesius</i> (Sw.) Vain.	DQ883755	AY300835
<i>Diploschistes elixii</i> Lumbsch & Mangold	KF688541	—
<i>Diploschistes euganeus</i> (A. Massal.) J. Steiner	KF688544	KF688493
<i>Diploschistes muscorum</i> (Scop.) R. Sant.	AY485623	KC167077
<i>Diploschistes scruposus</i> (Schreb.) Norman	AY641039	AF279389
<i>Diploschistes</i> sp.	EU126644	—
<i>Dyplolabia afzelii</i> (Ach.) A. Massal	KC020273	HQ639628
<i>Fissurina insidiosa</i> C. Knight & Mitt	DQ973083	DQ973045
<i>Glaucotrema glaucophaeum</i> (Kremp.) Rivas Plata & Lumbsch	JX420902	JX421502
<i>Glaucotrema glaucopallens</i> (Nyl.) Rivas Plata & Kalb	KF875518	AY605069
<i>Gomphillus calycioides</i> (Delise ex Duby) Nyl.	KF833356	KF833329
<i>Graphis scripta</i> (L.) Ach.	HM244793	DQ431937
<i>Gyalecta flotowii</i> Körb	HM244794	HM244764
<i>Gyalecta hypoleuca</i> (Ach.) Zahlbr	AY641060	AF465453
<i>Gyalecta jenensis</i> (Batsch) Zahlbr.	AY641043	AF465450
<i>Gyalecta truncigena</i> (Ach.) Hepp	HM244796	HM244766
<i>Gyalecta ulmi</i> (Sw.) Zahlbr.	AY641044	—
<i>Gyalideopsis vulgaris</i> (Müll. Arg.) Lücking	—	AY584649
<i>Leucodecton subcompunctum</i> (Nyl.) Frisch	KJ766978	JX421518
<i>Myriochapsa psoromica</i> (M.Cáceres, L.S.de Jesus & T.S.Vieira) M.Cáceres, Lücking & Lumbsch	JX420884	JX421461
<i>Myriotrema olivaceum</i> Fée	HM244799	EU075627
<i>Ocellularia allosporoides</i> (Nyl.) Patw. & Kulk.	JX420925	JX421544
<i>Ocellularia profunda</i> (Stirt.) Mangold, Elix & Lumbsch	JX420825	EU075636
<i>Odontotrem phacidioides</i> Nyl.	HM244803	HM244770
<i>Odontotrema phacidiellum</i> Nyl.	HM244802	HM244769
<i>Phaeographis intricans</i> (Nyl.) Staiger	JX420924	JX421601
<i>Phlyctis agelaea</i> (Ach.) Flot.	KC020280	AY853381
<i>Phlyctis argena</i> (Ach.) Flot.	KC020281	DQ986771
<i>Pleopsidium chlorophanum</i> (Wahlenb.) Zopf	DQ525460	AY58464
<i>Porina byssophila</i> P.M. McCarthy & Kantvilas	HM244809	—
<i>Porina epiphylla</i> Fée	KJ766942	—
<i>Porina lectissima</i> (Fr.) Zahlbr.	—	HM244774
<i>Porina nitidula</i> Müll. Arg.	—	KF833322
<i>Pycnotrema pycnoporellum</i> (Nyl.) Rivas Plata & Lücking	KF875513	JX421615
<i>Rhexophiale rhexoblephara</i> (Nyl.) Almq.	—	KJ766656
<i>Sagirolechia protuberans</i> (Ach.) A. Massal.	HM244812	KJ766655
<i>Schizoxylon albescens</i> Gilenstam, H. Döring & Wedin	HM244813	—
<i>Stegobolus anamorphus</i> (Nyl.) Frisch & Kalb	JX420913	JX421636
<i>Stegobolus subcavatus</i> (Nyl.) Frisch	JX420889	EU075641
<i>Stictis populorum</i> (Gilenstam) Gilenstam	HM244817	AY527327
<i>Thelenella antarctica</i> (I.M.Lamb) O.E. Erikss.	—	AY607739

TABLE 1. — Continuation.

ISOLATE	<i>rpb2</i>	LSU
<i>Thelotrema adjectum</i> Nyl.	JX420853	JX421641
<i>Thelotrema lepadinum</i> (Ach.) Ach.	DQ973085	AY300866
<i>Thelotrema porinoides</i> Mont. & Bosch	JX465345	JX421665
<i>Thelotrema subtile</i> Tuck.	JX465348	DQ871013
<i>Thelotrema suecicum</i> (H.Magn.) P.James	KC020284	AY300867
<i>Topeliopsis acutispora</i> Kalb	KF875515	JX421675
<i>Topeliopsis subdenticulata</i> (Zahlbr.) Frisch & Kalb	KF875517	EU126656
<i>Wirthiotrema decorticans</i> (Müll. Arg.) Frisch & Kalb	KC020285	EU075654

Claviradulomyces caseariae

L.L.Duarte, D.M.Macedo & R.W.Barreto, sp. nov.
(Fig. 2)

MYCOBANK NUMBER. — MB 811909.

HOLOTYPE. — **Brazil**. State of Minas Gerais, Fervedouro, Refúgio dos Gaudinos, on branches of *Casearia ulmifolia* Vahl ex. Vent. (Salicaceae), 23.I.2014, R.W.Barreto (VIC 42849, COAD 2348, GenBank LSU sequence MK931434, *rpb2* sequence MW191755).

ETYMOLOGY. — Referring to its host-genus *Casearia*.

DIAGNOSIS. — Differing from other species of *Claviradulomyces* by having septate conidia.

HABITAT AND DISTRIBUTION. — Inside lenticels on branches of a tree growing on the margin of a creek in remnants of the Atlantic tropical rainforest.

