A fungus-eat-fungus world: *Digitopodium*, with particular reference to mycoparasites of the coffee leaf rust, *Hemileia vastatrix*

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**Abstract**

*Digitopodium hemileiae* was described originally in 1930 as *Cladosporium hemileiae*; growing as a mycoparasite of the coffee leaf rust (CLR), *Hemileia vastatrix*, in a sample of diseased leaves of *Coffea canephora* collected in the Democratic Republic of Congo. No cultures from this material exist. More recently, the type material was re-examined and, based on morphological features, considered to be incorrectly placed in *Cladosporium*. The new genus *Digitopodium* was erected to accommodate this species. Interest in fungal antagonists of *H. vastatrix*, as potential biocontrol agents of CLR, led to comprehensive surveys for mycoparasites, both in the African centre of origin of the rust, as well as in its South American exotic range. Among the rust specimens from Ethiopia, one was found to be colonized by a fungus congeneric with, and similar to, *D. hemileiae*. Pure cultures obtained from the Ethiopian material enabled a molecular study and for its phylogenetic position to be elucidated, based on DNA sequence data from the ITS and LSU regions. Molecular data showed that two members of the recently erected genus *Hyalocladosporiella* (*Herpotrichiellaceae: Chaetothyriales*) are congeneric with *Digitopodium* from Ethiopia and morphologically similar to both *D. hemileiae* and the two Ethiopian isolates. These isolates were found to be morphologically and genetically identical to *H. tectonae*, described previously from Brazil. Thus, species of *Hyalocladosporiella* are re-allocated to *Digitopodium* here; including *D. tectonae*, and a novel species, *D. canescens*, recently found in Brazil growing as a mycoparasite of *Puccinia thaliae*. The potential use of *D. hemileiae* and *D. tectonae* for classical biological control of CLR is discussed.

**Keywords:** Classical biological control, Ethiopia, Fungicolous fungi, *Herpotrichiellaceae*, *Hyalocladosporiella*, New taxa, Phylogenetics

**INTRODUCTION**

*Hemileia vastatrix* is the most important pathogen of coffee plants worldwide, causing coffee leaf rust (CLR) (Zambolim, 2016, Talhinhas et al., 2017). The economic and social crisis provoked by CLR outbreaks of the past are well documented (Avelino et al. 2015, McCook & Vandermeer 2015). Since 2012, disastrous outbreaks of CLR have been destroying the livelihoods of the coffee growers in Central America (Avelino et al. 2015, Talhinhas et al. 2017) and have prompted mass migrations – refugee caravans – to Mexico and the USA (Ward et al. 2017).

Efforts in mitigating the impact of CLR have included a pioneering initiative towards the development of a classical biological control management strategy, based on the use of fungal natural enemies from the native range of coffee and *Hemileia vastatrix* in Africa. A number of mycoparasitic fungi of CLR have been reported previously (Carrion & Rico-Gray 2002, James et al. 2016). However, the latter records are all from the...
Americas, where coffee and *H. vastatrix* are exotic species. Such mycoparasites are interpreted, therefore, as generalists that have jumped from other fungal hosts and did not co-evolve as specialized parasites of the CLR fungus. Only two mycoparasites have been reported exclusively from the centre of origin of cultivated *Coffea* in Africa, namely: *Digitopodium hemileiae* (Steyaert 1930, Heuchert et al. 2005) and *Paranectriella hemileiae* (Pirozynski 1977). In order for any classical biocontrol agent to be introduced against its target in an exotic situation, it is critical to have its taxonomy fully elucidated (Scott 1995). This publication deals with a reappraisal of the taxonomy of *D. hemileiae* and related taxa, based on newly-collected specimens obtained during surveys for mycoparasites of *H. vastatrix* in Africa and of related material collected in Brazil.

