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## Mycodiversity in a micro-habitat: twelve *Cladosporium* species, including four new taxa, isolated from uredinia of coffee leaf rust, *Hemileia vastatrix*

C.M. Pereira<sup>1</sup>, S.S. Sarmiento<sup>1</sup>, A.A. Colmán<sup>1</sup>, K. Belachew-Bekele<sup>2</sup>, H.C. Evans<sup>3</sup>, R.W. Barreto<sup>1\*</sup>

<sup>1</sup>Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil

<sup>2</sup>Jimma Agricultural Research Center, Jimma, Oromia Region, Ethiopia

<sup>3</sup>CAB International, Bakeham Lane, Egham, Surrey TW20 9TY, UK

\*Corresponding author: rbarreto@ufv.br

### Key words:

biological control

*Cladosporiaceae*

*Coffea*

mycoparasites

new species

phylogenetics

**Abstract:** During surveys in the centres of origin of the coffee leaf rust (CLR), *Hemileia vastatrix* in Africa, as well as in its exotic range in Brazil, 23 isolates of the genus *Cladosporium* were obtained from uredinial pustules. Using a phylogenetic analysis of all isolates involving a combination of partial sequences of the internal transcribed spacer region of rDNA (ITS) and two gene regions: actin (*act*) and translation elongation factor-1α (*tef1*), 12 species were delimited; including four new species – *Cladosporium chlamydosporiformans*, *C. hemileiicola*, *C. mucilaginosum* and *C. setoides*. GCPSR criteria were employed for species recognition, supported by morphological and cultural characters. The potential of these purported mycoparasites is discussed in the context of biological control of CLR in Latin America.

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## INTRODUCTION

Coffee leaf rust (CLR), caused by *Hemileia vastatrix*, is the most important disease of coffee worldwide (Zambolim 2016, Koutouleas *et al.* 2019) and the historical impacts due to CLR have been well documented (McCook 2006). More recently, disastrous outbreaks of CLR have been destroying the livelihoods of coffee growers in Central and northern South America (Avelino *et al.* 2015, Talhinhos *et al.* 2017); prompting mass migrations to North America (Ward *et al.* 2017). This humanitarian crisis led World Coffee Research (WCR) to initiate a programme – funded by USAID and targeted at smallholder farmers – to mitigate the impact of CLR in Central America. The main emphasis is on developing resistant coffee varieties through plant breeding, but alternative management strategies are also being considered, including biological control using mycoparasitic or antagonistic fungi (Rodríguez *et al.* 2021). The present paper is part of a series documenting the fungi recorded, both as mycoparasites and endophytes, during surveys in the African centres of origin of *Coffea* and *Hemileia vastatrix*, as well as in their exotic range in South America (Crous *et al.* 2018, Colmán *et al.* 2021, Guterres *et al.* 2021, Rodríguez *et al.* 2021, Salcedo-Sarmiento *et al.* 2021, Kapeua-Ndacnou *et al.* 2023a, b). Here, we name and describe the isolates of *Cladosporium* resulting from these surveys in Africa and Brazil.

## MATERIALS AND METHODS

### Sampling, isolation and preservation

The *Cladosporium* isolates included in this study were all obtained from material collected in Brazil, Kenya and Ethiopia. A detailed description of the procedures adopted during the field studies is provided in Colmán *et al.* (2021) and Rodríguez *et al.* (2021). In the case of purported or cryptic mycoparasites of CLR, rust-infected leaves were scanned in the field with a × 40 hand lens to detect evidence of colonies overgrowing *H. vastatrix* uredinia. Leaves with evidence of mycoparasitic associations were dried in a plant press until later examination in the laboratory using a dissecting microscope (Olympus SZX7). Isolations were made by transferring spores or other fungal structures from colonized uredinia onto plates containing potato-dextrose agar (PDA) with a sterilized fine-point needle.

For long-term storage, a representative pure culture of each isolate was transferred to flasks containing silica-gel or a 10 % glycerol solution maintained at 80 °C, as described by Dhingra & Sinclair (1995) and Gonçalves *et al.* (2016). Fungal cultures were deposited in the culture collection ‘Coleção Octávio de Almeida Drumond’ (COAD) of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. Colonies of each isolate were metabolically inactivated and dried cultures were deposited in the UFV herbarium (VIC).

The origin of each isolate, as well as their COAD and VIC codes are presented in Table 1.

## Morphological characterization

Isolates were cultivated on malt extract agar (MEA), oatmeal agar (OA), PDA, and synthetic nutrient-poor agar (SNA) media for 14 d at  $25 \pm 2$  °C in the dark. Cultural characteristics were described following the terminology proposed by Crous *et al.* (2019), and colors were rated according to Rayner (1970).

The slide-culture method (Mafia & Alfenas 2016) was used for microscopic observations and preparation of illustrations. *Cladosporium* isolates were transferred to the sides of small PDA blocks placed in the centre of sterile glass slides and a sterile coverslip was placed over the block. The material was kept in an incubator, adjusted to  $25 \pm 2$  °C, under a light regime of 12 h, for 5–7 d. The PDA blocks were then removed and the coverslips and slides containing fungal structures were carefully mounted in lactoglycerol or lactofuchsin for examination.

Slides were observed and microphotographs were taken using a light microscope (Olympus BX-51) equipped with a digital color camera (Olympus Q-COLOR3). Biometric data were collected from at least 30 representative structures from each isolate and conidia were ranked by following the terminology of Schubert *et al.* (2007).

## DNA extraction, amplification and sequencing

Isolates were transferred to plates containing potato dextrose (PD) broth and maintained in an incubator, adjusted to  $25 \pm 2$  °C, under a light regime of 12 h, for 7 d. The mycelium was then harvested and placed on sterile filter paper to dry. Dried mycelium was transferred to sterile 1.5 ml centrifuge microtubes containing three stainless steel beads and macerated with a mechanical disruptor of cells (model L Beader 3 – Loccus Biotecnologia, Cotia, SP, Brazil), set at 4 000 rpm for two cycles of 10 s each. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's protocol.

Polymerase chain reactions (PCR) were performed for genomic regions of interest for the genus *Cladosporium* (Bensch *et al.* 2018) for each sample. The internal transcribed spacer regions and intervening 5.8S rRNA gene (ITS) of the rDNA was amplified with the primer pair ITS-5/ ITS-4 (White *et al.* 1990), and part of the actin (*act*) and the translation elongation factor 1-alpha (*tef1*) genes were amplified with the primer pairs ACT-512F/ ACT-783R (Carbone & Kohn 1999), and EF1-728F and EF1-986R (Carbone & Kohn 1999), respectively.

The cycling conditions used during each PCR followed the temperatures and times described for each pair of primers (Rosado *et al.* 2019). The PCR products were analyzed on agarose gels stained with GelRed™ (Biotium Inc., Hayward, CA, USA) and visualized under UV light to verify the purity and size of the amplicons. Then, PCR products were purified with ExoSAP-IT™ (Amersham Biosciences, Arlington Heights, IL, USA) following the manufacturer's instructions and sent to Macrogen Inc. (Seoul, South Korea, <http://www.macrogen.com>) to be sequenced.

## Sequencing and phylogenetic analysis

To visualize and evaluate electropherograms, correct possible nucleotides on ambiguous positions and assemble consensus sequences (contigs), the software SeqAssem v. 07/2008 (Hepperle 2021) was used. The sequences generated from the

isolates under study were deposited in GenBank (accession codes are presented in Table 1) and preliminarily compared with other published sequences through a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to verify the taxonomic identity of each isolate. For the phylogenetic inferences, sequence data included in this study were obtained from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) (Table 1).

The sequences were aligned with the MUSCLE algorithm implemented in the software MEGA X v. 10.2.2 (Kumar *et al.* 2018). Initially, *act*, ITS, and *tef1* alignments were combined using the software SequenceMatrix v. 1.8 (Vaidya *et al.* 2011), and an analysis was performed with all described *Cladosporium* species, using *Cercospora beticola* CBS 116456 as an outgroup taxon (data not shown). After determining to which species complex each isolate under study belongs, a new alignment was made for each locus using sequences of the *C. cladosporioides* and *C. sphaerospermum* species complex, and with *C. herbarum* cultures CBS 121621 and CBS 300.49 as the outgroup taxon.

To define the phylogenetic relationship between the *Cladosporium* isolates under study and other species, two phylogenetic methods were used: the Maximum Likelihood analysis (ML) and the Bayesian Inference (BI). The ML analysis was performed using the program RAxML-HPC v. 8.2.12, implemented in the CIPRES portal (Miller *et al.* 2010). Maximum likelihood was performed with 1 000 bootstrap replicates and bootstrap support (BS) values were determined.

