A new species of the lenticel fungal genus *Claviradulomyces* (*Ostropales*) from the Brazilian Atlantic forest tree *Xylopia sericea* (*Annonaceae*)

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Abstract: *Claviradulomyces xylopiae* sp. nov. is introduced for a fungus occurring in association with abnormal (enlarged, spongy) lenticels of *Xylopia sericea* (*Annonaceae*), a common tree of the Atlantic forest and Cerrado ecosystems in Brazil. This is the second species described in the genus and, although it is morphologically distinct from the type species, *C. dabeicola* from West Africa, it possesses the same characteristics. Apothecial ascomata have periphysoids and paraphyses that are inflated apically (clavate), and ornamented with denticles (raduliform). Furthermore, similar to the type species, it also has long-cylindric or acerose, aseptate ascospores and conidia. An additional asexual morph was produced in culture and is described. Molecular studies of *C. dabeicola* and the new species confirmed a placement in *Ostropales*, although a relationship to *Odontotremataceae* was not supported. Both species were consistently in association with abnormal lenticular development on their woody hosts. It remains to be ascertained, however, if these are the causal agents of the bark disorders, or, simply, opportunistic colonisers. The finding of the second species in the genus *Claviradulomyces* on a plant from a distantly related family to that of the host of *C. dabeicola* (*Erythroxylaceae*) for the genus on a different continent suggests that fungi in this genus may be common on lenticels of other woody plants, and could even have a pantropical distribution. It is possible that fungi in the genus have remained unreported until now because lenticels have remained neglected as a habitat surveyed by mycologists.

Key words:
Ascomycota
mycobiota
Ostropales
phylogeny
plant disease
taxonomy

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INTRODUCTION

Xylopia sericea (*Annonaceae*) is a fast-growing native tree of the Brazilian Atlantic forest and Cerrado ecosystems (Lorenzi 1992), known locally as pimenteiro. During a collection of entomopathogenic fungi associated with armoured scale insects in the canopy of *X. sericea* (Fig. 1A), a high proportion of the pruned branches showed abnormal development of lenticels: the latter appearing as prominent eruptions along the branches (Fig. 1B–D). A discomycete fungus was observed consistently colonising the lenticular tissues, which was identified provisionally as similar to *Claviradulomyces dabeicola* (Evans *et al.* 2010). On closer inspection, morphological differences were found that distinguished the fungus on *X. sericea* from *C. dabeicola*. The fungus was isolated into pure culture, from the sexual morph and from the purported asexual morph, allowing for molecular data to be generated to confirm the sexual relationship. A detailed description of the new species and a discussion of *Claviradulomyces* phylogeny and its placement within *Ostropales* are presented.

MATERIALS AND METHODS

Isolates and morphology

Stems bearing pronouncedly developed lenticels were collected from the canopy of *Xylopia sericea* with the aid of a pruning pole (Fig. 1) from two sites in the municipality of Viçosa (state of Minas Gerais, Brazil): at the edge of a well preserved stretch of Atlantic rainforest,and a roadside stand adjacent to farmland. Samples were air-dried and specimens were deposited in the collections of the Universidade Federal de Viçosa (VIC) and of the CBS-KNAW Fungal Biodiversity Centre Herbarium (CBS).

Isolations were performed either by transferring sporocarps to sterile distilled water agar (DWA), breaking them open with fine forceps, and streaking the spores across the agar surface to await germination, when germinating spores (ascospores, conidia) were selected with the aid of a sterile fine-pointed needle under a stereo-microscope with transmitted light and placed on potato carrot agar (PCA), or by direct transfer of ascomata or pycnidia onto plates