**MATERIAL AND METHODS**

Surveys involved scientists from Ethiopia, Brazil, and the UK and were concentrated in areas where *Coffea arabica* still occurs in the wild or is cultivated in semi-wild conditions, as in Ethiopia. At each selected site, coffee plants were examined for rust pustules – with particular attention to collecting rust colonies overgrown by other fungi, or appearing to be abnormal (unusual colour, poor sporulation). Specimens were dried in a plant press for later processing in the laboratory (preliminary identification and isolation). The dried samples were processed within 2 weeks of collection after transport to laboratories in the UK or Brazil. Mono-conidial cultures were obtained by direct isolation of the fungi by aseptic transfer of fungal propagules from colonized tissue with a sterile fine point needle onto potato dextrose-agar (PDA) plates. Pure cultures were preserved temporarily in potato carrot-agar (PCA) slants and long-term preservation was in silica-gel and in 10% glycerol at -80 °C, as described in Dhingra & Sinclair (1995). Pure cultures were deposited in the culture collection of the Universidade Federal de Viçosa (COAD) and dried specimens were deposited in the herbarium of the Universidade Federal de Viçosa (VIC).

Culture characteristics were described based on colonies formed on 2% malt extract-agar (MEA), PDA, and oatmeal-agar (OA) for 7 d at 25 ± 2 °C under a 12 h light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). Colony colour terminology followed Rayner (1970).

Morphology was described based on the structures formed on colonized rust pustules on dried specimens, complemented with observations made on slide cultures, as described in Waller et al. (1998); colonies being formed on blocks of synthetic nutrient poor-agar (SNA) (Nirenberg, 1981) for 14 d, under the conditions mentioned above. Slide cultures and fungal structures obtained directly from rust pustules were mounted in lactoglycerol or lactofuchsin and the microscope slides were examined under a light microscope, Olympus BX 53 (Olympus, Melville, NY, USA), connected to an Olympus Q-color 5 camera (Olympus, Center Valley, PA, USA). Conidial morphology was based on shape, colour, and presence or absence of septation. Biometric data were generated from the observation of at least 30 structures.

DNA was extracted from single spore isolates cultivated on potato dextrose liquid medium at 25 °C for 5 d. Total genomic DNA was extracted from approximately 50–80 mg of mycelium. Mycelial masses were disrupted with a L-Beader 3 (Locus Biotechnologia, Cotia, SP, Brazil) adjusted to a speed of 4000 rpm, 2 cycles of 10 s each. DNA extraction was carried using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's recommendations.

DNA PCR amplifications were performed with the primer pairs LR0R/LR5 (Vilgalys & Hester 1990) and ITS4/ITS5 (White et al. 1990) for the partial 28S rDNA (LSU) and ITS/5.8 nr-DNA (ITS) regions. The polymerase chain reactions (PCR) were performed using a total volume of 12 μL in reactions with mixture containing 30 μg DNA, 0.5 μm of each primer and 1X Master mix Dream-Taq DNA polymerase, as recommended by the manufacturer (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). The amplification was performed for LSU with an initial denaturing at 94 °C at 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension initial at 72 °C for 30 s, and 7 min final extension at 72 °C. The PCR products were purified by using an ExoSAP-IT purification kit (Amersham Biosciences, Arlington Heights, IL, USA), according to the manufacturer’s recommendations. Amplified fragments were sequenced by Macrogen (Seoul, South Korea, http://www.macrogen.com).

**Phylogenetic analyses**

The nucleotide sequences obtained from forward and reverse primers were used to obtain consensus sequences using SeqAssem (SeqentiX—Digital DNA Processing, Klein Raden, Germany) (Hepperle, 2004). Complementary sequences used in the analyses were obtained from GenBank (http:// www.ncbi.nlm.nih.gov) (Table 1). The alignment performed using MUSCLE implemented in the MEGA 7 (Kumar et al. 2016). The aligned sequences were manually corrected where needed. The consensus sequences were deposited in GenBank (Table 1) and taxonomic novelties in MycoBank (Crous et al. 2004).