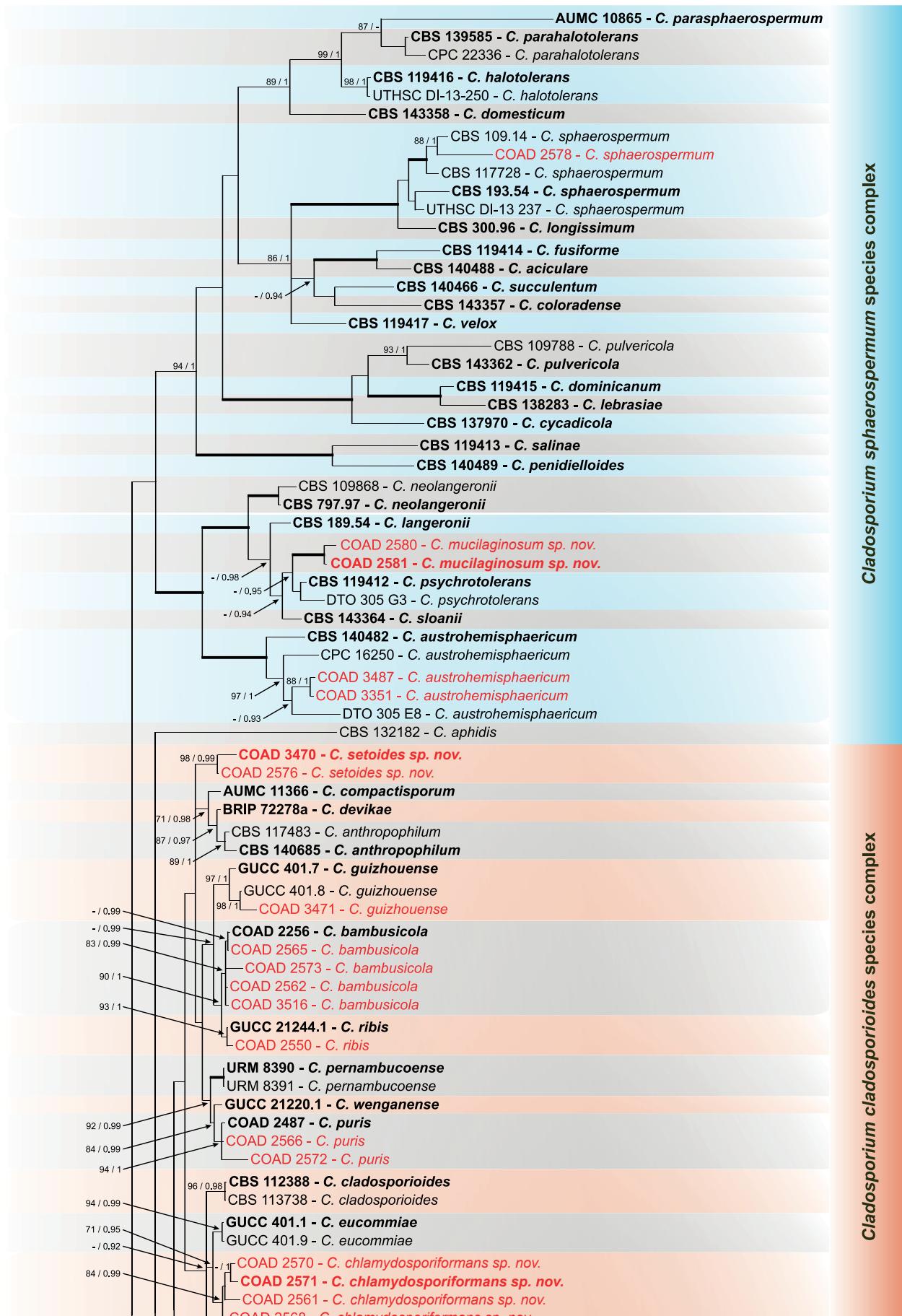
Prior to the BI analyses, the best nucleotide substitution model for each locus was determined. For this, the jModelTest2 v. 2.1.6 (Darriba *et al.* 2012), implemented in the CIPRES Science Gateway portal (Miller *et al.* 2010), was utilized. For the *act* and *tef1* loci, the best substitution models were HKY+I+G and GTR+I+G, respectively. The model SYM+I+G was applied to the ITS region.

For the BI analysis, MrBayes v. 3.2.7a (Ronquist & Huelsenbeck 2003), also present in the CIPRES portal (Miller *et al.* 2010), was used. In order to search for the best tree topology, the Markov chain Monte Carlo (MCMC) method was applied. Two simultaneous and independent analyses were started, each with four chains. In each analysis, trees were randomly generated up to 5 M generations, with sampling every 500 generations, resulting in 10 000 trees sampled. The first 2 500 trees (25 %) were discarded in the burning phase before setting the consensus tree. Through the 7 500 remaining trees from each analysis, the consensus tree was obtained and posterior probability (PP) values were determined. These methods were carried out for each locus individually (data not shown), for a combined dataset of *act* and *tef1* loci (data not shown), and for a combination of *act*, ITS, and *tef1* loci. The resulting trees of concatenated analysis, combining *act*, ITS, and *tef1* loci, were visualized in TreeGraph 2 v. 2.15.0-887 (Stöver & Müller 2010), their topologies were compared, and the BI topology was adopted. The tree was edited in Inkscape v. 1.3 (<https://inkscape.org/>), and BS and PP values higher than 70 % or 0.95, respectively, were maintained.

## RESULTS

### Sequencing and phylogenetic analysis

For *act*, ITS, and *tef1*, amplicons ranging between 153–242, 472–545 and 205–371 bp, respectively, were generated. An



**Fig. 1.** Phylogenetic tree based on a Bayesian Inference (BI) analysis from the combined ITS, *act*, and *tef1* sequence alignment dataset of the *Cladosporium sphaerospermum* and *C. cladosporioides* species complexes. Numbers above the nodes indicate Maximum Likelihood bootstrap support values (BS) (left, BS  $\geq$  70 %) and Posterior Probabilities (PP) from BI (right, PP  $\geq$  0.9), lower values are indicated as "-". Fully supported nodes with 100 % BS and with 1.00 PP are shown as thick lines. The tree is rooted to *Cladosporium herbarum* isolates (CBS 121621 and CBS 300.49). Ex-type strains are highlighted in bold. Isolates from the present study are presented in red.

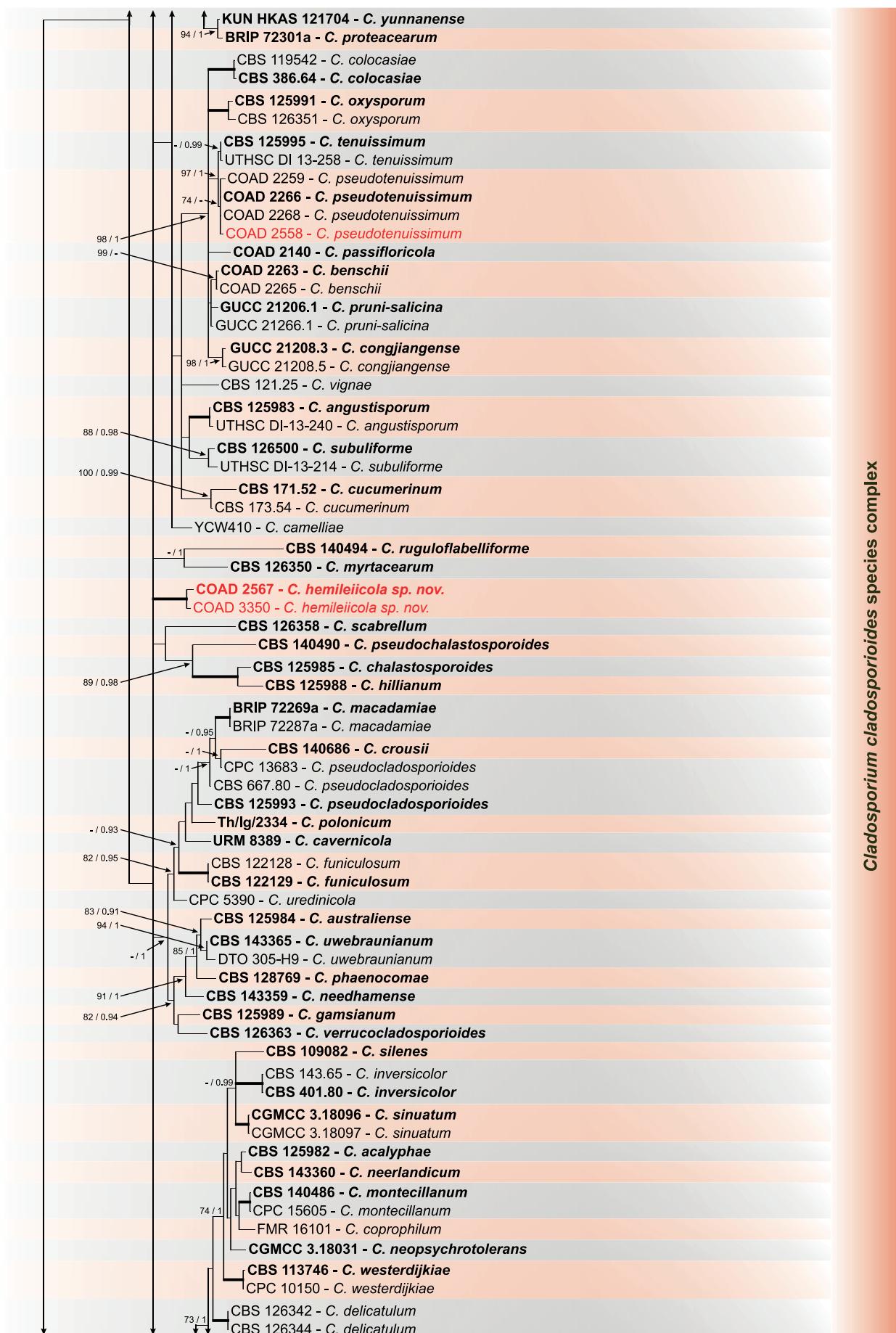


Fig. 1. (Continued).