DESCRIPTION

Asexual morph in abnormal hypertrophied lenticels with spongy tissue present on living branches. Conidiomata pycnidial, semi-immersed, isolate, mostly one per lenticel, globose to subglobose 50–70 µm diam; walls pale brown, *textura intricata* at base, changing into olivaceous to light brown *textura angularis* in the upper half, ostiolate, rostrate; neck subulate 50–105 µm long and c. 20 µm diam, walls pale brown, *textura porrecta*, ending in a short, narrower tip of bristle-like hyphae, smooth. Conidiophores normally reduced to conidiogenous cells, hyaline, suboblate to somewhat lageniform, straight to slightly curved, (–10) 12.5–15 × 2.5 µm, 0–1 septate, hyaline, smooth. Conidiogenous cells hyaline, holoblastic and smooth. Conidia fusoid-filiform to narrowly acerose, 14.5–30 × 1.5–2.5 µm, attenuated toward the ends, apex rounded, base subtruncate, guttulate, 0–2 septate, guttulate, hyaline, smooth. Sexual morph not observed.

Culture characteristics

Very slow growing (2.2–3.9 cm diam after 40 days), colonies dry, flat or effuse, slightly depressed, with lobed or entire margins, aerial mycelium scarce and immersed at periphery; pale purplish grey, pigmenting the medium; ending with a white periphery; not sporulating.

NOTES

Claviradulomyces caseariae sp. nov. has the most divergent morphology among the old and newly described species for of *Claviradulomyces* in terms of its asexual stage. It has larger conidiophores, (–10) 12.5–15 × 2.5 µm, and is the only species described until now having septate conidia.

Claviradulomyces machaeriae

D.M.Macedo & R.W.Barreto, sp. nov.
(Fig. 3)

MYCOBANK NUMBER. — MB 838369.

HOLOTYPE. — **Brazil**. State of Rio de Janeiro, Nova Friburgo, Riograndina, on branches of *Machaerium* sp. (Fabaceae), 7.IV.2014, R.W.Barreto (VIC 42840; culture COAD 1936; GenBank LSU sequence KP975453).

ETYMOLOGY. — Refers to its host-genus *Machaerium*.

DIAGNOSIS. — Similar to *C. xylopieae* but having a pale brown to olivaceous pycnidial body instead of dark brown as in *C. xylopieae*.

HABITAT AND DISTRIBUTION. — Inside lenticels on branches of *X. sericea* in a secondary Atlantic Forest and on lenticels on branches of *Machaerium* sp. in ruderal (roadside) situation.

DESCRIPTION

Asexual morph on living branches bearing abnormal hypertrophied lenticels with spongy tissue. Conidiomata pycnidial, commonly several per lenticel, subglobose, 50–95 µm diam, basal narrow layer of subhyaline, walls subhyaline to olivaceous, *textura intricata* at base, changing into pale brown *textura angularis* in upper half, smooth, ostiolate, rostrate; neck long, 140–340 × 20–37 µm, slightly curved, dark brown to olivaceous becoming subhyaline at the tip, of *textura porrecta*. Conidiophores reduced to conidiogenous cells, holoblastic, suboblate to somewhat lageniform, straight, 5–8 × 1.5–2 µm, hyaline, smooth. Conidia fusoid-filiform, 25–35 × 2–3 µm, straight to slightly curved, attenuated toward the acute tip, aseptate, guttulate, subhyaline to hyaline, smooth. Sexual morph not observed.

Culture characteristics

Very slow-growing (1.5–2.5 cm diam after 40 days). Colonies dry or moist, flat to slightly raised centrally, margins even or