Phylogenetic analyses were reconstructed by means of methods based on an analysis of Bayesian Inference (BI) of the combined LSU/ITS alignments using the Markov chain Monte Carlo (MCMC) algorithm. Models of
nucleotide substitution for each gene region were determined using jModeltest 2.1.7 (Darriba et al. 2012). The likelihood values were calculated and the models were selected according to the Akaike Information Criterion (AIC). The BI analysis was completed with Mr. Bayes v.3.2.6 (Ronquist et al. 2012). Simulations were carried out with 10 million random generations and samples of tree were taken every 1000 generations. The first 2500 trees were discarded from the analysis, resulting in 10,000 trees. The phylogenetic analysis of the concatenated alignment was performed on the CIPRES web portal (Miller et al. 2010). The phylogenetic tree was viewed and edited with Figtree v 1.4.3 (Rambaut, 2016). Sequence alignments were deposited in TreeBASE – Study S27398.

RESULTS
Phylogenetic relationships were inferred using combined ITS and LSU sequences. Bayesian Inference analysis (BI) was performed combining ITS and LSU loci for the Digitopodium isolates and selected taxa, plus two isolates serving as outgroups (Cladosporium uredinicola and C. cladosporioides), totalling 27 isolates (Table 1). The phylogenetic analysis indicated that members now assigned to Digitopodium do not belong in the Cladosporiaceae. Instead, they form a clade together with isolates of Hyalocladosporiella spp. (Chaetothyriales – Herpotrichiellaceae) (Fig. 1). Sequences for isolates COAD 2928, COAD 2639, COAD 2640 and COAD 2641 were generated and included in the study.

TAXONOMY
A comparison of the morphology of the fungus in the Ethiopian specimen showed that its general characters fitted within the morphological description given for the genus in Heuchert et al. (2005), based on the re-examination of the type material of Cladosporium hemileiae. The combination of the morphological evidence
and the results of the molecular analysis (Fig. 1), led to the recognition that *Hyalocladosporiella* represents a younger, heterotypic synonym for *Digitopodium*, although differences in the characteristics of the conidia in the Ethiopian collection to the type of *D. hemileiae* indicated that these represent a separate species. Morphologically and genetically, the Ethiopian material is closest to *Hyalocladosporiella tectonae*, which is now transferred to *Digitopodium*, and, provisionally, maintained as a separate species, pending new collections of *D. hemileiae* suitable for epitypification, and once its phylogenetic characterization is available.

**Digitopodium** U. Braun et al., *Schlechtendalia* **13**: 65 (2005)

**Synonym:** *Hyalocladosporiella* Crous & Alfenas, *Persoonia* **32**: 237 (2014).

**Description:** Mycelium branched, septate hyaline, smooth. Conidiophores uniform or dimorphic, solitary or in loose groups. Microconidiophores (when present) erect, subcylindrical, straight to geniculate-sinuous, septate, brown to olivaceous brown, smooth. Macroconidiophores erect to slightly curved, plurisepaete, pale to dark brown, smooth, cylindrical, flexuous, unbranched or sometimes branched, either with well-developed basal digitate rhizoids or poorly developed or even absent. Conidiogenous cells holoblastic, integrated, terminal, subcylindrical, proliferating sympodially, but geniculations mostly not evident, smooth, brown. Conidiogenous...
**Digitopodium hemileiae** (Steyerta) U. Braun et al. 2005

**Note:** Hyalocladosporiella Crous & Alfenas is placed as a synonym of *Digitopodium* for the first time.

**Digitopodium hemileiae** (Steyerta) U. Braun, Heuchert & K. Schub., *Schlechtendalia* 13: 66 (2005)

**Basionym:** *Cladosporium hemileiae* Steyaert, *Bull. Soc. Roy. Bot. Belgique* 63: 47 (1930).

**Type:** Democratic Republic of Congo: formerly Zaire): Prov. Orientale, Biaro, near Kisangani (Stanleystown), on pustules of *Hemileia vastatrix* (Pucciniales) on *Coffea canephora*, Oct. 1929, R.L. Steyaert (BPI 426854 – holotype).