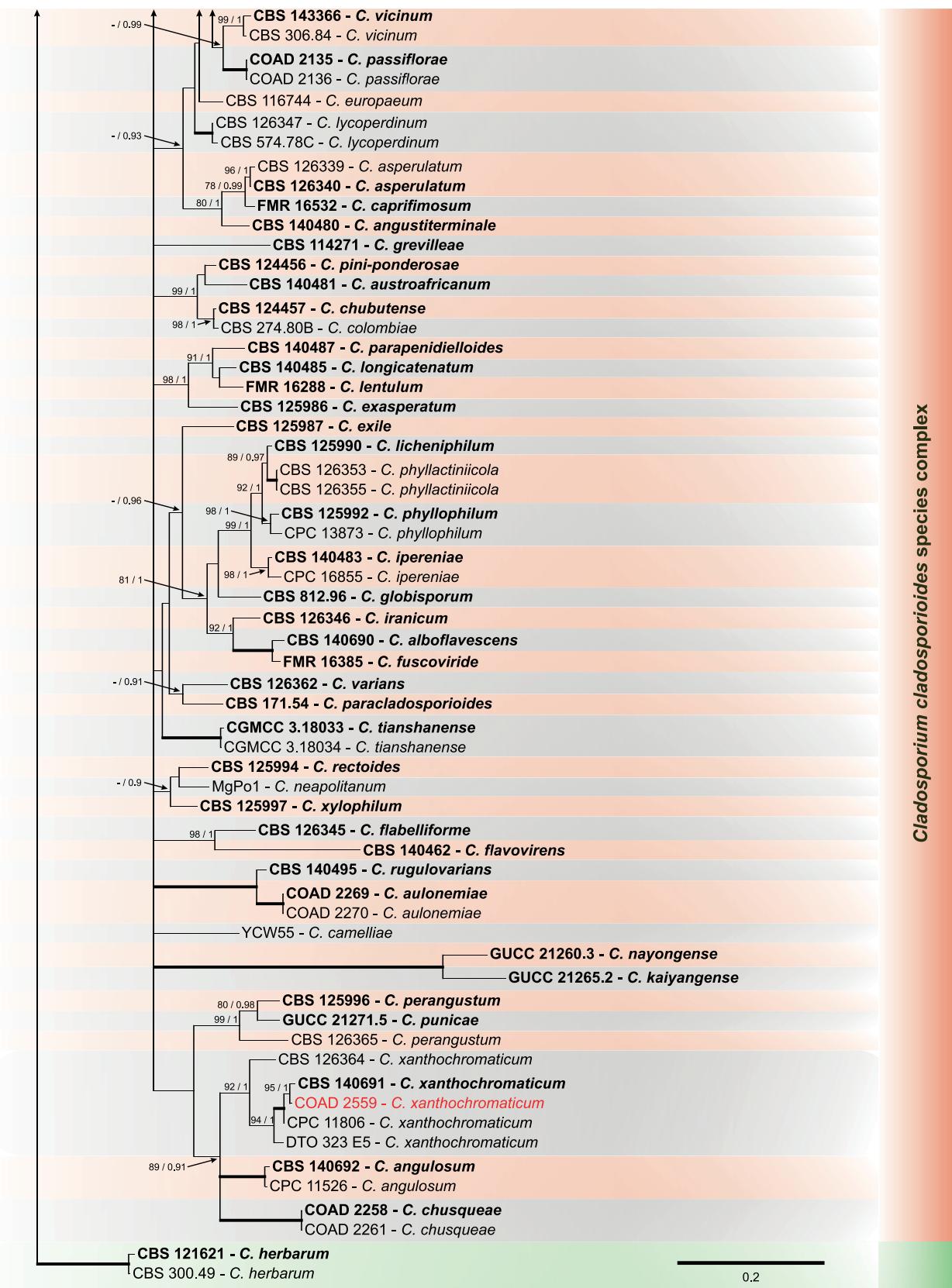


Fig. 1. (Continued).

initial inference with isolates from the three *Cladosporium* species complexes (*C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum*) revealed that all *Cladosporium* isolates evaluated here belong either to the *C. cladosporioides* or *C. sphaerospermum* species complexes. A new analysis was made considering just species belonging to these two complexes.

The final alignment consisted of 197 taxa, including the 23 *Cladosporium* isolates resulting from the present study and two *C. herbarum* isolates used as an outgroup taxon. The combination of *act*, *ITS*, and *tef1* regions was represented by 1 232 characters, including gaps (*act*: 267; *ITS*: 513; *tef1*: 452 sites), from which 511 were conserved sites (*act*: 74; *ITS*: 390; *tef1*: 47 sites), and 489 sites were potentially informative for parsimony (*act*: 140; *ITS*: 61; *tef1*: 288 sites).

The phylogenetic tree (Fig. 1) revealed that of the 23 *Cladosporium* isolates obtained from uredinia of *H. vastatrix*, 18 belong to the *C. cladosporioides* species complex, and the other five to the *C. sphaerospermum* species complex.

Within the *C. cladosporioides* species complex, four isolates clustered with *C. bambusicola* (COAD 2562, 2565, 2573, and 3516), whilst isolate COAD 2550 formed a distinct group defined as *C. ribis*. One isolate (COAD 3471) formed a clade with two isolates of *C. guizhouense*, and another isolate (COAD 2558) grouped with three isolates of *C. pseudotenuissimum*. Isolate COAD 2559 corresponded to *C. xanthochromaticum*, forming a clade with isolates of this species. Two isolates, COAD 2566 and COAD 2572, formed a clade with one isolate of *C. puris*.

The other eight isolates clustered in clades different from known species of this complex, indicating that they represent new species. *Cladosporium chlamydosporiformans* sp. nov. (COAD 2561, 2568, 2570, and 2571) formed a monophyletic group closely related to *C. eucommiae*. *Cladosporium hemileicola* sp. nov. (COAD 2567 and 3350) formed a new clade with high support value within this species complex. Finally, *Cladosporium setoides* sp. nov. (COAD 2576 and 3470) formed a well-supported clade closely related to a group containing *C. anthropophilum*, *C. compactisporum* and *C. devikae*.

Within the *C. sphaerospermum* species complex, two isolates (COAD 3351 and 3487) grouped in the *C. austrohemisphaericum* clade and another isolate (COAD 2578) clustered in the *C. sphaerospermum* monophyletic group. *Cladosporium mucilaginosum* sp. nov. is represented by two isolates (COAD 2580 and 2581), which are closely related to the *C. psychrotolerans* and *C. langeronii* clade, but form a distinct and well-supported group.

## Taxonomy

***Cladosporium austrohemisphaericum*** Bensch et al., Stud. Mycol. **82**: 42. 2015. MycoBank MB 814626.

**Materials examined:** Ethiopia, Oromia Region, Jimma, University farm, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 30 May 2017, H.C. Evans & K.B. Bekele (culture COAD 3487); Agaro, Center, Jimma University, on uredinia of *Hemileia vastatrix* from leaves of *Coffea arabica*, 3 May 2017, H.C. Evans & K.B. Bekele (culture COAD 3351).

For a detailed description and illustrations, see Bensch et al. (2015, 2018).

**Notes:** The two isolates evaluated here form a subclade within a well-supported superior monophyletic group of *C. austrohemisphaericum*. The cultural characteristics of these two isolates are similar to those described by Bensch et al. (2015, 2018) but presented some biometric discrepancies to the original description, but these are interpreted here as resulting from the effect of their growth in different media from that used in the original description (SNA). For instance, conidiophores were wider, 2.5–5.5 µm vs. 2.5–3.5 µm than those in the original description and the conidiogenous cells were somewhat shorter, 8.5–34.5 µm long vs. (6–)13–45(–60) µm. In addition, the ramoconidia, both primary and secondary, were also shorter (primary ramoconidia 6–22 µm long vs. 12–36 µm; secondary ramoconidia 2.5–8.5 µm long vs. (8–)10–27(–30) µm). This species has a wide distribution and host range in the Southern Hemisphere and has been isolated from air samples, plant material and fruits of different hosts in Australia, New Zealand and South Africa (Bensch et al. 2015, 2018). Nevertheless, this appears to be the first record from Ethiopia (and, therefore, from the Northern Hemisphere), as well as the first report as a mycoparasite and of its occurrence on uredinia of *H. vastatrix*.

***Cladosporium bambusicola*** P.P. Costa et al., Phytotaxa **560**: 15. 2022. MycoBank MB 824725.

**Materials examined:** Brazil, Minas Gerais, Araponga, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 3 May 2015, R.W. Barreto (culture COAD 2562); Minas Gerais, Três Pontas, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, Sep. 2015, A.A. Colmán (culture COAD 2565); Espírito Santo, Conceição da Barra, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, Sep. 2015, A.A. Colmán (culture COAD 2573); Minas Gerais, Monte Carmelo, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 12 Jul. 2022, R.W. Barreto (culture COAD 3516).

For a detailed description and illustrations, see Costa et al. (2022).

**Notes:** The four isolates formed a large and well-supported clade with two isolates of *C. bambusicola* and have a similar morphology to that described for this species in Costa et al. (2022); although some differences were noted. The conidiophores of the CLR isolates were longer (up to 427 µm long on the CLR samples vs. up to 179 µm in the original description) and conidial sizes differed: ramoconidia and intercalary conidia being smaller than those reported (ramoconidia: 7–28 × 1.5–4.5 µm vs. 33.9–39.9 × 4.3–5.8 µm; intercalary conidia: 3.5–7 × 2–3.5 µm vs. 4.1–10.8 × 2.6–5.2 µm), and terminal conidia being slightly thinner (2–3.5 µm wide vs. 3.3–5.7 µm). Originally, *C. bambusicola* was obtained from decayed leaves of *Aulonemia amplissima* in the coffee-growing region of Minas Gerais (Costa et al. 2022). More recently, this species was isolated from uredinia of another rust fungus (*Austropuccinia psidii*) on leaves of *Corymbia* sp. and on fruits of *Psidium guajava* (Silva et al. 2023). This is the first record of *C. bambusicola* on *H. vastatrix* and seems to indicate that this species is well adapted to grow on uredinia of the *Pucciniales*. Thus far, *C. bambusicola* has only been reported from Minas Gerais, Brazil.