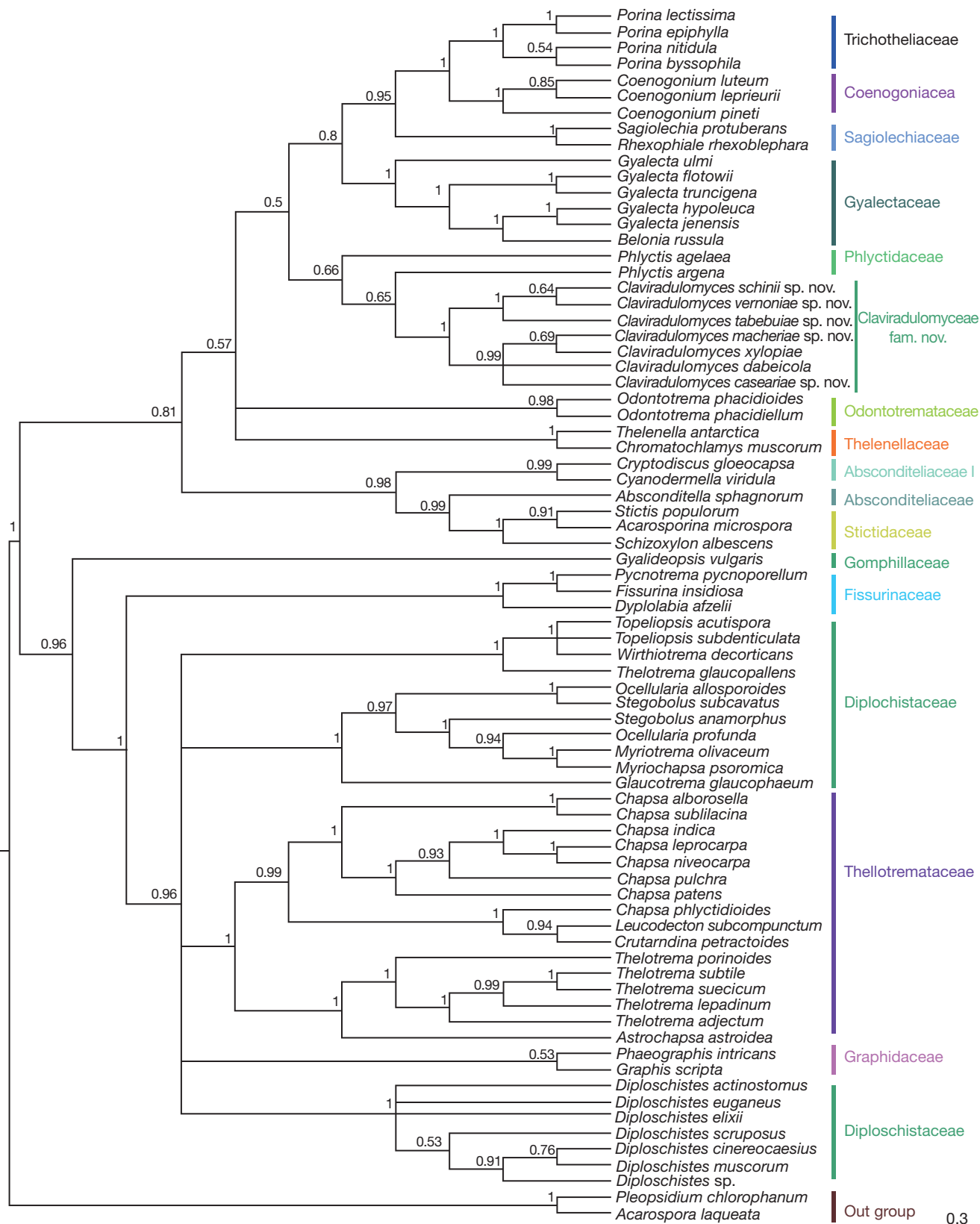


FIG. 1. — Phylogenetic tree of the *Claviradulomyces* isolates inferred by Bayesian analysis of nuclear LSU and *rpb2* sequences. Bayesian posterior probabilities are given at the nodes and the accession numbers are presented together with the species names. The newly proposed family Claviradulomyceae is indicated. The tree is rooted with *Pleopsidium chlorophanum* (Wahlenb.) Zopf and *Acarospora laqueata* Stizenb. ex Flagey.



FIG. 2. — *Claviradulomyces casearia* L.L.Duarte, D.M.Macedo & R.W.Barreto sp. nov. (VIC 42849, holotype): **A, B**, *Casearia ulmifolia* Vahl ex. Vent. branches with abnormal lenticels colonized by *C. casearia*; **C**, pycnidium with rostrate ostiole neck; **D**, conidiophores with attached immature conidia. Scale bars: C, 20 μ m; D, 10 μ m.

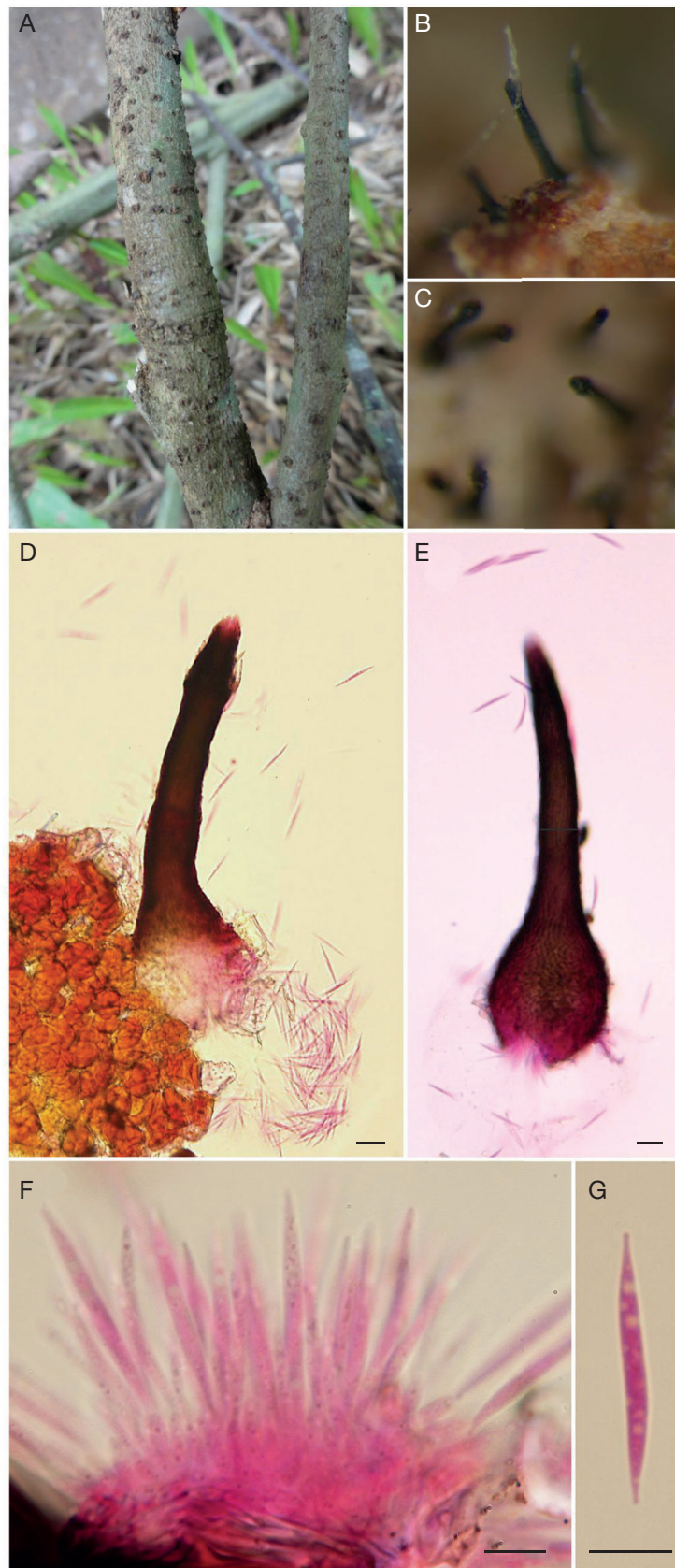


FIG. 3. — *Claviradulomyces machaeriae* D.M.Macedo & R.W.Barreto, sp. nov.: **A**, branch of *Machaerium* sp. showing abnormal lenticels colonized by *C. machaeriae* sp. nov. (VIC 42840); **B**, **C**, pycnidia on lenticels. **D**, **E**, pycnidia; **F**, group of immature conidia attached to the conidiogenous cells; **G**, conidium. Scale bars: B-E 20 µm; F, G, 10 µm.