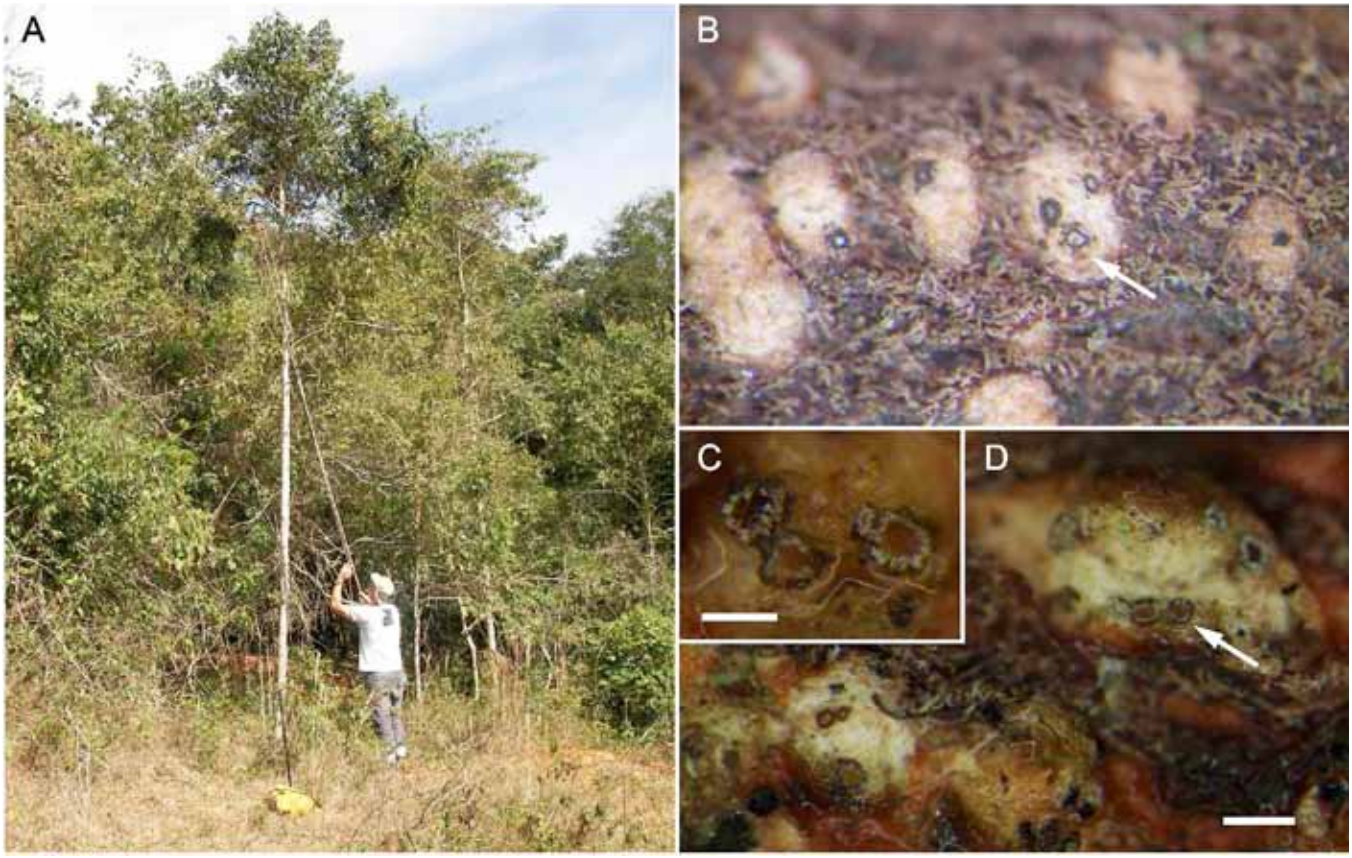

Fig. 1. A. Collecting on *Xylopia sericea* at type locality of *Claviradulomyces xylopiae* – margin of Atlantic forest, Mata do Seu Nico, Fazenda Bonsucesso, Viçosa, state of Minas Gerais Brazil. **B–D.** Close-up of bark of *X. sericea* colonised by *C. xylopiae* showing hypertrophied lenticels bearing bearing apothecial ascomata, showing fully opened habit (C–D) after 24 h in a humid chamber. Bars: C = 0.5 cm; D = 1 cm.

containing vegetable broth agar (VBA), as described in Pereira *et al*. (2003). Representative cultures were deposited in the CBS-KNAW Fungal Biodiversity culture collection. Colony characters were noted on malt extract agar (MEA) and PCA, either in the dark or with a 12 h light/ 12 h dark regime, at 25 ºC. Colony colour was assessed according to Rayner (1970). Morphological observations were made in lactic acid or lacto-fuchsin from hand sections of sporocarps or those teased from the lenticels and macerated.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) 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 (SSU), ITS1, 5.8S rRNA gene, ITS2 and the first 900 bases at the 5' end of the 28S rRNA (LSU) genes. The primers ITS4 (White *et al*. 1990) and LSU1Fd (Crous *et al*. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions followed the methods of Crous *et al*. (2006, 2009b).

Sequences were compared with those from *Claviradulomyces dabeicola* (Evans *et al.* 2010) and from the taxa treated by Baloch *et al*. (2010). LSU and mtSSU sequences from the two *Claviradulomyces* spp. were concatenated and incorporated into the alignments of Baloch *et al*. (2010) using Geneious (Drummond *et al*. 2011). Data were analysed with Bayesian phylogenetic methods using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), with gaps treated as missing data, applying the GTR+I+G model for both genes, the models selected using the AIC method in MrModelTest v. 2.3 (Nylander 2004). The data set was run with two chains for 10 M generations, and trees sampled every 1000 generations. Convergence of all parameters was checked using the internal diagnostics of the standard deviation of split frequencies and performance scale reduction factors (PSRF), and then externally with Tracer v. 1.5 (Rambaut & Drummond 2007). On this basis, the first 25 % of generations were discarded as burnin. Bayesian posterior probabilities were obtained from 50 % majority rule consensus trees.