**Table 1.** Cladosporium species, strain information, and GenBank accession numbers for taxa used in the phylogenetic trees. Type specimens are indicated in bold, and GenBank accession numbers of sequences generated in this study are highlighted with an underline.

| Species   | Strain numbers    | Host / Substrate  | Collection Sites |                 |                 | GenBank accession numbers    |                              | References |
|---|-------------------|---|------------------|-----------------|-----------------|------------------------------|------------------------------|------------|
|   |                   |   | act              | ITS             | tef1            | act                          | ITS                          |            |
| <b>Cladosporium cladosporioides species complex</b> |                   |   |                  |                 |                 |                              |                              |            |
| <i>C. acalyphae</i>                                 | <b>CBS 125982</b> | <i>Acalypha australis</i>   | South Korea      | HM148481        | HM147994        | HM148235                     | Bensch et al. (2010)         |            |
|   | <b>CBS 140690</b> | Bronchoalveolar lavage fluid of animal                                      | USA              | LN834604        | LN834516        | Sandoval-Denis et al. (2016) |                              |            |
| <i>C. angulosum</i>                                 | <b>CBS 140692</b> | Bronchoalveolar lavage fluid of human                                       | USA              | LN834609        | LN834521        | Sandoval-Denis et al. (2016) |                              |            |
|   | CPC 11526         | <i>Acacia mangium</i>   | Thailand         | HM148616        | HM148127        | HM148371                     | Bensch et al. (2018)         |            |
| <i>C. angustisporum</i>                             | <b>CBS 125983</b> | <i>Alloxylon wickhamii</i>  | Australia        | HM148482        | HM147995        | HM148236                     | Bensch et al. (2010)         |            |
|   | UTHSC DI-13-240   | Human, toe nail   | USA              | LN834540        | LN834356        | LN834452                     | Sandoval-Denis et al. (2015) |            |
| <i>C. angustiterminale</i>                          | <b>CBS 140480</b> | <i>Banksia grandis</i>  | Australia        | KT600575        | KT600379        | KT600476                     | Bensch et al. (2015)         |            |
| <i>C. anthropophilum</i>                            | CBS 117483        | Unknown   | USA              | HM148494        | HM148007        | HM148248                     | Sandoval-Denis et al. (2016) |            |
|   | <b>CBS 140685</b> | Bronchoalveolar lavage fluid of human                                       | USA              | LN834621        | LN834437        | LN834533                     | Sandoval-Denis et al. (2016) |            |
|   | CPC 11122         | <i>Phytolacca americana</i>   | South Korea      | HM148506        | HM148019        | HM148260                     | Sandoval-Denis et al. (2016) |            |
| <i>C. asperulatum</i>                               | CBS 126339        | <i>Eucalyptus</i> leaf litter   | India            | HM148484        | HM147997        | HM148238                     | Bensch et al. (2010)         |            |
|   | <b>CBS 126340</b> | <i>Protea susannae</i>  | Portugal         | HM148485        | HM147998        | HM148239                     | Bensch et al. (2010)         |            |
| <i>C. aulonemiae</i>                                | <b>COAD 2269</b>  | Decayed leaf of <i>Aulonemia amplissima</i>                                 | Brazil           | MT373119        | MZ318427        | MT680198                     | Costa et al. (2022)          |            |
|   | COAD 2270         | Decayed leaf of <i>Aulonemia amplissima</i>                                 | Brazil           | MT373120        | MZ318428        | MT680199                     | Costa et al. (2022)          |            |
| <i>C. austriaiense</i>                              | <b>CBS 125984</b> | <i>Eucalyptus moluccana</i>   | Australia        | HM148486        | HM147999        | HM148240                     | Bensch et al. (2010)         |            |
| <i>C. austroafricanum</i>                           | <b>CBS 140481</b> | Leaf litter   | New Zealand      | KT600577        | KT600381        | KT600478                     | Bensch et al. (2015)         |            |
| <i>C. bambusicola</i>                               | <b>COAD 2256</b>  | Decayed leaf of <i>Aulonemia amplissima</i>                                 | Brazil           | MT373125        | MZ318433        | MT680204                     | Costa et al. (2022)          |            |
|   | COAD 2562         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Brazil           | <u>OP598123</u> | <u>OP535371</u> | <u>OP676082</u>              | This study                   |            |
|   | COAD 2565         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Brazil           | OP598124        | OP535372        | OP676083                     | This study                   |            |
|   | COAD 2573         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Brazil           | <u>OP598121</u> | <u>OP535369</u> | <u>OP676080</u>              | This study                   |            |
|   | COAD 3516         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Brazil           | <u>OR669144</u> | <u>OR666147</u> | <u>OR669145</u>              | This study                   |            |
| <i>C. benschii</i>                                  | <b>COAD 2263</b>  | Decayed leaf of <i>Aulonemia amplissima</i>                                 | Brazil           | MT373128        | MZ318436        | MT680207                     | Costa et al. (2022)          |            |
|   | COAD 2265         | Decayed leaf of <i>Aulonemia amplissima</i>                                 | Brazil           | MT373129        | MZ318437        | MT680208                     | Costa et al. (2022)          |            |
| <i>C. brigadirensis</i>                             | COAD 2257         | Decayed leaf of <i>Chusquea urelytra</i>                                    | Brazil           | MT373127        | MZ318435        | MT680206                     | Costa et al. (2022)          |            |
| <i>C. camelliae</i>                                 | YCW410            | <i>Camellia sinensis</i>  | China            | OP588395        | OP558377        | OP586704                     | Lv et al. (2023)             |            |
|   | YCW55             | <i>Camellia sinensis</i>  | China            | OP588408        | OP558390        | OP586717                     | Lv et al. (2023)             |            |

Table 1. (Continued).

| Species                                 | Strain numbers | Host / Substrate   | Collection Sites |            |             | GenBank accession numbers |   | References |
|---|----------------|--|------------------|------------|-------------|---------------------------|---|------------|
|   |                |  | <i>act</i>       | <i>ITS</i> | <i>tef1</i> |                           |   |            |
| <i>C. caprifimosum</i>                  | FMR 16532      | From goat dung   | Spain            | LR813205   | LR813198    | LR813210                  | Iturrieta-González <i>et al.</i> (2021) |            |
| <i>C. cavernicola</i>                   | URM 8389       | Air sample in a bat cave   | Brazil           | MZ555746   | MZ518829    | MZ555733                  | Pereira <i>et al.</i> (2022)            |            |
| <i>C. chalastosporoides</i>             | CBS 125985     | Fruiting bodies of <i>Teratosphaeria proteaearboreae</i> on leaves of <i>Protea nitida</i> | South Africa     | HM148488   | HM148001    | HM148242                  | Bensch <i>et al.</i> (2010)             |            |
| <i>C. chlamydosporiformans</i> sp. nov. | COAD 2561      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>                | Brazil           | OP598122   | OP535370    | OP676081                  | This study                              |            |
|   | COAD 2570      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>                | Brazil           | OP598116   | OP535364    | OP676075                  | This study                              |            |
|   | COAD 2571      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>                | Kenya            | OP598126   | OP535374    | OP676085                  | This study                              |            |
|   | COAD 2568      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>                | Ethiopia         | OP598130   | OP535378    | OP676089                  | This study                              |            |
| <i>C. chubutense</i>                    | CBS 124457     | <i>Pinus ponderosa</i>   | Argentina        | FJ936165   | FJ936158    | FJ936161                  | Bensch <i>et al.</i> (2010)             |            |
| <i>C. chusqueae</i>                     | COAD 2258      | Decayed leaf of <i>Chusquea urelytra</i>   | Brazil           | MT373122   | MZ318430    | MT680201                  | Costa <i>et al.</i> (2022)              |            |
|   | COAD 2261      | Decayed leaf of <i>Chusquea urelytra</i>   | Brazil           | MT373124   | MZ318431    | MT680203                  | Costa <i>et al.</i> (2022)              |            |
|   | CBS 113738     | Grape bud  | USA              | HM148491   | HM148004    | HM148245                  | Bensch <i>et al.</i> (2010)             |            |
|   | CBS 112388     | <i>Pisum sativum</i>   | Germany          | HM148490   | HM148003    | HM148244                  | Bensch <i>et al.</i> (2010)             |            |
| <i>C. cladosporoides</i>                | CBS 386.64     | <i>Colocasia esculenta</i>   | Taiwan           | HM148555   | HM148067    | HM148310                  | Bensch <i>et al.</i> (2010)             |            |
|   | CBS 119542     | <i>Colocasia esculenta</i>   | Japan            | HM148554   | HM148066    | HM148309                  | Bensch <i>et al.</i> (2010)             |            |
|   | AUMC 11366     | Air sample   | Egypt            | OL514010   | MN826822    | —                         | Moharram <i>et al.</i> (2022)           |            |
| <i>C. compactisporum</i>                | GUCC 21208.3   | On leaves of <i>Passiflora edulis</i>  | China            | OP863094   | OP852675    | OP859042                  | Yang <i>et al.</i> (2023)               |            |
| <i>C. congianense</i>                   | GUCC 21208.5   | On leaves of <i>Passiflora edulis</i>  | China            | OP863095   | OP852676    | OP859043                  | Yang <i>et al.</i> (2023)               |            |
| <i>C. coprophilum</i>                   | FMR 16101      | From unidentified herbivore dung   | Spain            | LR813204   | LR813199    | LR813211                  | Iturrieta-González <i>et al.</i> (2021) |            |
| <i>C. crousei</i>                       | CBS 140686     | Bronchoalveolar lavage fluid of human  | USA              | LN834615   | LN834431    | LN834527                  | Sandoval-Denis <i>et al.</i> (2016)     |            |
| <i>C. cucumerinum</i>                   | CBS 171.52     | <i>Cucumis sativus</i>   | Netherlands      | HM148561   | HM148072    | HM148316                  | Bensch <i>et al.</i> (2010)             |            |
|   | CBS 173.54     | <i>Cucumis sativus</i>   | Netherlands      | HM148563   | HM148074    | HM148318                  | Bensch <i>et al.</i> (2010)             |            |
| <i>C. delicatulum</i>                   | CBS 126342     | Indoor air   | Denmark          | HM148568   | HM148079    | HM148323                  | Bensch <i>et al.</i> (2010)             |            |
|   | CBS 126344     | <i>Tilia cordata</i>   | Germany          | HM148570   | HM148081    | HM148325                  | Bensch <i>et al.</i> (2010)             |            |
| <i>C. devikae</i>                       | BRIP 72278a    | From flower blight of <i>Macadamia integrifolia</i>  | Australia        | MZ344212   | MZ303808    | MZ344193                  | Prasannath <i>et al.</i> (2021)         |            |
| <i>C. eucommiae</i>                     | GUCC 401.1     | Fallen leaves of <i>Eucommia ulmoides</i>  | China            | OL519775   | OL587465    | OL504966                  | Wang <i>et al.</i> (2022)               |            |
|   | GUCC 401.9     | Fallen leaves of <i>Eucommia ulmoides</i>  | China            | ON383337   | ON334729    | —                         | Wang <i>et al.</i> (2022)               |            |
| <i>C. europeum</i>                      | CBS 116744     | <i>Acer pseudoplatanus</i>   | Germany          | HM148540   | HM148053    | HM148294                  | Bensch <i>et al.</i> (2018)             |            |
| <i>C. exasperatum</i>                   | CBS 125986     | <i>Eucalyptus tintinnans</i>   | Australia        | HM148579   | HM148090    | HM148334                  | Bensch <i>et al.</i> (2010)             |            |