**Description:** see Heuchert et al. (2005) for a complete description.

**Notes:** Heuchert et al. (2005) examined the type material of *Cladosporium hemileiae* and published a comprehensive description and illustration based on the holotype. *Digitopodium hemileiae* and *Hyalocladosporiella tectonae* are morphologically very close, but there are some obvious differences in the formation, pigmentation, and width of the conidia, which clearly places them into separate species. *Digitopodium hemileiae* is characterized by having conidia formed in simple chains (primary and secondary ramoconidia lacking), consistently pigmented (not hyaline), and 5–7 μm wide [vs conidia formed in branched chains (primary and secondary ramoconidia present), secondary ramoconidia, intermediary and terminal conidia colourless, much narrower, 2–3.5 μm wide]. The values of the conidial widths of *Digitopodium hemileiae* and *D. tectonae* do not even overlap.

**Digitopodium tectonae** (Crous & Alfenas) A. Colmán & R. W. Barreto, **comb. nov.** (Fig. 2)

**Morphology of structures formed in vitro (slide cultures):** Conidiophores 150–310 × 3.5–7.5 μm, septate, with digitate/rhizoidal base, 2–16 × 2–5 μm; conidiogenous cells 14–33 × 3–3.5 μm. Primary ramoconidia 25–30 × 3–3.5 μm, 1–3-septate, plus 1–3 hila. Secondary ramoconidia 20–26 × 3–3.5 μm, septate, with 1–4 hila. Intermediary conidia (10–)12–16 × (20–)2.5–3.5 μm, aseptate, with 2 hila. *Terminal conidia* 12–14 × 2–3 μm, aseptate, with one hilum.

**Culture characteristics:** Slow growing (12–18 mm diam after 7 d, at 25 °C), edge entire, low convex to umbonate, aerial mycelium either sparse or dense, either felt, cottony or floccose, whitish to smoke-grey to grey olivaceous, reverse grey olivaceous; sporation abundant (OA), scarce (PDA), to absent (MEA).

**Type:** Brazil: Mato Grosso, Verde Novo, Colider, on leaves of *Tectona grandis* (Lamiaceae), Apr. 2013, A.C. Alfenas (CBS H-21702 – holotype; CBS 137989 – ex-type living culture; ITS sequence GenBank KJ869142; LSU sequence GenBank KJ869199).
Notes: Digtopodium hemileiae was originally described as Cladosporium hemileiae by Steyaert (1930). Much later this taxon was included in a revision of fungicolous Cladosporium species and redescribed as belonging to a newly erected genus Digtopodium by Heuchert et al. (2005), based on the feature of it having some of its conidiophores bearing distinct short digitate rhizoids at the base and lacking cladosporioid (coronate) conidial scars. During the field survey in Ethiopia, fresh material of Digtopodium on H. vastatrix pustules was collected. When compared with the description given in Heuchert et al. (2005), it was found that the morphology of the fungus from Ethiopia was similar to that described for D. hemileiae. Nevertheless, D. hemileiae lacks ramoconidia and produces broader conidia than found in the Ethiopian specimen (5– 7 μm vs 2– 3.5 μm diam). Additionally, conidia of D. hemileiae are consistently pigmented, contrary to what was observed in the Ethiopian material. The morphological similarity between D. hemileiae, the type species of Digtopodium was evident, and the collection of the mycoparasite on CLR in Ethiopia, material. The morphological similarity between D. hemileiae and D. tectonae is undoubtedly not restricted to Hemileia, as previously assumed, and may have a broad host range within the Pucciniales. Although poorly documented, undoubtedly due to little attention being paid to such mycoparasitic fungi, D. tectonae seems to have a wide pantropical distribution; the only two existing records now being from distant locations: East Africa (Ethiopia) and South America (Brazil).