RESULTS

Taxonomy

Claviradulomyces xylopiae R.W. Barreto, H.C. Evans, P.R. Johnst., **sp. nov.**
MycoBank MB801140
(Figs 1–5)

Etymology: derived from *Xylopia*, the generic name of the host plant.

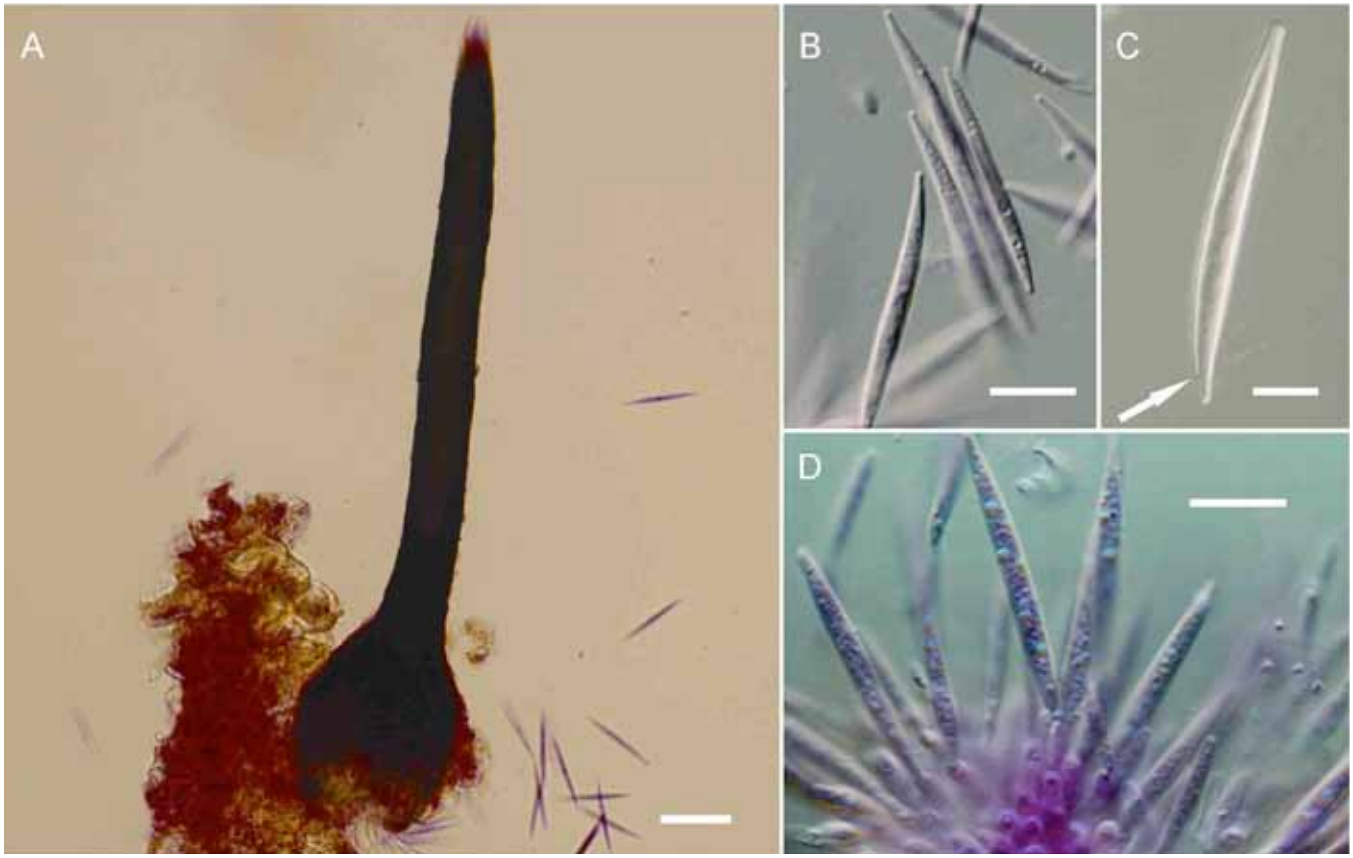

Fig. 2. *Claviradulomyces xylopiae* asexual morph (VIC 31417 mounted in lactofuchsin). **A.** Pycnidium with long rostrate ostiole. **B, C.** Conidia [note subtle heel at base of conidium in **C** (arrowed)]. **D.** Group of immature conidia attached to conidiogenous cells. Bars: A = 50 µm; B = 15 µm; C = 5 µm; D = 10 µm,