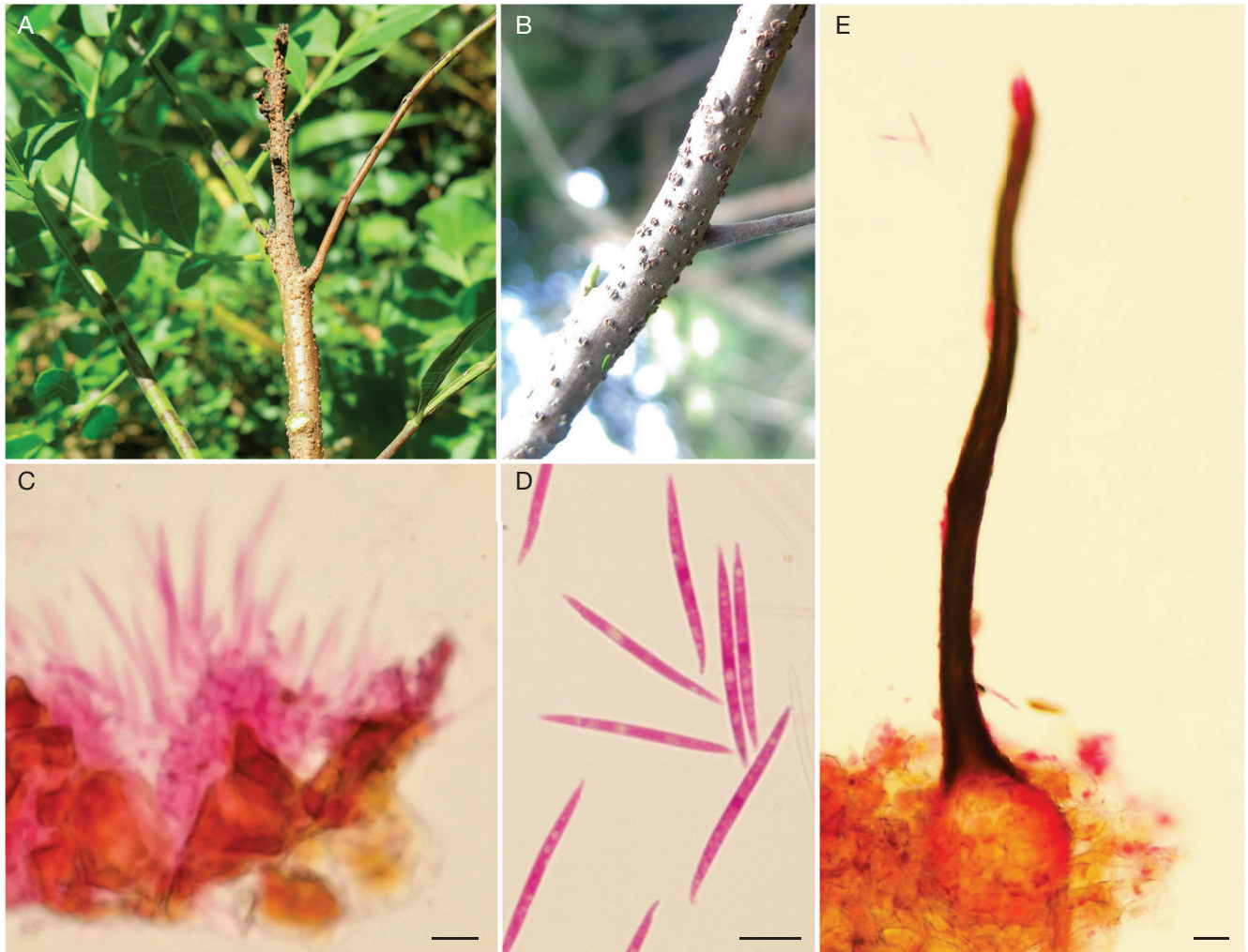


FIG. 4. — *Claviradulomyces schini* sp. nov. (VIC 42845, holotype): **A**, *Schinus terebinthifolia* Raddi. Individual branch with dieback symptom and abnormal lenticels colonized by *C. schini*; **B**, close-up of hypertrophied lenticels; **C**, conidiophores with immature conidia still attached; **D**, conidia; **E**, pycnidium with sinuose and rostrate-vermiform ostiole neck. Scale bars: B, C, 10 µm; E, 30 µm.

lobate, aerial mycelium scarce, mostly dark brick with cinnamon edges or ochreous centrally followed by a dark mouse grey band, ending with a narrow honey periphery or velvet isabelline mycelium centrally and powdery buff mycelium at the periphery; not sporulating.

NOTES

Claviradulomyces machaeriae D.M.Macedo & R.W.Barreto, sp. nov. is morphologically similar to *C. xylopieae*. However, the color of the pycnidial body is olivaceous in *C. machaeriae* D.M.Macedo & R.W.Barreto, sp. nov. and dark brown in *C. xylopieae*. The neck is bigger in *C. machaeriae* D.M.Macedo & R.W.Barreto, sp. nov. in comparison with *C. xylopieae*. The combination of morphological and molecular, using *rpb2* confirm the fungus on *Machaerium* as a distinct species, although close to *C. xylopieae*.