Table 1. (Continued).

| Species                         | Strain numbers | Host / Substrate   | Collection Sites | GenBank accession numbers |          |                                     | References                              |
|---------------------------------|----------------|--|------------------|---------------------------|----------|-------------------------------------|---|
|                                 |                |  |                  | act                       | ITS      | tef1                                |   |
| <i>C. exile</i>                 | CBS 125987     | Chasmothecia of <i>Phyllactinia guttata</i> on leaves of <i>Corylus avellana</i> | USA              | HM148580                  | HM148091 | HM148335                            | Bensch <i>et al.</i> (2010)             |
| <i>C. flabelliforme</i>         | CBS 126345     | <i>Melaleuca cajuputi</i>  | Australia        | HM148581                  | HM148092 | HM148336                            | Bensch <i>et al.</i> (2010)             |
| <i>C. flavovirens</i>           | CBS 140462     | Man, toenail   | USA              | LN834624                  | LN834536 | Sandoval-Denis <i>et al.</i> (2016) |   |
| <i>C. funiculosum</i>           | CBS 122128     | <i>Ficus carica</i>  | Japan            | HM148582                  | HM148093 | HM148337                            | Bensch <i>et al.</i> (2010)             |
|                                 | CBS 122129     | <i>Vigna umbellata</i>   | Japan            | HM148583                  | HM148094 | HM148338                            | Bensch <i>et al.</i> (2010)             |
| <i>C. fuscoviride</i>           | FMR 16385      | From garden soil   | Spain            | LR813206                  | LR813200 | LR813212                            | Iturrieta-González <i>et al.</i> (2021) |
| <i>C. gamsianum</i>             | CBS 125989     | <i>Strelitzia</i> sp.  | South Africa     | HM148584                  | HM148095 | HM148339                            | Bensch <i>et al.</i> (2010)             |
| <i>C. globisporum</i>           | CBS 812.96     | Meat stamp   | Sweden           | HM148585                  | HM148096 | HM148340                            | Bensch <i>et al.</i> (2010)             |
| <i>C. grevilleae</i>            | CBS 114271     | <i>Grevillea</i> sp.   | Australia        | JF770473                  | JF770450 | JF770472                            | Crous <i>et al.</i> (2011)              |
| <i>C. guizhouense</i>           | GUCC 401.7     | Fallen leaves of <i>Eucommia ulmoides</i>  | China            | OL519780                  | OL579741 | OL504965                            | Wang <i>et al.</i> (2022)               |
|                                 | GUCC 401.8     | Fallen leaves of <i>Eucommia ulmoides</i>  | China            | ON383338                  | ON334728 | ON383470                            | Wang <i>et al.</i> (2022)               |
|                                 | COAD 3471      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>      | Ethiopia         | OP598134                  | OP535382 | OP676093                            | This study                              |
| <i>C. hemileiicola</i> sp. nov. | COAD 2567      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>      | Brazil           | OP598128                  | OP535376 | OP676087                            | This study                              |
|                                 | COAD 3350      | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>      | Ethiopia         | OP598133                  | OP535381 | OP676092                            | This study                              |
| <i>C. hillianum</i>             | CBS 125988     | Leaf mold of <i>Typha orientalis</i>   | New Zealand      | HM148586                  | HM148097 | HM148341                            | Bensch <i>et al.</i> (2010)             |
| <i>C. inversicolor</i>          | CBS 143.65     | Leaf of <i>Tilia</i> sp.   | Netherlands      | HM148589                  | HM148100 | HM148344                            | Bensch <i>et al.</i> (2010)             |
|                                 | CBS 401.80     | Leaf of <i>Triticum aestivum</i>   | Netherlands      | HM148590                  | HM148101 | HM148345                            | Bensch <i>et al.</i> (2010)             |
| <i>C. ipereniae</i>             | CBS 140483     | <i>Puya</i> sp.  | Chile            | KT600589                  | KT600394 | KT600491                            | Bensch <i>et al.</i> (2015)             |
|                                 | CPC 16855      | <i>Arctostaphylos pallida</i>  | USA              | KT600590                  | KT600395 | KT600492                            | Bensch <i>et al.</i> (2015)             |
| <i>C. iranicum</i>              | CBS 126346     | Leaf of <i>Citrus sinensis</i>   | Iran             | HM148599                  | HM148110 | HM148354                            | Bensch <i>et al.</i> (2010)             |
| <i>C. kaiyangense</i>           | GUCC 21265.2   | On decaying fruit of <i>Eriobotrya japonica</i>                                  | China            | OP859045                  | OP852665 | OP863097                            | Yang <i>et al.</i> (2023)               |
| <i>C. lentulum</i>              | FMR 16288      | From unidentified leaf litter  | Spain            | LR813209                  | LR813203 | LR813215                            | Iturrieta-González <i>et al.</i> (2021) |
|                                 | CBS 125990     | <i>Phaeophyscia orbicularis</i> and <i>Physcia</i> sp.                           | Germany          | HM148600                  | HM148111 | HM148355                            | Bensch <i>et al.</i> (2010)             |
| <i>C. licheniphilum</i>         | CBS 140485     | Unknown plant  | Australia        | KT600598                  | KT600403 | KT600500                            | Bensch <i>et al.</i> (2015)             |
| <i>C. longicatenatum</i>        |                |  | Russia           | HM148604                  | HM148115 | HM148359                            | Bensch <i>et al.</i> (2010)             |
| <i>C. lycooperdinum</i>         | CBS 574.78C    | <i>Aureobasidium caulinorum</i>  | Canada           | HM148601                  | HM148112 | HM148356                            | Bensch <i>et al.</i> (2010)             |
|                                 | CBS 126347     | Galls of <i>Apiosporina morbosa</i> on <i>Prunus</i> sp.                         | Australia        | MZ344214                  | MZ303810 | MZ344195                            | Prasannath <i>et al.</i> (2021)         |
| <i>C. macadamiae</i>            | BRIP 72269a    | From flower blight of <i>Macadamia integrifolia</i>                              | Australia        | MZ344215                  | MZ303811 | MZ344196                            | Prasannath <i>et al.</i> (2021)         |
|                                 | BRIP 72287a    | From flower blight of <i>Macadamia integrifolia</i>                              | Mexico           | KT600406                  | KT600504 | KT600504                            | Bensch <i>et al.</i> (2015)             |
| <i>C. montecillanum</i>         | CBS 140486     | Pine needles   |                  |                           |          |                                     |   |

Table 1. (Continued).