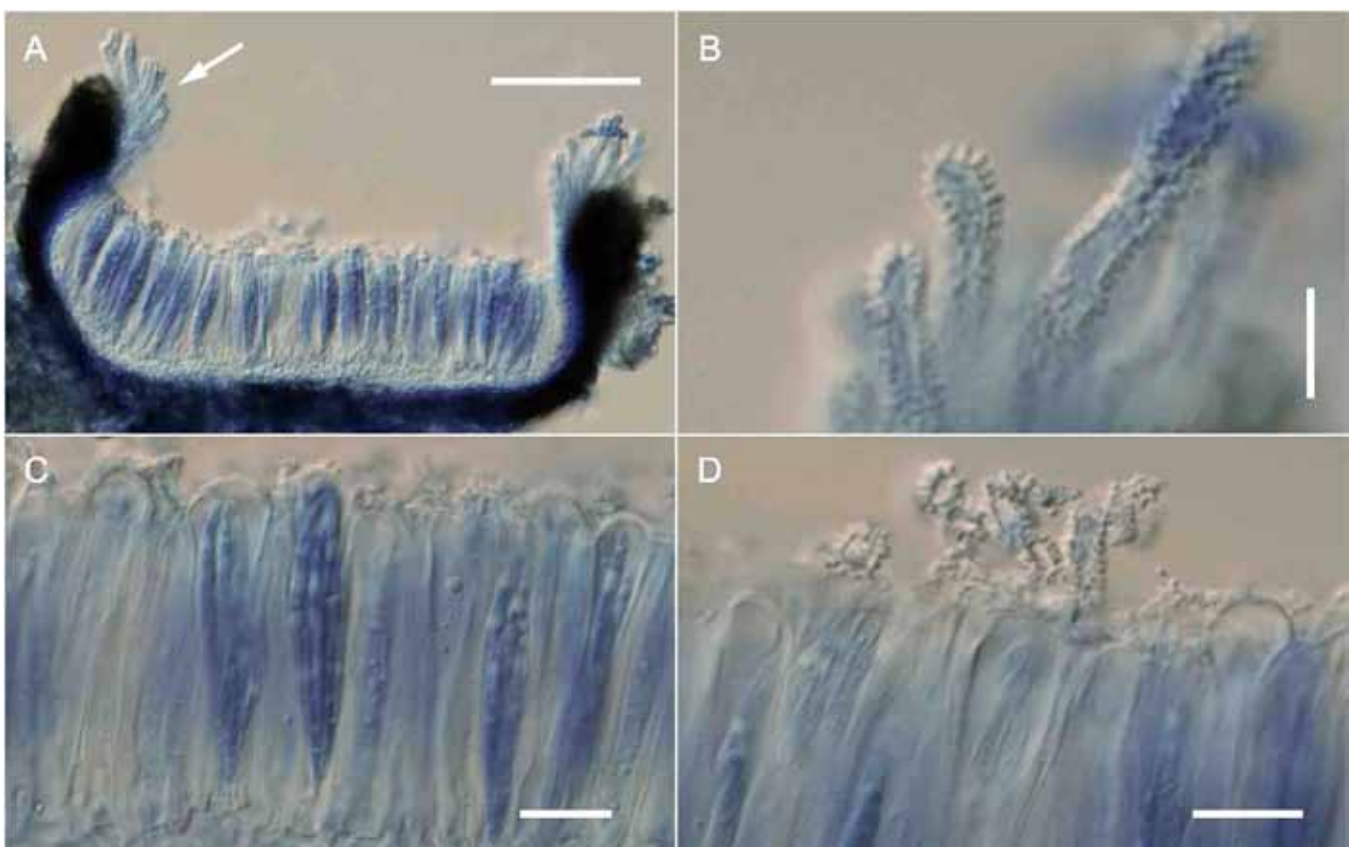

Fig. 3. *Claviradulomyces xylopiae* sexual morph (VIC 31417 mounted in lactic acid-cotton blue). **A.** Cross section of fully opened, apothecial ascoma (note group of denticulate periphysoids at the margins of apothecium). **B.** Close-up of periphysoids. **C.** Hymenium with parallel asci and paraphyses. **D.** Muricate and denticulate paraphyses extending above the top of asci. Bars: A = 15 µm; B = 10 µm; C = 10 µm; D = 15 µm.