Claviradulomyces schini

L.L.Duarte, D.M.Macedo & R.W.Barreto, sp. nov.
(Fig. 4)

MYCOBANK NUMBER. — MB 811918.

HOLOTYPE. — **Brazil**. State of Minas Gerais, Viçosa, on branches of *Schinus terebinthifolius* Raddi (Anacardiaceae), 12.XII.2013, R.W.Barreto (VIC 42845; ex-type culture COAD 1937; GenBank LSU sequence KP975456).

ADDITIONAL SPECIMENS EXAMINED. — **Brazil**. State of Minas Gerais, Viçosa, on branches of *S. terebinthifolius*, 22.VI.2013, R.W.Barreto (VIC 42842).

ETYMOLOGY. — Referring to its host-genus *Schinus*.

DIAGNOSIS. — Similar to *C. xylopieae* but producing pycnidia with vermiform (narrow, long and sinuose) ostiole necks.

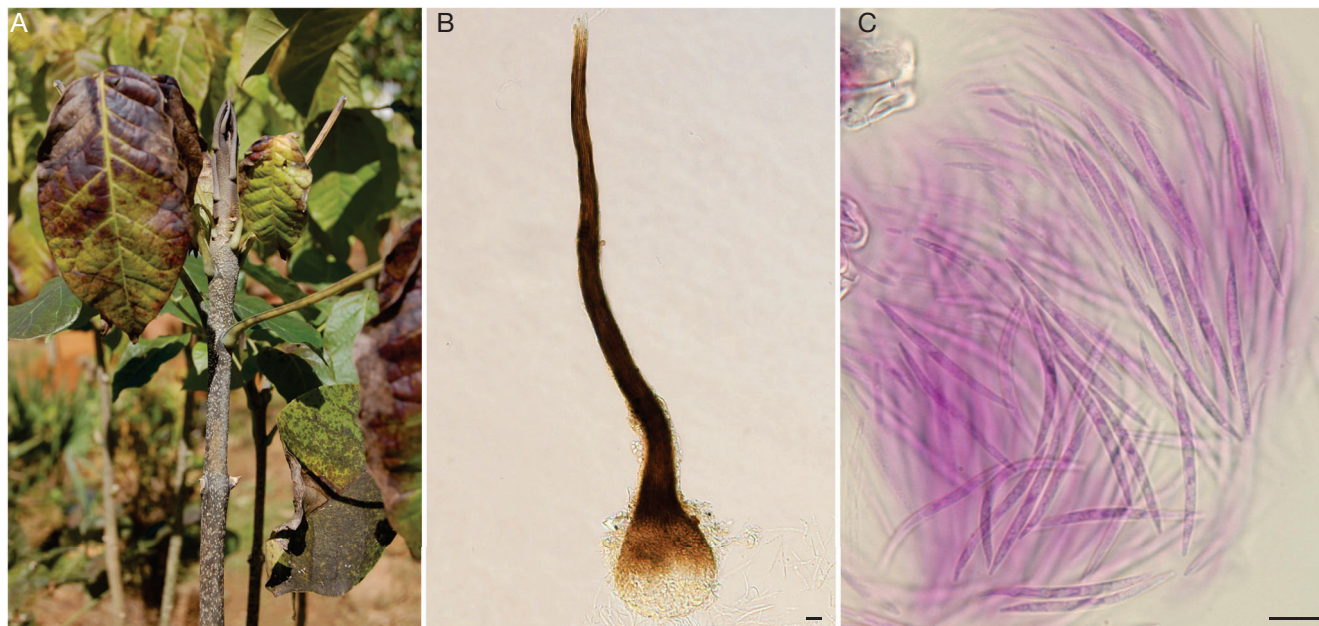


FIG. 5. — *Claviradulomyces tabebuiae* sp. nov. (VIC 42848, holotype): **A**, *Tabebuia roseo-alba* (Ridl.) Sandwith stem with abnormal lenticels colonized by *C. tabebuiae* sp. nov.; **B**, pycnidium with rostrate-vermiform ostiole neck; **C**, group of conidia. Scale bars: B, 30 μ m; C, 10 μ m.

HABITAT AND DISTRIBUTION. — Inside lenticels on branches of *S. terebinthifolius* (Brazilian pepper tree) in secondary forest or ruderal situations.

DESCRIPTION

Asexual morph on living, often defoliated branches bearing abnormal hypertrophied spongy lenticels. Conidiomata pycnidial, semi-immersed, mostly isolate, globose, 55–125 μ m diam, walls subhyaline to yellowish, *textura intricata* at the base, changing into light brown *textura globulosa* in the upper half, ostiolate, rostrate: neck, long, sinuous, vermiform, 245–385 $\times$ 32 μ m, dark brown, *textura porrecta* becoming subhyaline towards the tip, smooth. Conidiophores commonly reduced to conidiogenous cells, lageniform, straight to slightly curved, 4.5–6 $\times$ 1.5–2 μ m, 0–1 septate, hyaline, smooth. Conidia straight to slightly curved, fusoid-filiform to narrowly acerose, 18–31 $\times$ 1–2.5 μ m, attenuated toward the ends, apices acute to subacute, aseptate, guttulate, hyaline, smooth. Sexual morph not observed.

Culture characteristics

Slow growing (5.5 cm diam after 40 days), colonies flat, adpressed on the medium (in the dark) or raised and moist (under alternating light), aerial mycelium velvety, dense becoming scarce towards periphery, uniformly olivaceous – black or greenish black with narrow dull green peripheral ring; spermogonia (formed on PCA), subsphaerical, 62.5–115 $\times$ 47.5–87.5 μ m, spermatia in white creamy masses, globose to subglobose, 2–3 $\times$ 2–2.5 μ m, hyaline, smooth. No conidia or ascospores produced in any medium.