Additional specimens examined: Ethiopia: Oromia Region, Bale Mountains, Harenna Forest, Mayate Coffee Village, Jan. 2018, K.B. Belachew & H.C. Evans, on pustules of Hemileia vastatrix (Pucciniales) on Coffea arabica (Rubiaceae), (VIC47361); living culture COAD 2640, LSU sequence of COAD 2640 GenBank MK829190 and ITS sequence of COAD 2640 GenBank MK829187; Details as above, living culture COAD 2641, LSU sequence of COAD 2641 GenBank MK829193 and ITS GenBank MK829189. Brazil: Mato Grosso, Varzea Grande, on pustules of Olivea tectonae (Pucciniales) on Tectona grandis (Lamiaceae), Feb. 2019, R. Alfenas (VIC 47183; living culture COAD 2639), LSU sequence of COAD 2639 GenBank MK829188 and ITS sequence of COAD 2639 GenBank MK829191.

*Digtopodium cannae* (T.K.A. Kumar) A. Colmán & R. W. Barreto, comb. nov.

MycoBank, MB 832331

*Basionym:* Hyalocladosporiella cannae T.K.A. Kumar, Persoonia 39: 307 (2017).

*Type:* India: Kerala, Kozhikode, on leaves of Canna indica (Cannaceae), 20 Aug. 2014, T.K.A. Kumar (CAL 1342 – holotype).

*Description:* For a complete description see Kumar (in Crous et al. 2017). Notes: Based on molecular and morphological characters, H. cannae is reallocated to Digtopodium and the new combination D. cannae is made. Besides having molecular differences from D. hemileiae, D. cannae does not appear to have well-developed digitate rhizoids, as in D. hemileiae and D. tectonae. If it does, then these were overlooked (Crous et al. 2017). There is mention of a possible connection between the newly described fungus and Puccinia thaliae but with no certainty. Based on the phylogenetic analysis and the new combination made here, it seems that Digtopodium species are obligate mycoparasites of Pucciniales.

*Digtopodium canescens* A. Colmán & R. W. Barreto, sp. nov. (Fig. 3)

MycoBank, MB 832332

*Etymology:* Named after the distinctly greyish colonies formed over the rust host pustules.

*Diagnosis:* Similar to the other species of Digtopodium spp. but having wider micro- and macroconidiophores than in D. cannae, and differing from D. hemileiae and D. tectonae, in having shorter conidiophores of two kinds – solitary (microconidiophores) or in fascicles (macroconidiophores) – and also lacking well-developed digitate rhizoids at the base of conidiophores as in D. hemileiae and D. tectonae. Type: Brazil, Minas Gerais, Coronel Pacheco, on rust colonies of Puccinia thaliae on leaves of Canna × generalis, 10 Dec. 2018, R.W. Barreto (VIC 47182 – holotype; COAD 2928 ex-type living culture; LSU sequence GenBank MK829192.

*Description:* Mycelium immersed and superficial on Puccinia thaliae pustules, olivaceous grey, branched, septate, 1–3 μm wide. Conidiophores either solitary
(microconidiophores) or in loose fascicles (macroconidiophores). Microconidiophores erect, subcylindrical, almost straight to geniculate-sinuous, (35–) 40–74 × 3–6 μm, 1–4-septate, pale brown to olivaceous brown, smooth, thick-walled. Conidiogenous cells integrated, terminal, subcylindrical, 9–26 × 2.5–4 μm, somewhat thick-walled, pale brown, smooth. Conidiogenous loci sympodially arranged, slightly thickened and darkened. Macroconidiophores erect, cylindrical, flexuous, geniculate, (25) 40–140 × 3–5 μm, sparingly branched, 3–14-septate, dark brown, smooth, thick-walled, rhizoid bases present but poorly developed. Conidiogenous cells integrated, terminal, subcylindrical, 16–40 × 3.5–4 μm, dark brown to brown, smooth, wall slightly thickened. Conidiogenous loci sympodially arranged, slightly thickened and darkened. Primary ramoconidia ellipsoid to cylindrical, 9–34 (– 40) × 3.0–4 μm, 0–2-septate, wall slightly thickened, hyaline to pale olivaceous grey; hila 2–3 per conidium, slightly thickened and darkened, smooth. Secondary ramoconidia in branched chains, ellipsoid to cylindrical, (11–)15–30 (– 35) × 2.5–3 μm, 0–1-septate, hyaline, smooth, guttulate, wall slightly thickened, hila 2–3 per conidium, thickened and darkened. Intermediary conidia fusoid-ellipsoid, 11–25 × 2.5–3 μm, 0–1-septate, guttulate, hyaline, smooth, thin-walled, hila 1–2 per conidium, slightly thickened and darkened. Terminal conidia