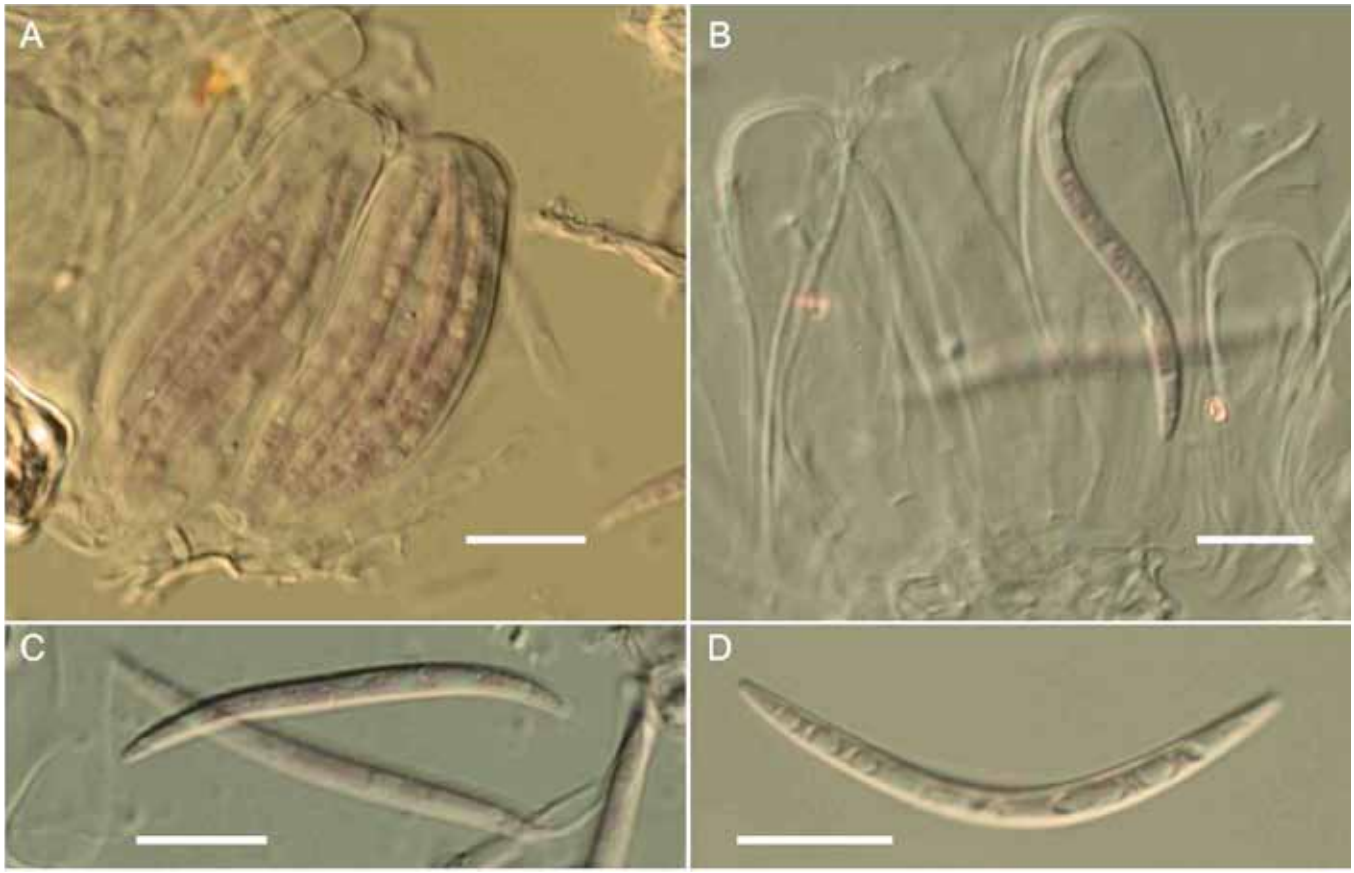

Fig. 4. *Claviradulomyces xylopiae* asci and ascospores (VIC 31417 mounted in lactofuchsin). **A.** Mature asci containing parallel to somewhat spirally arranged ascospores. **B.** Single vermiform ascospore within ascus. **C, D.** Ascospores. Bars: A = 40 µm; B = 35 µm; C = 10 µm; D = 10 µm.

Diagnosis: Similar to *Clavariadulomyces dabeicola,* from which it is distinguished by the longer periphysoids (16–39 µm, shorter asci (35–50 µm), a longer ostiolar neck in the asexual state, shorter conidia (14–34.5 µm) with a discrete heel region, and molecular sequence data.

Type: **Brazil**: *Minas Gerais*: Viçosa, Piúna, on living branches of *Xylopia sericea* (*Annonaceae*), 11 June 2010, *R.W. Barreto* (VIC31417 — holotype; CBS 133260 and CBS 133261 – ex-holotype cultures).

Paratype: **Brazil**: *Minas Gerais*: Viçosa, "Mata do Seu Nico", Fazenda Bonsucesso, on living branches of *Xylopia sericea,* 20 May 2010, *H.C. Evans & R.W. Barreto* (VIC 31416).