| Species                           | Strain numbers | Host / Substrate  | Collection Sites |            |             | GenBank accession numbers |                                 | References |
|-----------------------------------|----------------|---|------------------|------------|-------------|---------------------------|---------------------------------|------------|
|                                   |                |   | <i>act</i>       | <i>ITS</i> | <i>tef1</i> |                           |                                 |            |
| <i>C. myrtacearum</i>             | CPC 15605      | <i>Taraxacum</i> sp.  | Mexico           | KT600603   | KT600407    | KT600505                  | Bensch <i>et al.</i> (2015)     |            |
| <i>C. nayongense</i>              | CBS 126350     | <i>Corymbia foelscheana</i>   | Australia        | HM148606   | HM148117    | HM148361                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. neapolitanum</i>            | GUCC 21260.3   | Leaves of <i>Prunus pseudocerasus</i>   | China            | OP859054   | OP852669    | OP853106                  | Yang <i>et al.</i> (2023)       |            |
| <i>C. needhamense</i>             | MgPo1          | From receptacle of <i>Micromeria graeca</i> flower                              | Italy            | MK416051   | MK387890    | MK416094                  | Zimowska <i>et al.</i> (2021)   |            |
| <i>C. neerlandicum</i>            | CBS 143359     | Indoor air sample   | USA              | MF473991   | MF473142    | MF473570                  | Bensch <i>et al.</i> (2018)     |            |
| <i>C. neopsychrotolerans</i>      | CBS 143360     | Swab sample   | Netherlands      | KP702010   | KP701887    | KP701764                  | Bensch <i>et al.</i> (2018)     |            |
| <i>C. oxytropis</i>               | CGMCC 3.18031  | From the rhizosphere soil of <i>Saussurea involucrata</i>                       | China            | KX938366   | KX938383    | KX938400                  | Ma <i>et al.</i> (2017)         |            |
| <i>C. paracallosporoides</i>      | CBS 125991     | Soil, near the terracotta army  | China            | HM148607   | HM148118    | HM148362                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. paracallosporoides</i>      | CBS 126351     | Indoor air  | Venezuela        | HM148608   | HM148119    | HM148363                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. parapenidielloides</i>      | CBS 171.54     | —   | —                | HM148609   | HM148120    | HM148364                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. passiflorae</i>             | CBS 140487     | <i>Eucalyptus</i> sp.   | Australia        | KT600606   | KT600410    | KT600508                  | Bensch <i>et al.</i> (2015)     |            |
| <i>C. passiflorae</i>             | COAD 2135      | Leaves of <i>Passiflora edulis</i>  | Brazil           | MH729795   | MH682175    | MH724943                  | Rosado <i>et al.</i> (2019)     |            |
| <i>C. passiflorica</i>            | COAD 2136      | Leaves of <i>Passiflora edulis</i>  | Brazil           | MH729796   | MH682176    | MH724944                  | Rosado <i>et al.</i> (2019)     |            |
| <i>C. passiflorica</i>            | COAD 2140      | Fruits of <i>Passiflora edulis</i>  | Brazil           | MH729800   | —           | MH724948                  | Rosado <i>et al.</i> (2019)     |            |
| <i>C. perangustum</i>             | CBS 125996     | <i>Cussonia</i> sp.   | South Africa     | HM148610   | HM148121    | HM148365                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. perambucoense</i>           | CBS 126365     | Chamothecia of <i>Phyllactinia guttata</i> on leaves of <i>Corylus avellana</i> | USA              | HM148612   | HM148123    | HM148367                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. phaenocoma</i>              | URM 8390       | Air sample in a bat cave  | Brazil           | MZ555745   | MZ518828    | MZ555732                  | Pereira <i>et al.</i> (2022)    |            |
| <i>C. phylactiniicola</i>         | URM 8391       | Air sample in a bat cave  | Brazil           | MZ555747   | MZ518830    | MZ555734                  | Pereira <i>et al.</i> (2022)    |            |
| <i>C. phyllophilum</i>            | CBS 128769     | Leaf bracts of <i>Phoenocoma prolifera</i>                                      | South Africa     | JF499881   | JF499837    | JF499875                  | Crous <i>et al.</i> (2011)      |            |
| <i>C. pini-ponderosae</i>         | CBS 126353     | Chamothecia of <i>Phyllactinia guttata</i> on leaves of <i>Corylus avellana</i> | USA              | HM148640   | HM148151    | HM148395                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. phylactiniicola</i>         | CBS 126355     | Chamothecia of <i>Phyllactinia guttata</i> on leaves of <i>Corylus avellana</i> | USA              | HM148642   | HM148153    | HM148397                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. polonicum</i>               | CBS 125992     | <i>Taphrina</i> sp. on <i>Prunus cerasus</i>                                    | Germany          | HM148643   | HM148154    | HM148398                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. proteacearum</i>            | CPC 13873      | On <i>Teratosphaeria proteae-artoreae</i> on <i>Protea arborea</i>              | South Africa     | HM148644   | HM148155    | HM148399                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. ponderosa</i>               | CBS 124456     | <i>Pinus ponderosa</i>  | Argentina        | FJ936167   | FJ936160    | FJ936164                  | Bensch <i>et al.</i> (2010)     |            |
| <i>C. polonicum</i>               | Th/lg/2334     | From gall of <i>Asphondyilla serpylli</i> on <i>Thymus vulgaris</i>             | Poland           | MK416055   | MK387894    | MK416098                  | Zimowska <i>et al.</i> (2021)   |            |
| <i>C. proteacearum</i>            | BRIP 72301a    | From flower blight of <i>Macadamia integrifolia</i>                             | Australia        | MZ344213   | MZ303809    | MZ344194                  | Prasannath <i>et al.</i> (2021) |            |
| <i>C. pruni-salicina</i>          | GUCC 21206.1   | On leaves of <i>Prunus salicina</i>   | China            | OP863092   | OP852683    | OP859041                  | Yang <i>et al.</i> (2023)       |            |
| <i>C. pseudochalastosporoidea</i> | CBS 140490     | On leaves of <i>Prunus salicina</i>   | China            | OP863093   | OP852684    | —                         | Yang <i>et al.</i> (2023)       |            |
|                                   | CBS 667.80     | Pine needles  | Mexico           | KT600611   | KT600415    | KT600513                  | Bensch <i>et al.</i> (2015)     |            |
|                                   | CBS 125993     | <i>Malus sylvestris</i>   | Italy            | HM148654   | HM148165    | HM148409                  | Bensch <i>et al.</i> (2010)     |            |
|                                   |                | Outside air   | Netherlands      | HM148647   | HM148158    | HM148402                  | Bensch <i>et al.</i> (2010)     |            |

Table 1. (Continued).

| Species                      | Strain numbers       | Host / Substrate  | Collection Sites |          |          | GenBank accession numbers |          | References                   |
|------------------------------|----------------------|---|------------------|----------|----------|---------------------------|----------|------------------------------|
|                              |                      |   | act              | ITS      | tef1     | HM148417                  | HM148662 |                              |
| <i>C. pseudotenuissimum</i>  | CPC 13683            | <i>Eucalyptus platycarpus</i>   | Australia        | HM148417 |          |                           |          | Bensch et al. (2010)         |
|                              | <b>COAD 2266</b>     | Decayed leaf of <i>Chusquea urelytra</i>  | Brazil           | MT373132 | MZ318439 | MT680211                  |          | Costa et al. (2022)          |
|                              | COAD 2259            | Decayed leaf of <i>Chusquea anelytrooides</i>                                       | Brazil           | MT373130 | MZ318438 | MT680209                  |          | Costa et al. (2022)          |
|                              | COAD 2268            | Decayed leaf of <i>Chusquea urelytra</i>  | Brazil           | MT373134 | MZ318441 | MT680213                  |          | Costa et al. (2022)          |
|                              | COAD 2558            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Brazil           | OP598118 | OP535366 | OP676077                  |          | This study                   |
| <i>C. punicae</i>            | <b>GUCC 21271.5</b>  | On leaves of <i>Punica granatum</i>   | China            | OP863108 | OP852672 | OP859056                  |          | Yang et al. (2023)           |
| <i>C. puris</i>              | <b>COAD 2487</b>     | Submerged litter in streams   | Brazil           | MK249980 | MK253337 | MK293777                  |          | Freitas et al. (2021)        |
|                              | COAD 2566            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Brazil           | OP598125 | OP535373 | OP676084                  |          | This study                   |
|                              | COAD 2572            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Brazil           | OP598127 | OP535375 | OP676086                  |          | This study                   |
| <i>C. rectoides</i>          | <b>CBS 125994</b>    | <i>Vitis flexuosa</i>   | South Korea      | HM148683 | HM148193 | HM148438                  |          | Bensch et al. (2010)         |
| <i>C. ribis</i>              | <b>GUCC 21244.1</b>  | On leaves of <i>Ribes burejense</i>   | China            | OP863098 | OP852666 | OP859046                  |          | Yang et al. (2023)           |
|                              | COAD 2550            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Brazil           | OP598117 | OP535365 | OP676076                  |          | This study                   |
| <i>C. ruguloflabiliforme</i> | <b>CBS 140494</b>    | <i>Diatrapaceae</i> sp. on <i>Aloe</i> sp.  | South Africa     | KT600655 | KT600458 | KT600557                  |          | Bensch et al. (2015)         |
| <i>C. rugulovarians</i>      | <b>CBS 140495</b>    | Leaf sheaths of unidentified Poaceae  | Brazil           | KT600656 | KT600459 | KT600558                  |          | Bensch et al. (2015)         |
| <i>C. scabrellum</i>         | <b>CBS 126358</b>    | <i>Ruscus hypoglossum</i>   | Slovenia         | HM148685 | HM148195 | HM148440                  |          | Bensch et al. (2010)         |
| <i>C. setoides</i> sp. nov.  | COAD 2576            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Ethiopia         | OP598132 | OP535380 | OP676091                  |          | This study                   |
|                              | COAD 3470            | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i>         | Ethiopia         | OP598131 | OP535379 | OP676090                  |          | This study                   |
| <i>C. silenes</i>            | <b>CBS 109082</b>    | Stems of <i>Silene maritima</i>   | England          | EF679506 | EF679354 | EF679429                  |          | Crous et al. (2011)          |
| <i>C. sinuatum</i>           | CGMCC 3.18096        | From alpine soil  | China            | KX938368 | KX938385 | KX938402                  |          | Ma et al. (2017)             |
|                              | CGMCC 3.18097        | From alpine soil  | China            | KX938369 | KX938386 | KX938403                  |          | Ma et al. (2017)             |
| <i>C. subuliforme</i>        | <b>CBS 126500</b>    | <i>Chamaedorea metallica</i>  | Thailand         | HM148686 | HM148196 | HM148441                  |          | Bensch et al. (2010)         |
|                              | UTHSC DI-13-214      | Bronchoalveolar lavage fluid of human   | USA              | LN834578 | LN834394 | LN834490                  |          | Sandoval-Denis et al. (2015) |
| <i>C. tenuissimum</i>        | <b>CBS 125995</b>    | <i>Lagerstroemia</i> sp.  | USA              | HM148687 | HM148197 | HM148442                  |          | Bensch et al. (2010)         |
|                              | UTHSC DI-13-258      | Human, thoracentesis fluid  | USA              | LN834588 | LN834404 | LN834500                  |          | Sandoval-Denis et al. (2015) |
| <i>C. tianshanense</i>       | <b>CGMCC 3.18033</b> | From the rhizosphere soil of <i>Saussurea involucrata</i>                           | China            | KX938364 | KX938381 | KX938398                  |          | Ma et al. (2017)             |
|                              | CGMCC 3.18034        | From the rhizosphere soil of <i>Saussurea involucrata</i>                           | China            | KX938365 | KX938382 | KX938399                  |          | Ma et al. (2017)             |
| <i>C. uredinicola</i>        | CPC 5390             | On <i>Cronartium fusiforme</i> f. sp. <i>quercum</i> on <i>Quercus nigra</i> leaves | —                | HM148712 | AY251071 | HM148467                  |          | Bensch et al. (2010)         |
| <i>C. uwebrauniannum</i>     | <b>CBS 143365</b>    | Indoor air sample   | Netherlands      | MF474156 | MF473306 | MF473729                  |          | Bensch et al. (2018)         |
|                              | DTO 305-H9           | House dust  | New Zealand      | MF474157 | MF473307 | MF473730                  |          | Bensch et al. (2018)         |
| <i>C. varians</i>            | <b>CBS 126362</b>    | <i>Catalpa bungei</i>   | Russia           | HM148715 | HM148224 | HM148470                  |          | Bensch et al. (2010)         |