![Image](image_url)
limoniform to pyriform to tear-drop-shaped, ellipsoid or fusoid, (5-) 8–16 × 2.5–3 μm, aseptate, hyaline, guttulate, smooth, thin-walled, hila slightly thickened and darkened.

*Morphology of structures formed in vitro (slide cultures):* Microconidiophores (35–) 40–74 × 3–6 μm, 1–4-septate conidiogenous cells 9–26 × 2.5–4 μm. *Macroconidiophores* 65–180 × 2–5 μm, 3–16-septate, conidiogenous cells 8–25 × 2.5–3 μm. *Primary ramoconidia* 10–28 (– 36) × 2.5–3 μm, 0–2-septate, hila 2–3. *Secondary ramoconidia* 18–37 (– 40) × 2.5–3 μm, 0–2-septate, hila 2; intermediary conidia 15–30 × 2.5–3 μm, 0–2 septate, hila 2. *Terminal conidia* 8–13 × 3–4 μm, aseptate, with one hilum.
Culture characteristics: Slow growing (15–18 mm diam after 12 d at 25 °C), edge entire, low convex to umbo-
nate, aerial mycelium either sparse or dense, felted, cot-
tony or floccose, olivaceous grey to pale olivaceous grey 
at periphery, olivaceous black; sporulation abundant 
(OA) and (PDA) to absent (MEA).

Notes: Digitopodium canescens is described here as a 
new species from Brazil. Morphologically it resembles D. 
cannae but can readily be distinguished by the size of its 
ramoconidia and conidiophores. In D. canescens, the mi-
cro- and macroconidiophores are wider than in D. can-
nae. Digitopodium canescens also differs from D. 
hemileiae and D. tectonae in having conidiophores of 
two kinds, viz. solitary (microconidiophores) or in loose 
fascicles (macroconidiophores), and not having well-
developed digitate rhizoids at the conidiophile base. 
Phylogenetically, it forms a clade together with other 
Digitopodium species in the Herpotrichiellaceae, but is 
sufficiently morphologically distinct to confirm it as a 
separate species.

DISCUSSION

The taxonomy of the genus Cladosporium (Cladosporia-
ceae) has recently undergone a major revision (Bensch 
et al. 2012, 2015). The genus previously encompassed 
more than 850 species. A broad molecular phylogenetic 
study – including sequences of the internal transcribed 
spacer regions ITS1 and ITS2, the 5.8S nrDNA, as well 
as partial actin and translation elongation factor 1-α 
gene sequences, of multiple isolates – has shown that 
only 169 species are true Cladosporium species (Bensch 
et al. 2012). Many of the existing names were not veri-
fied because of the lack of available cultures. Following 
this publication, many other isolates of Cladosporium 
have been reported from a wide range of substrates (soil, 
clinical and indoor samples) from around the world, in-
creasing this number of accepted species to 234, includ-
ing a number of new species (Crous et al. 2014, Bensch 
et al. 2012, 2015, Braun et al. 2015, Sandoval-Denis et al. 
2015, Razafinarivo et al. 2016, Ma et al. 2017, Marin-
Felix et al. 2017).