Description: *Internal mycelium* intra- and intercellular, 1–2 µm diam, septate, branched, hyaline. *Ascomata* erumpent from spongy tissues of lenticels on bark of living branches; apothecial when mature and turgid, but perithecium-like and opening by a large, round pore when dry; sessile, urceolate, 0.12–0.25 mm diam, wall black, extending above the surface of the hymenium, partially covering the hymenium when dry, opening pore lined with a whitish fringe of periphysoids. In vertical section, lower part of ascomatal wall often ill-formed and restricted to a loose hyphal layer from which asci and paraphyses arise, 5–15 µm thick, composed of tangled hyphae 1–2 µm diam; upper part of wall dark brown to 37 µm thick, narrowing and becoming paler towards the base and there 10–12 µm wide. *Periphysoids* lining the upper wall above the level of the hymenium, cylindrical or club-shaped, sinuose or curved, 16–39 µm long and 3–4 µm wide along the axis, often slightly swollen in the upper part up to 6 µm, wall hyaline along most of the length, bearing abundant blunt denticles, arising from short brown smooth basal stalks. *Paraphyses* 1–2 µm wide, to 60 µm long, apex swollen and bulbous, to 5–7 µm wide and bearing abundant blunt denticles, imparting a mace-like or muricate appearance, extending beyond the asci but usually prostrate over the hymenial surface. *Asci* parallel, clavate with a broadly rounded to somewhat flattened apex, becoming ellipsoidal when free of the hymenium, without a basal stalk, 35–50 × 5–10 µm, apex non-amyloid, 8-spored. *Ascospores* in single fascicles extending to the base of the ascus, parallel to spirally or partly spirally arranged in the upper half, cylindrical to vermiform, attenuating towards the sub-acute ends, straight to slightly curved or sigmoid, sometimes strongly curved at the apices, (21–) 28–43 × 2–3 µm, aseptate, hyaline, smooth, strongly guttulate. *Asexual morph*: formed separately or in combination with the sexual morph in the same lenticel. *Conidiomata* pycnidial, semi-immersed, globose, 76–137 µm diam; thin walled, walls 4–15 µm thick, with long cylindrical ostiolate necks, 110–360 × 24–46 µm, composed of parallel hyphae 1–3 µm wide, often reduced to a narrower cylinder of bristle-like hyphal tips at the apex, 15–21 ×15 µm. *Conidiophores* usually reduced to conidiogenous cells, occasionally consisting of a small stalk