Table 1. (Continued).

| Species   | Strain numbers      | Host / Substrate  | Collection Sites   |            | GenBank accession numbers |             | References                          |
|---|---------------------|---|--------------------|------------|---------------------------|-------------|-------------------------------------|
|   |                     |   | <i>act</i>         | <i>ITS</i> | <i>tef1</i>               | <i>tef1</i> |                                     |
| <i>C. verrucocladodosporioides</i>                        | <b>CBS 126363</b>   | <i>Rhus chinensis</i>   | South Korea        | HM148717   | HM148226                  | HM148472    | Bensch <i>et al.</i> (2010)         |
| <i>C. vicinum</i>   | <b>CBS 143366</b>   | Indoor air sample   | USA                | MF474161   | MF473311                  | MF473734    | Bensch <i>et al.</i> (2018)         |
|   | CBS 306.84          | Urediniospores of <i>Puccinia allii</i>                                     | England            | HM148544   | HM148057                  | HM148299    | Bensch <i>et al.</i> (2018)         |
| <i>C. vignae</i>  | CBS 121.25          | <i>Vigna unguiculata</i>  | USA                | HM148718   | HM148227                  | HM148473    | Bensch <i>et al.</i> (2010)         |
| <i>C. wenganense</i>                                      | <b>GUCC 21220.1</b> | On leaves of <i>Prunus persica</i>  | China              | OP863101   | OP852682                  | OP859049    | Yang <i>et al.</i> (2023)           |
| <i>C. westerdijkiae</i>                                   | <b>CBS 113746</b>   | Bing cherry fruits  | USA                | HM148548   | HM148061                  | HM148303    | Bensch <i>et al.</i> (2018)         |
| <i>C. xanthochromaticum</i>                               | <b>CBS 140691</b>   | Fatoua villosa  | South Korea        | HM148549   | HM148062                  | HM148304    | Bensch <i>et al.</i> (2018)         |
|   | CPC 10150           | Bronchoalveolar lavage fluid of human                                       | USA                | LN834599   | LN834415                  | LN834511    | Sandoval-Denis <i>et al.</i> (2016) |
|   | CBS 126364          | <i>Erythrophleum chlorostachys</i>  | Australia          | HM148611   | HM148122                  | HM148366    | Sandoval-Denis <i>et al.</i> (2016) |
|   | CPC 11806           | <i>Streptitzia</i> sp.  | South Africa       | HM148618   | HM148129                  | HM148373    | Sandoval-Denis <i>et al.</i> (2016) |
|   | DTO 323-E5          | Indoor air  | China              | MF474171   | MF473321                  | MF473744    | Bensch <i>et al.</i> (2018)         |
|   | COAD 2559           | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Brazil             | OP598120   | OP535348                  | OP676079    | This study                          |
| <i>C. xylophilum</i>                                      | <b>CBS 125997</b>   | <i>Picea abies</i>  | Russia             | HM148721   | HM148230                  | HM148476    | Bensch <i>et al.</i> (2010)         |
| <i>C. yunnanense</i>                                      | KUN HKAS 121704     | On leaves of <i>Paris polystylioides</i>                                    | China              | OL466937   | OK338502                  | OL825680    | Xu <i>et al.</i> (2021)             |
| <b><i>Cladosporium sphaerospermum</i> species complex</b> |                     |   |                    |            |                           |             |                                     |
| <i>C. aciculare</i>                                       | <b>CBS 140488</b>   | <i>Syzygium corynanthum</i>   | Australia          | KT600607   | KT600411                  | KT600509    | Bensch <i>et al.</i> (2015)         |
| <i>C. aphidis</i>   | CBS 132182          | On dead carcasses of aphids   | Germany            | JN906997   | JN906984                  | JN906984    | Bensch <i>et al.</i> 2012           |
| <i>C. austromispshaericum</i>                             | <b>CBS 140482</b>   | <i>Lagunaria patersonia</i> , black mould on fruit surface                  | New Zealand        | KT600578   | KT600382                  | KT600479    | Bensch <i>et al.</i> (2015)         |
|   | DTO 305-E8          | House dust  | New Zealand        | MF473785   | MF472935                  | MF473362    | Bensch <i>et al.</i> (2018)         |
|   | CPC 16250           | <i>Cussonia thyrsiflora</i>   | South Africa       | —          | KT600383                  | KT600480    | Bensch <i>et al.</i> (2015)         |
|   | COAD 3351           | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Ethiopia           | OP598137   | OP535385                  | OP676096    | This study                          |
|   | COAD 3487           | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Ethiopia           | OP598129   | OP535377                  | OP676088    | This study                          |
|   | <b>CBS 143357</b>   | Indoor air sample   | USA                | MF473795   | MF472945                  | MF473372    | Bensch <i>et al.</i> (2018)         |
|   | <b>CBS 137970</b>   | Leaves of <i>Cycas media</i>  | Australia          | KJ869227   | KJ869236                  | KJ869236    | Bensch <i>et al.</i> (2018)         |
|   | <b>CBS 143358</b>   | Indoor air sample   | USA                | MF473805   | MF472955                  | MF473382    | Bensch <i>et al.</i> (2018)         |
|   | <b>CBS 119415</b>   | Hypersaline water, salt lake  | Dominican Republic | EF101368   | DQ780353                  | JN906986    | Bensch <i>et al.</i> (2018)         |
| <i>C. coloradense</i>                                     | <b>CBS 119414</b>   | Hypersaline water, saltern  | Slovenia           | EF101372   | DQ780388                  | JN906988    | Bensch <i>et al.</i> (2018)         |
| <i>C. cycadicola</i>                                      | <b>CBS 119416</b>   | Hypersaline water, salterns   | Namibia            | EF101397   | DQ780364                  | JN906989    | Bensch <i>et al.</i> (2018)         |

Table 1. (Continued).