Unlike several other taxa that have been excluded 
from Cladosporium and re-allocated to other genera in 
Bensch et al. (2012), most of the fungicolous “Cladospor-
ium-like” species included in the revision of Heuchert 
et al. (2005) were left out of that reappraisal. Among 
these was the monotypic genus Digitopodium proposed 
by Heuchert et al. (2005) to accommodate C. hemileiae. 
This fungus was originally collected in the Democratic 
Republic of Congo and recognized by Steyaert (1930) as 
a mycoparasite of Hemileia vastatrix (Steyaert 1930, 
Heuchert et al. 2005). The precise taxonomic and phylo-
genetic status of D. hemileiae remained uncertain be-
cause of the lack of pure cultures accompanying the 
type. Digitopodium hemileiae was regarded as differing 
from Cladosporium in having inconspicuous or subcon-
spicuous, non-corneal conidiogenous scars (loci) on 
the conidiogenous cells; conidiogenous cells which are 
not conspicuously symodial (not geniculate); and the 
base of the conidiophores having short digitate rhizoid-
like protuberances (Heuchert et al. 2005).

Appropriate collections of D. hemileiae from Central 
Africa that can be used for epitypification purposes, in-
cluding cultures and retrieved sequence data for the 
phylogenetic characterization, are still lacking, but the 
striking morphological and ecological similarity between 
D. hemileiae and Hyalocladosporiella tectonae allowed 
us to consider the latter genus congenic with Digitopo-
dium and to propose the new combination D. tectonae. 
The case of Digitopodium–Cladosporium adds to the 
numerous examples of genetically unrelated hyphomy-
cetes which have acquired through convergent evolution 
a striking morphological similarity, only revealed now, 
through the use of molecular tools. The morphological 
differences between D. hemileiae and D. tectonae in co-
nidial proliferation, pigmentation and conidial width do 
not support the concept of a single species, but indicate 
that there are two different species occurring on CLR. 
This case is comparable to D. cannae and D. canescens, 
two closely allied, but morphologically and genetically 
distinct species mycoparasitic on the rust of Canna spe-
cies, Puccinia thaliae.

Other mycoparasitic species of Cladosporium need to 
be recollected, isolated and reappraised in order to verify 
whether they also represent members of the genus Digi-

topodium, for example, the numerous collections and 
isolates uncritically assigned to and recorded as “Clados-
porium uredinicola”. Specimens have been collected and 
recorded under that name as mycoparasites of several 
rust species, namely: Chrysocyclus cestri (syn. Puccinia 
cestri, Spegazzini 1912), Cronartium quercuum (Mor-
gan-Jones & McKemy 1990), P. horiana (Sheta 1996), P. 
araujiae (Anderson et al., 2015), P. melanocephala (Ryan 
& Wilson 1981), P. puta (Barros et al. 1999), and P. vio-
lae (Traquair et al. 1984). Although some C. uredinicola 
records are phylogenetically proven to be genuine spe-
cies of Cladosporium, with corone conidiogenous loci 
(Bensch et al. 2012), it is possible that some of these re-

cords of mycoparasites were based on misidentification 
and may pertain to Digitopodium. There is also a clear 
need to recollect, epitify and sequence C. uredinicola, 
originally described from Argentina by Spegazzini 
(1912).

Isolates of all species assigned to Digitopodium, includ-
ing Hyalocladosporiella, form a strongly supported monophyletic clade in our phylogenetic tree (Fig. 1). It was also found that Digitopodium is relatively close to 
several species of Metulocladosporiella obtained from
Musa spp. (Crous et al. 2006). Rhizoid-like structures were also described for Metulocladosporiella spp. Perhaps such structures may, in some instances, represent a useful morphological marker for the recognition of Cladosporium-like fungi that actually belong in the Herpotrichiellaceae.