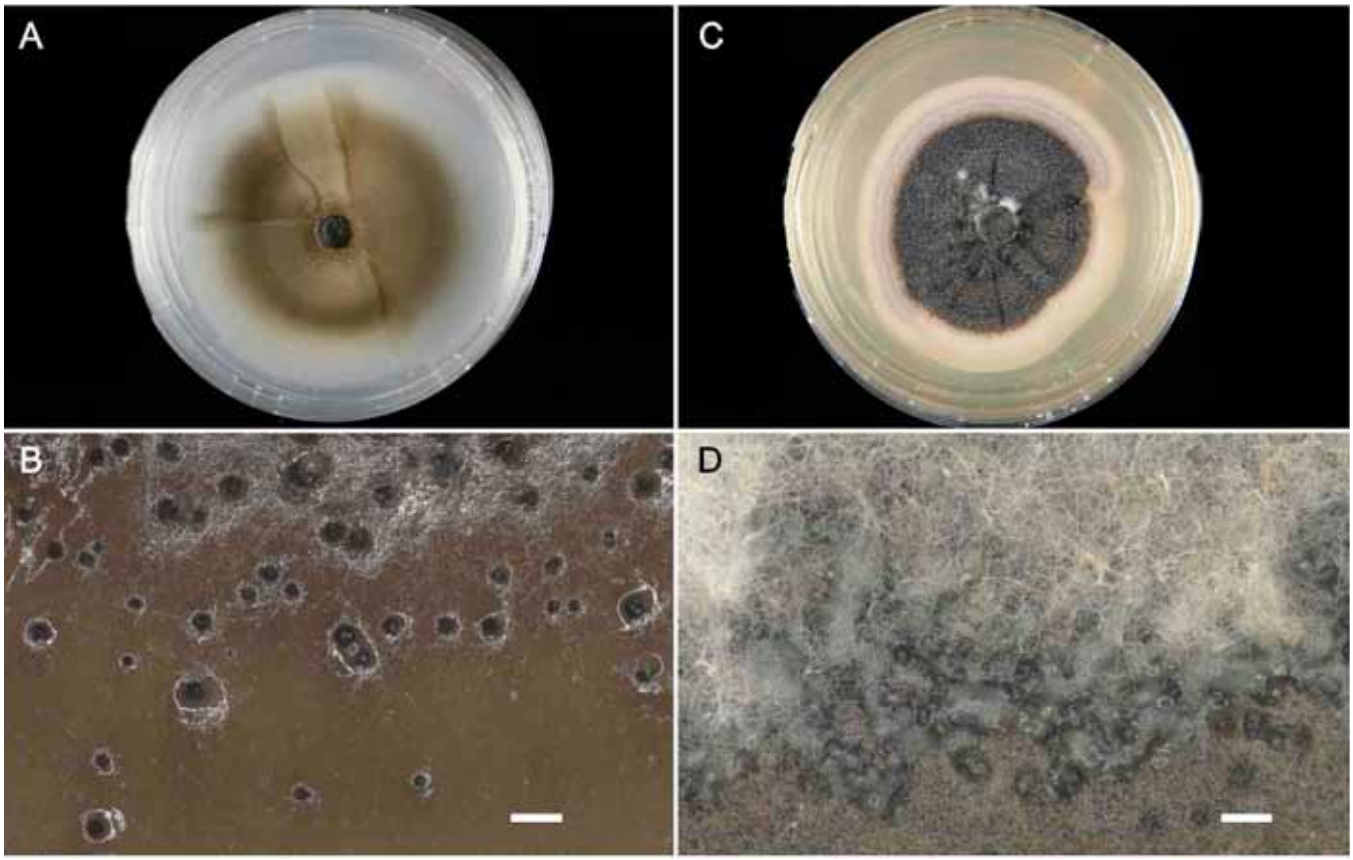

Fig. 5. *Claviradulomyces xylopiae* in culture . **A.** Colony formed on PCA under 12 h daily light regime. **B.** Colony formed on MEA under 12 h daily light regime. **C.** Close-up of margin of colony formed on PCA (Note groups of black pycnidia, isolated and in groups). **D.** Close-up of colony formed on MEA (Note mucilaginous mass of conidia oozing from black spermogonia). Bars = 2 mm.

of one or two cells. *Conidiogenous cells* lining the pycnidial wall, holoblastic, seemingly monoblastic, subcylindrical, lageniform or oblong, straight or curved, solitary, occasionally branched, 4.5–12.5 × 1.5–2 µm, hyaline, smooth. *Conidia* probably mucilaginous, acerose to narrowly cymbiform, mostly straight or slightly curved or sigmoid, attenuated towards a basal subtle heel continuing as a short cylindrical penducle and ending in a rounded base, aseptate, guttulate, hyaline, smooth, 14–34.5 × 2–3.5 µm.

Cultures: Slow growing, to 76 mm diam after 23 d, either totally immersed or flat to slightly raised centrally, with radiating grooves of compressed medium, immersed at periphery; felt-like or entirely slimy centrally, comprising a dense, rosy vinaceous mat of dark brown monilioid hyphae within a pale hyphal matrix; embedded, black setose pycnidia densely formed on PCA in light, producing a white-creamy ooze of cylindrical to oval hyaline conidia (2−4 × 1.5−2 µm) and oblong hyaline spermatia (1.5 × 1 µm); pycnidia fewer and sterile in the dark.

Phylogenetic analysis

The sequences generated from both collections were identical (GenBank accession numbers ITS JX843524; LSU JX843525; SSU JX843526). The two *Claviradulomyces* spp. formed a strongly supported clade within the *Ostropales*, but the family level relationship within the order was not resolved (Fig. 6).

DISCUSSION

Claviradulomyces xylopiae represents a novel species on an indigenous Brazilian plant distantly related to *Erythroxylum mannii*, the host of the type species, *C. dabeicola*, in West Africa (Evans *et al*. 2010). This suggests that fungi in this genus could have a pantropical distribution, perhaps as endophytic colonisers of tropical woody plants, with the ability to sporulate on the lenticular tissues of living plants. This somewhat cryptic niche has not traditionally been explored by mycologists, and this could explain the absence of previous records of this genus. However, it remains to be proven whether or not these fungi are benign endophytes, or acting as systemic pathogens that promote abnormal lenticel growth to facilitate sporulation, or opportunistic invaders of trees with bark disorders.

Claviradulomyces xylopiae has considerable similarity to *C. dabeicola,*and has the same muricate periphysoids, which characterise the genus (Evans *et al.* 2010). It can, however, be separated from the type species by the longer periphysoids, 16–39 µm compared to 6.5–14 µm, and shorter asci, 35–50 µm compared with 60–75 µm. Nevertheless, the most significant morphological divergences are to be found in the asexual state of the two species. The ostiolar neck in the new species is long, sometimes reaching up to three times the length of the pycnidial body, whereas in *C. dabeicola* the neck is reduced to a short protrusion. Conidia in *C. xylopiae* are also shorter, 14–34.5 µm in length, than in *C. dabeicola*