NOTES

Claviradulomyces schini sp. nov. is similar to *C. tabebuiae* sp. nov. but can be differentiated from that species by having shorter conidia, 18–31 μ m as compared to 32–40 μ m of *C. tabebuiae* sp. nov., and by having its conidiophores reduced to lageniform conidiogenous cells.

Claviradulomyces tabebuiae

L.L.Duarte, D.M.Macedo & R.W.Barreto, sp. nov.
(Fig. 5)

MYCOBANK NUMBER. — MB 811920.

HOLOTYPE. — **Brazil.** Minas Gerais, Viçosa, Fazenda Antuérpia Mineira, on branches of *Tabebuia roseo-alba* (Ridl.) Sandwith (Bignoniaceae), 18. XII..2013, R. W.Barreto (VIC 42848; ex-type culture COAD 1935; GenBank LSU sequence KP975454, *rpb2* sequence MW191754).

ETYMOLOGY. — Referring to its host-genus *Tabebuia*.

DIAGNOSIS. — Differs from other known species of *Claviradulomyces* by forming larger pycnidia (70–230 μ m diam) and very long (up to 540 μ m) rostra.

HABITAT AND DISTRIBUTION. — Inside lenticels on branches of ornamental tree *Tabebuia roseo-alba* at a commercial nursery.

DESCRIPTION

Asexual morph mostly in abnormal hypertrophied spongy lenticels of defoliated living branches. Conidiomata pycnidial, semi-immersed, subglobose to globose, isolated, 70–230 μ m diam, walls subhyaline to olivaceous, *textura intricata* at

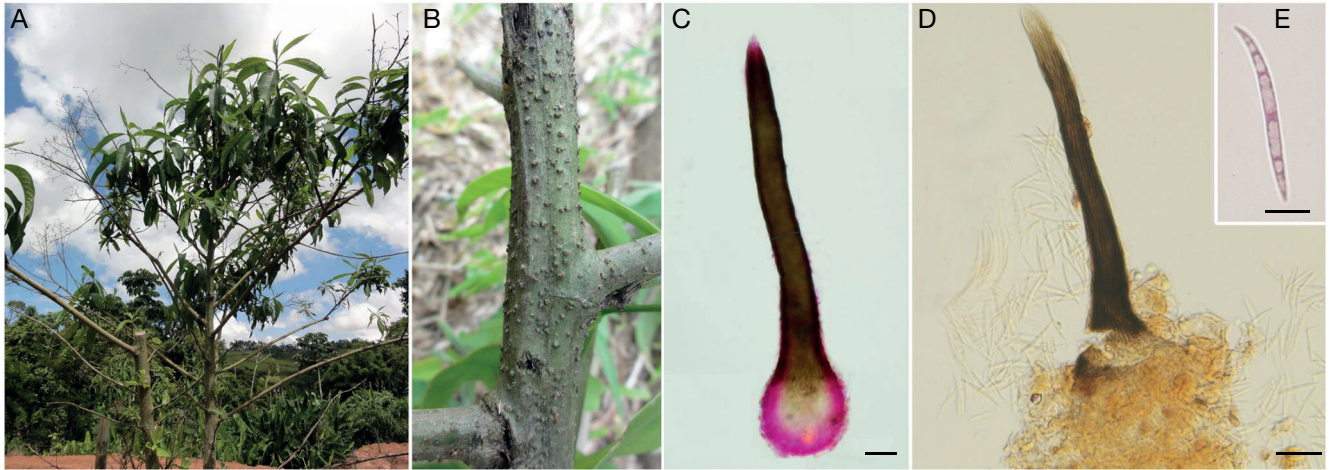


FIG. 6. - *Claviradulomyces vernoniae* sp. nov. (VIC 42847, holotype): **A**, *Vernonia* sp. defoliated stem with abnormal lenticels colonized by *C. vernoniae*; **B**, close-up of abnormal lenticels; **C**, **D**, pycnidium with rostrate ostiole neck; **E**, detail of conidium. Scale bars: C, D, 30 µm; E, 10 µm.

base, changing into pale brown *textura angularis* in upper half, smooth, ostiolate, rostrate; neck long sinuous, vermiform, 160–540 × 37 µm, medium to dark brown becoming light brown at the tip, *textura porrecta*. Conidiophores reduced to conidiogenous cells, holoblastic, suboblate to somewhat lageniform, straight to slightly curved, 3.5–5 × 1.5–2 µm, hyaline, smooth. Conidia fusoid-filiform to narrowly acerose, 32–40 × 2–2.5 µm, straight to slightly curved, attenuated toward the acute tip, subtly heeled at the base ending in very short narrow stalk, aseptate, guttulate, subhyaline to hyaline, smooth. Sexual morph not observed.

Culture characteristics

Very slow growing (1.5–2 cm diam after 40 days), colonies dry, flat to slightly depressed, with lobed or entire margins, aerial mycelium scarce and immersed at periphery; either buff centrally, followed by an isabelline band or evenly buff or honey, darkening the culture medium around the colony; not sporulating.

NOTES

Claviradulomyces tabebuiae sp. nov., produces large pycnidia which bear long vermiform rostra, 160–540 µm, (the longest amongst the members of the genus). Additionally, it produces the smallest conidiophores, 3.5–5 × 1.5–2 µm known for the genus.

Claviradulomyces vernoniae

L.L.Duarte, D.M.Macedo & R.W.Barreto, sp. nov.
(Fig. 6)

MYCOBANK NUMBER. — MB 811921.

HOLOTYPE. — **Brazil**. State of Minas Gerais, Viçosa, campus of Universidade Federal de Viçosa – near the Experimental Barn, on branches of *Vernonia* sp. (Asteraceae), 14.XII.2013, R.W.Barreto

(VIC 42847; ex-type culture COAD 1934; GenBank LSU sequence KP975455, *rpb2* sequence MW191753).