It is surprising that among the four species of Digitopodium now recognized, one pair of species (D. cannea and D. canescens) was found on a single rust host, Puccinia thalaei, but on different continents, whereas another pair of Digitopodium species (D. hemileiae and D. tectonae) was found on another rust host, H. vastatrix in Africa, in addition to the occurrence of the latter species on Olivea tectonae in Brazil. This was also an unexpected finding and suggests that, unless there are specialized infraspecific genotypes of D. tectonae, there is no potential for this species to be deployed as a classical biological control agent in the Americas, since it already occurs in the New World without having ever been recorded on H. vastatrix nor producing any noticeable control of CLR. Of course, the records from the state of Mato Grosso may represent a recent occurrence of the mycoparasite, possibly introduced together with the exotic rust species O. tectonae. The latter was recorded for the first time in Brazil by Cabral et al. (2010) on the exotic timber tree Tectona grandis. As the state of Mato Grosso is distant from the coffee producing areas of Brazil, where H. vastatrix commonly occurs, the fungus may not have been able, as yet, to spread to these areas; or, perhaps, because surveys of mycoparasites of H. vastatrix in Brazil have to date involved only limited sampling, concentrated in the south-eastern states, D. tectonae may also be present on CLR but has remained undetected. For the moment, it seems appropriate to give priority to fungal antagonists of H. vastatrix other than D. tectonae as potential classical biological control agents of CLR. A rich diversity of mycoparasites of CLR, as well as endophytic mycoparasites within coffee plants, exists in Africa and thus there are alternative candidates for use in classical introductions (Rodríguez et al., 2020). Nevertheless, D. hemileiae is worthy of recollecting for taxonomic purposes, as well as for its potential application as a classical biological control agent within Africa.

CONCLUSIONS

Digitopodium – formerly a monotypic genus accommodating the dematiaceous Cladosporium-like species, D. hemileiae – is expanded here with the description of the new species D. cannea and the synonymization of the genus Hyaloocladosporiella, containing two species, with the recombination of H. tectonae and H. cannea into Digitopodium. The molecular appraisal of all three species for which DNA sequences were or became available placed Digitopodium clearly in the Herpotrichiellaceae.

It is conjectured that other Cladosporium-like fungi presently maintained in Cladosporium and related taxa may in fact belong to Digitopodium; and that particular attention should be given to species of Cladosporium mycoparasitic on members of the Pucciniomycotina. Circumstantial evidence indicates that Digitopodium is a specialist genus of species adapted to a mycoparasitic lifestyle on rust hosts and that there may be some degree of host specificity involved.

From our on-going evaluation of the fungi associated with coffee leaf rust, it is concluded that there exists a diverse and largely undocumented guild of mycoparasites attacking Hemileia vastatrix, especially in its African centre of origin, of which Digitopodium is a minor component (Rodríguez et al., 2020, Authors unpubl.). In general, mycoparasites have been poorly studied and, thus far, few have been exploited as biocontrol agents of plant pathogens. We posit that the mycoparasite niche will prove to be a vault of hidden fungal taxa filling part of the void between the number of species of fungi known to science and the progressively increasing estimates of total fungal species (Hawksworth & Lücking 2017). Exploring this niche should expand our knowledge of the magnitude of the fungal component of global biodiversity and, potentially, also provide novel, sustainable tools for plant-disease management.

Abbreviations

AIC: Akaike Information Criterion; BI: Bayesian Inference; CLR: Coffee leaf rust; COAD: Coleção Octávio de Almeida Drummond — official name for the culture collection of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil); MEA: Malt extract-agar; OA: Oatmeal-agar; PDA: Potato dextrose-agar; PCR: Polymerase chain reactions; SNA: Synthetic nutrient poor-agar; VIC: Herbarium Universidade Federal de Viçosa

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Authors’ contributions

Adans A. Colmán and Sara S. Salcedo processed the samples, isolated the fungi, and described their morphology and produced and analysed the molecular data. Rikele Belachew-Beleké, Harry C. Evans and Robert W. Barreto planned and conducted the surveys and collected the specimens which served as basis for the work. Uwe Braun critically reviewed the work and made significant corrections in the interpretation of the data. All authors contributed in the preparation and also read and approved the final manuscript.

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All data generated or analysed during this study are included in this published article. Materials, not involving intellectual property rights or other similar restrictions, will be available from the authors via request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
We consent to publication.

Competing interests
The authors declare that they have no competing interests.

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