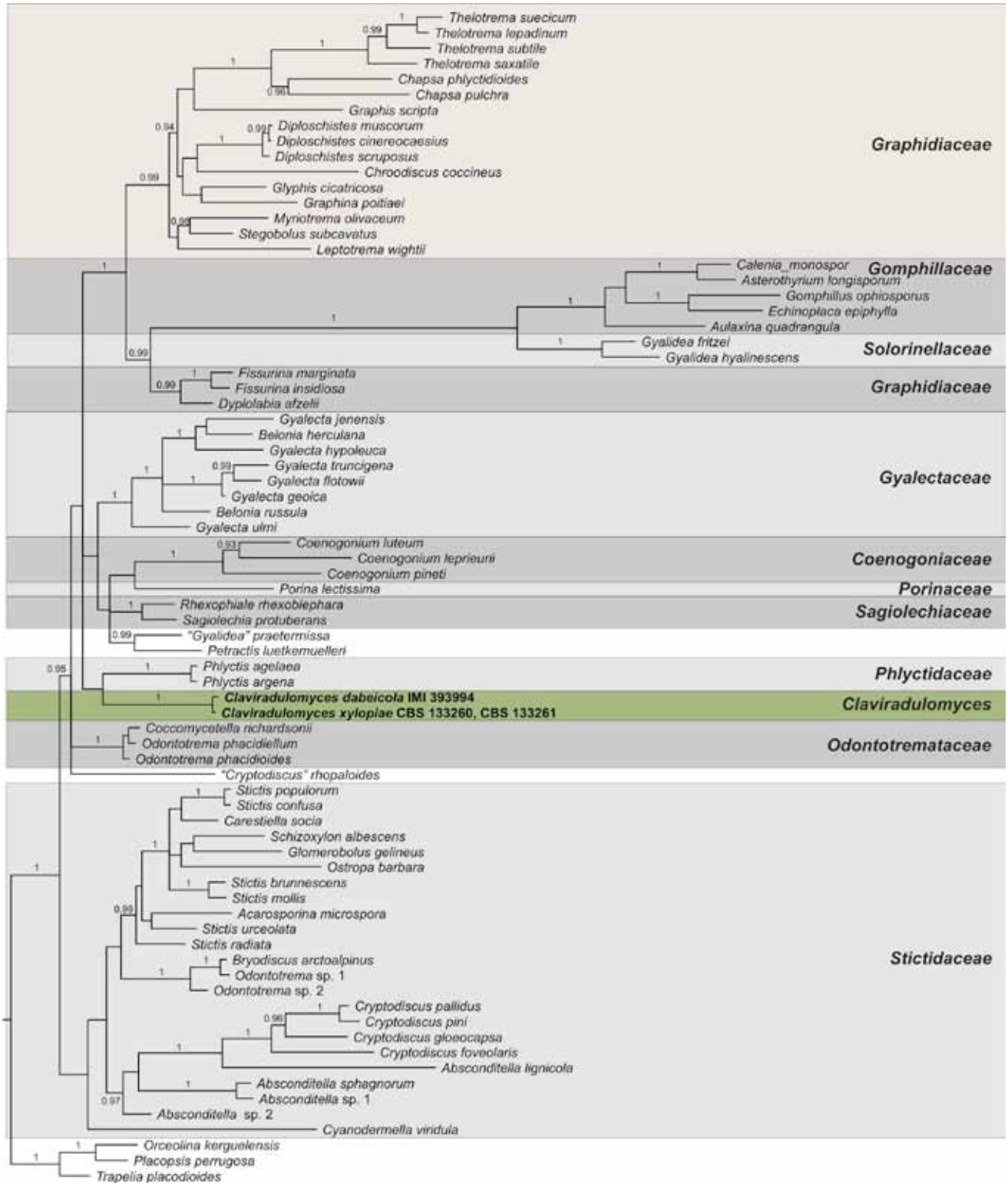

Fig. 6. Bayesian analysis (50 % majority rule consensus tree) of nucLSU and mtSSU sequences. Bayesian posterior probabilities are shown where above 90 %. Sequences for all taxa except *Claviradulomyces dabeicola* (GenBank records records GQ337897, GQ337900) and *C. xylopiae* (GenBank LSU JX843525; SSU JX843526) are from Baloch *et al*. (2010). The clade labels follow Baloch *et al.* (2010).

where they measure 33–42 µm, and also have a discrete heel region not seen in *C. dabeicola.*

Evans *et al*. (2010) referred *Claviradulomyces* to *Odontotremataceae* based on morphology. At that time, no DNA sequences of *Odontotrematcaeae* had been published. A subsequent comprehensive phylogenetic study of the *Ostropales* (Baloch *et al*. 2010) allowed a re-evaluation of the phylogenetic position of *Claviradulomyces*, which we now regard as "*incertae sedis*" within *Ostropales*. The morphological similarity with the *Odontotremataceae* is not reflected in the phylogenetic position of these purported woody plant endophytes.

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