ADDITIONAL SPECIMENS EXAMINED. — **Brazil**. State of Rio de Janeiro, Nova Friburgo, Riograndina, on branches of *Vernonia* sp., 11.XII.2013, R.W.Barreto (VIC 42844). Ibid. 07.IV.2014, R.W.Barreto (VIC 42839).

ETYMOLOGY. — Refers to its host-genus *Vernonia*.

DIAGNOSIS. — Similar to *C. xylopiæ* but having a paler subhyaline to yellowish brown pycnidial body instead of dark brown as in *C. xylopiæ*.

HABITAT AND DISTRIBUTION. — Inside lenticels on branches of *Vernonia* sp. growing in ruderal situations (abandoned pastures and roadsides) at localities in the states of Minas Gerais and Rio de Janeiro (Brazil).

DESCRIPTION

Asexual morph on living branches bearing abnormal hypertrophied lenticels with spongy tissue. Conidiomata pycnidial, semi-immersed, isolate, subglobose to globose, 75–100 µm diam, walls subhyaline, *textura intricata* at the base, yellowish brown *textura angularis* and then dark brown at the upper part basal to the neck, ostiolate; neck slightly curved to sinuose, with a broad base, 170–190 µm long and *c.* 29 µm diam, walls dark brown, *textura porrecta*, progressively tapering towards the apex, ended in acute pale brown tip, smooth, Conidiophores mostly reduced to holoblastic conidiogenous cells, lageniform, straight to slightly curved, 5–7.5 × 1.5–2 µm, 0–1 septate, hyaline, smooth. Conidia fusoid-filiform to narrowly acerose, straight to curved, 17–35 × 2–3 µm, attenuated toward the acute tip, subtly heeled at the base ending in narrow stalk, aseptate, guttulate, hyaline, smooth. Sexual morph not observed.

Culture characteristics

Very slow-growing (1.5–2 cm diam after 40 days), colonies grooved or adpressed on the medium, dry or moist, aerial

KEY TO THE SPECIES OF *CLAVIRADULOMYCES* (BASED ON ASEQUAL MORPH CHARACTERS ONLY)

1. Pycnidium with short ostiole neck (shorter than pycnidium diam)..... *Claviradulomyces dabeicola*
— Pycnidium with long ostiole neck (equal or longer than pycnidium diam) 2
2. Ostiole neck vermiform (narrow, long and sinuous) 3
— Ostiole neck non-vermiform 4
3. Conidia less than 31 µm long..... *Claviradulomyces schini* sp. nov.
— Conidia longer than 31 µm *Claviradulomyces tabebuiae* sp. nov.
4. Conidia septate *Claviradulomyces caseariae* sp. nov.
— Conidia aseptate..... 5
5. Pycnidium body mostly pale brown to olivaceous *Claviradulomyces vernoniae* sp. nov.
— Pycnidium body dark brown 6
6. Ostiole neck curved..... *Claviradulomyces machaeriae* sp. nov.
— Ostiole neck straight *Claviradulomyces xylopieae*

mycelium either abundant and cottony or scarce white centrally and buff at the edge or olivaceous grey to iron grey with a very narrow white edge, pigmenting the medium in brown; not sporulating.

NOTES

Claviradulomyces vernoniae sp. nov. is morphologically similar to *C. xylopieae*, with only minor biometric differences found between these two species. Nevertheless, the color of the pycnidial body is a useful feature for distinguishing between *C. vernoniae* sp. nov. and *C. xylopieae* sp. nov. It is subhyaline to yellowish in *C. vernoniae* sp. nov. and more evenly dark brown in *C. xylopieae* sp. nov. The combination of morphological and molecular evidence are regarded here as sufficient to justify the proposition of the new species.

For a comparison between the species of *Claviradulomyces* a key to species identification is presented below.

DISCUSSION

Naming of fungal taxa reflecting their evolutionary relationships is a cornerstone of modern mycology, but the application of species concepts to asexual fungal taxa can be very controversial, especially when the morphological markers are scarce and overlapping (Harrington & Rizzo 1999; Stewart *et al.* 2014). Although the two species of *Claviradulomyces* firstly described (*C. dabeicola* and *C. xylopieae*) produced elaborate apothecia, it was found that morphological characters of the sexual morph provided little ground for species segregation (only ascus and peryphoid length being of some use in the case of those two species). The most significant morphological differences found between *C. dabeicola* and *C. xylopieae* were in their asexual structures (Barreto *et al.* 2012). It was not possible to anticipate if this would also apply to species to be found and included in the genus. Fortunately, morphology

of the pycnidial stage was, sufficiently divergent to provide a useful basis for species differentiation for all five novel species of *Claviradulomyces* proposed herein. Size and shape of the conidiophores and conidogenous cells, size of conidia and color of pycnidium, as well as the shape and length of the neck alone or in combination, were adequate for distinction between species. Host-specialization, at least to the host family-level, also seems to exist, and may be of taxonomic relevance, as each of seven species of *Claviradulomyces* was found in distinct host family: *C. caseariae* sp. nov. on a member of the Salicaceae, *C. dabeicola* on an Erythroxylaceae, *C. machaeriae* D.M.Macedo & R.W.Barreto, sp. nov. on a member of the Fabaceae, *C. schini* sp. nov. on an Anacardiaceae, *C. tabebuiae* on a Bignoniaceae, *C. vernoniae* sp. nov. on an Asteraceae and *C. xylopieae* on a member of the Annonaceae. As knowledge about this genus is expanded, a more clear understanding of the host-specificity issue will naturally emerge. Unfortunately, at this stage, there are no results of cross inoculation experiments available to further verify the host range of the species belonging in this genus. Such studies would be of relevance for this group of fungi, to clarify the ecological relations of *Claviradulomyces* to its habitat and niche.

Recently, the classification of orders and families of the main subclasses of Lecanoromycetes were reappraised and information drawn from the analysis of the *rpb2* gene region proved fundamental for reorganizing these groups of taxa (Kraichak *et al.* 2018). The flaws of classifications based on LSU- only phylogenies were corrected and a better resolved and more natural classification became available portraying the relationships between the Lecanoromycetes families.

The Lecanoromycetes is a class of fungi traditionally viewed by mycologists as composed mostly of lichen-forming species (Kirk *et al.* 2008). Currently, it is divided in two subclasses, Lecanoromycetidae and Ostropomycetidae. Lecanoromycetidae is very large and includes more than 10.000 accepted species of lichen-forming species placed in

eight orders (Lücking *et al.* 2017). The other subclass – the Ostropomycetidae – was only recognized recently (Lücking *et al.* 2017). In comparison with Lecanoromycete this is a smaller subclass (c. 5000 spp.) (Reeb *et al.* 2004). The Ostropomycetidae represent a morphologically and ecologically diverse assemblage of ascomycetes (Schmitt *et al.* 2005, 2009, 2012). It includes six lichen-forming orders (Arctomiales, Aeomycetales, Hymeneliales, Pertusariales, Sarrameanales and Trapeliales) and one order – Ostropales – including lichenized and non-lichenized fungi (Lücking *et al.* 2017). The Ostropales includes 10 families, namely: Coenogoniaceae, Graphidaceae, Gyalectaceae, Myeloconidaceae, Odontotremataceae, Phaneromycetaceae, Phlyctidaceae, Porinaceae, Sagirolechiaceae, and Stictidaceae (Baloch *et al.* 2010; Lumbsch & Huhndorf 2010; Rivas Plata *et al.* 2012). Although Coenogoniaceae, Gyalectaceae, Porinaceae and Sagirolechiaceae are regarded as monophyletic, the affinity among the families of Ostropales is regarded as remaining uncertain (Miadlikowska *et al.* 2014). Although the placement of the lenticel fungi in the Ostropales was considered as strongly supported by the phylogenetic analysis of Barreto *et al.* (2012), the family level relationship of *Claviradulomyces* was considered as remaining unresolved.

When *Claviradulomyces* was first described it was readily recognized, based on morphological and molecular features, as belonging to the order Ostropales (Evans *et al.* 2010). It was firstly thought to belong to the Odontotremataceae because of its morphology (Evans *et al.* 2010). Later phylogenetic analysis of the Odontotremataceae divided it in two groups *Odontotremae* s. str. (Baloch *et al.* 2010) and a second clade nested with the Stictidaceae (Baloch *et al.* 2013). Nevertheless, the suggested placement of *Claviradulomyces* in the Odontotremataceae was shown to be mistaken after a phylogeny reconstruction based at LSU and SSU sequences placed it as “incertae sedis” within the Ostropales (Barreto *et al.* 2012). This study indicated that *Claviradulomyces* is only distantly related to the Odontotremataceae. At the time it was thought that, if a larger number of taxa sharing the same morphological, ecological and molecular features was found it might be justifiable to treat this group at a supra-generic, possibly as a distinct family. The results of the novel phylogenetic study, presented herein, consistently showed that *Claviradulomyces* belongs to a separate, well resolved and well supported clade within the Ostropales, occupying a basal position within the order (Fig. 1). It was hence considered best to erect the new family Claviradulomyceae fam. nov. to accommodate this group of fungi.

The new isolates of *Claviradulomyces* are associated with botanical families distantly related to each other, as suggested by Barreto *et al.* (2012). This seems to confirm that *Claviradulomyces* represents a diverse and possibly large assemblage of fungi that has a wide distribution, perhaps being ubiquitous in woody plants. Possibly such diversity remained concealed because of the small size of *Claviradulomyces* and their adaptation to a little explored habitat, the plant lenticels. This work provides significant evidence in favor of the earlier conjecture of Barreto *et al.* (2012).

It is now hoped that other mycologists in Brazil and abroad will be encouraged to examine plant lenticels more closely and it is expected that, as a result, a plethora of additional species of *Claviradulomyces* will be found. It is also hoped that the true nature of the association between *Claviradulomyces* and their hosts will be also unraveled. Although field observations suggested, in some instances, that colonization of plants by *Claviradulomyces* results in defoliation and branch die-back of host plants, such as in the case of the type-species of the genus, and also, in *C. schini* sp. nov., the cause-effect of this association remains to be tested (although with considerable practical difficulties). *Claviradulomyces* grows very slowly in culture and most species do not produce ascospores or conidia *in vitro*. These would be the ideal inoculum for use in inoculation studies. In the specific case of *C. schini* sp. nov. there is a major stimulus for such studies being undertaken. The fungus was found in Brazil associated with prominent eruptions along heavily defoliated host branches and is possibly linked to a severe dieback disease of *S. terebinthifolia*. *Schinus terebinthifolia* (Brazilian pepper tree) is a major pantropical weed (Cronk & Fuller 1995) and the target of several programs of classical biological control involving natural enemies collected in the native range in Brazil, including fungal pathogens (Faria *et al.* 2008; Macedo *et al.* 2013). In case pathogenicity of *C. schini* sp. nov. to Brazilian pepper tree is proven as well as its host-specificity, it may become of major importance for use as a classical biological control agent to mitigate the highly environmentally damaging invasions of natural ecosystems in Florida, Hawaii, Australia and many other regions.

The lenticel fungi investigated fit well into Wedin *et al.* (2004) expectations of new exciting discoveries awaiting to be made in novel studies of poorly studied groups of Ostropales. The Claviradulomyceae fam. nov., seems to represent an entirely new and overlooked fungal life-style and a group of fungi deserving the attention of mycologists both for their potential relevance as components of global mycodiversity and towards elucidating their ecological status.

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