| Species                          | Strain numbers    | Host / Substrate  | Collection Sites |          | GenBank accession numbers |            | References                          |
|----------------------------------|-------------------|---|------------------|----------|---------------------------|------------|-------------------------------------|
|                                  |                   |   | act              | ITS      | LN834558                  | LN834374   |                                     |
|                                  |                   |   |                  |          |                           |            | tef1                                |
|                                  | UTHSC DI-13-250   | Human, scalp  | USA              |          | LN834558                  | LN834374   | Sandoval-Denis <i>et al.</i> (2015) |
| <i>C. langeronii</i>             | <b>CBS 189.54</b> | Man, mycosis  | Brazil           | EF101357 | DQ780379                  | JN906990   | Bensch <i>et al.</i> (2018)         |
| <i>C. lebrasiæ</i>               | <b>CBS 132823</b> | Milk bread  | France           | KJ596631 | KJ596568                  | KJ596583   | Bensch <i>et al.</i> (2018)         |
| <i>C. longissimum</i>            | <b>CBS 300.96</b> | Soil along coral reef coast   | New Guinea       | EF101385 | DQ780352                  | EU570259   | Bensch <i>et al.</i> (2015)         |
| <i>C. mucilaginosum</i> sp. nov. | COAD 2580         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Ethiopia         | OP535384 | OP676095                  | This study |                                     |
|                                  | <b>COAD 2581</b>  | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Ethiopia         | OP535386 | OP676097                  | This study |                                     |
| <i>C. neolangeronii</i>          | CBS 109868        | Mortar of Muro Farnesiano   | Italy            | EF101362 | DQ780377                  | MF473571   | Bensch <i>et al.</i> (2018)         |
|                                  | <b>CBS 797.97</b> | Indoor environment  | Netherlands      | MF473992 | MF473143                  | —          | Bensch <i>et al.</i> (2018)         |
| <i>C. parahalotolerans</i>       | <b>CBS 139585</b> | Swab sample   | Netherlands      | KP702077 | KP701955                  | KP701832   | Bensch <i>et al.</i> (2018)         |
|                                  | CPC 223336        | Indoor air sample   | USA              | MF474000 | MF473152                  | MF473578   | Bensch <i>et al.</i> (2018)         |
| <i>C. paraspheospermum</i>       | <b>AUMC 10865</b> | Air sample  | Egypt            | OL514008 | MN826828                  | —          | Moharram <i>et al.</i> (2022)       |
| <i>C. penidielloides</i>         | <b>CBS 140489</b> | <i>Acacia verticillata</i>  | Australia        | KT600608 | KT600510                  | KT600510   | Bensch <i>et al.</i> (2015)         |
| <i>C. psychrotolerans</i>        | <b>CBS 119412</b> | Hypersaline water   | Slovenia         | EF101365 | DQ780386                  | JN906992   | Bensch <i>et al.</i> (2018)         |
| <i>C. pulvricola</i>             | DTO 305-G3        | House dust  | Australia        | MF474072 | MF473223                  | MF473645   | Bensch <i>et al.</i> (2018)         |
|                                  | CBS 109788        | Indoor air  | Canada           | MF474074 | MF473225                  | MF473647   | Bensch <i>et al.</i> (2018)         |
|                                  | <b>CBS 143362</b> | House dust  | New Zealand      | MF474075 | MF473226                  | MF473648   | Bensch <i>et al.</i> (2018)         |
| <i>C. saline</i>                 | <b>CBS 119413</b> | Hypersaline water, saltern  | Slovenia         | EF101390 | DQ780374                  | JN906993   | Bensch <i>et al.</i> (2018)         |
| <i>C. sloanii</i>                | <b>CBS 143364</b> | Swab sample   | Netherlands      | MF474103 | MF473253                  | MF473676   | Bensch <i>et al.</i> (2018)         |
| <i>C. sphaerospermum</i>         | <b>CBS 193.54</b> | Man, nails  | Netherlands      | EU570269 | DQ780343                  | EU570261   | Bensch <i>et al.</i> (2018)         |
|                                  | CBS 117728        | Wood  | USA              | EU570275 | AF393709                  | EU570268   | Bensch <i>et al.</i> (2018)         |
|                                  | CBS 109.14        | Leaf of <i>Carya illinoensis</i>  | USA              | EF101384 | DQ780350                  | EU570260   | Bensch <i>et al.</i> (2018)         |
|                                  | UTHSC DI-13-237   | Bronchoalveolar lavage fluid of human                                       | USA              | LN834574 | LN834390                  | LN834486   | Sandoval-Denis <i>et al.</i> (2015) |
|                                  | COAD 2578         | On uredinia of <i>Hemileia vastatrix</i> on leaves of <i>Coffea arabica</i> | Ethiopia         | OP535383 | OP676094                  | This study |                                     |
|                                  | <b>CBS 140466</b> | Dolphin, bronchus   | USA              | LN834618 | LN834434                  | LN834530   | Sandoval-Denis <i>et al.</i> (2016) |
| <i>C. velox</i>                  | <b>CBS 119417</b> | <i>Bambusa</i> sp.  | India            | EF101388 | DQ780361                  | JN906995   | Bensch <i>et al.</i> (2018)         |
| <b>Outgroup</b>                  |                   |   |                  |          |                           |            |                                     |
| <i>C. herbarum</i>               | <b>CBS 121621</b> | <i>Hordeum vulgare</i>  | Netherlands      | EF679516 | EF679440                  | EF679440   | Bensch <i>et al.</i> (2018)         |
|                                  | CBS 300.49        | <i>Biscutella laevigata</i>   | Switzerland      | EF679511 | EF679358                  | EF679434   | Bensch <i>et al.</i> (2018)         |

***Cladosporium chlamydosporiformans*** C.M. Pereira & R.W. Barreto, **sp. nov.** MycoBank MB 850456. Fig. 2.

**Etymology:** In reference to the abundant chlamydospores produced by this species in pure culture.

**Typus:** Kenya, Eastern Province, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 28 Jan. 2015, H.C. Evans (**holotype** VIC 47529, a dried metabolically inactive culture, culture ex-type COAD 2571).

**GenBank:** ITS = OP535374; act = OP598126; tef1 = OP676085.

**Mycelium** moderately abundant, hyphae sparingly branched, 3–6.5 µm wide, septate, without swelling, but with some constrictions at septa; light brown to pale brown, smooth to slightly verruculose, with somewhat thickened walls and sometimes covered by a mucilaginous layer. **Conidiophores** macronematous, solitary, 34.5–160 × 2.5–7 µm, arising terminal or laterally from hyphae, erect, occasionally sinuous, subcylindrical attenuated towards the apex, with a swollen bulbous base, dark brown at the base becoming progressively and distinctly lighter towards the light greyish-brown apex, unbranched but occasionally forming branches near the base, 2–7-septate, smooth, thick-walled, micronematous, lateral, 10.5–45 × 2.5–6 µm, cylindrical, subhyaline to pale brown, 1–2 apical loci, 1–3-septate. **Conidiogenous cells** integrated, terminal, subcylindrical, 1–5 sympodial proliferations, 11.5–56.5 × 2.5–6.5 µm, with 1–5 loci at the apex, conidiogenous loci protuberant, truncated, 0.5–2 µm diam, thickened and darkened-refractive. **Ramoconidia** cylindrical, 9–50.5 × 2–5.5 µm, 0–1-septate, with truncate base, brown, smooth, thin-walled. **Secondary ramoconidia** ellipsoid to oblong, 2–3 coronate hila, 4–10.5 × 2.5–5 µm, aseptate, brown, truncate base, smooth, thin-walled. **Conidia** abundant, in acropetal and branched chains, aseptate, light brown to brown, smooth, thin-walled; **intercalary conidia** limoniform, oval to ellipsoid, 5.5–12.5 × 2.5–5 µm, with hila sometimes protuberant and coronate; **terminal conidia** globose to ellipsoid or apiculate, 3–6 × 2–3.5 µm, hila not evident. **Chlamydospores** abundantly produced in hyphae, intercalary or terminally, forming clusters, occasionally solitary, brown to dark brown, thick-walled, globose to sub-globose, 6.5–9.5 µm diam.

**Culture characteristics:** Colonies on MEA up to 50 mm diam, flat and radially furrowed, margins undulate, aerial mycelium velvety, olivaceous black and grey olivaceous towards margins, reverse similar to surface. Colonies on OA up to 29 mm diam, flat and radially furrowed, with small central tufts, margins fimbriate, aerial mycelium somewhat felty, olivaceous black to grey olivaceous, reverse olivaceous black. Colonies on PDA up to 35 mm diam, flat, grainy at margin and centre, margins narrow, undulate, aerial mycelium granular to floccose, grey olivaceous to dull green towards the white edge, reverse olivaceous black. Colonies on SNA up to 40 mm diam, flat and radially furrowed, centrally tufted, margins wide, discretely undulate, aerial mycelium powdery, greenish olivaceous changing to dark herbage green to yellow-green towards the wide white margin, reverse olivaceous to greenish olivaceous. Sporulation moderate on MEA, and abundant on OA, PDA, and SNA.

**Additional material examined:** Brazil, Minas Gerais, Itutinga, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, Sep. 2015, A.A. Colmán (culture COAD 2561); Minas Gerais, Lambari, on uredinia

of *Hemileia vastatrix* from leaves of *Coffea arabica*, 19 Jun. 2015, A.A. Colmán (culture COAD 2570). Ethiopia, Oromia Region, Jimma, University of Jimma farm, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 30 May 2017, K.B. Bekele (culture COAD 2568).

**Notes:** *Cladosporium chlamydosporiformans* belongs to the *C. cladosporioides* species complex and clusters as a sister clade to *C. eucommiae*, a species isolated from leaves of *Eucommia ulmoides* in China. Some morphological differences between the two species are striking, such as the presence of constrictions at septa, the occurrence of primary ramoconidia and chlamydospores in *C. chlamydosporiformans* – seemingly absent from *C. eucommiae* (Wang et al. 2022). Other differences between the two species include wider conidiophores in *C. chlamydosporiformans* (up to 7 µm vs. up to 4.5 µm in *C. eucommiae*) and *C. chlamydosporiformans* having shorter secondary ramoconidia (4–10.5 µm) compared to *C. eucommiae* (5–25 µm). Whether or not *C. chlamydosporiformans* can be considered as a specialized mycoparasite of CLR is unknown, at present, but it is possible that it arrived in Brazil as a contaminant from its African centre of origin.

***Cladosporium guizhouense*** S.Y. Wang et al., MycoKeys 91: 160. 2022. MycoBank MB 842407.

**Material examined:** Ethiopia, Oromia Region, Illubabor Gore, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 30 Dec. 2017, K.B. Bekele (culture COAD 3471).

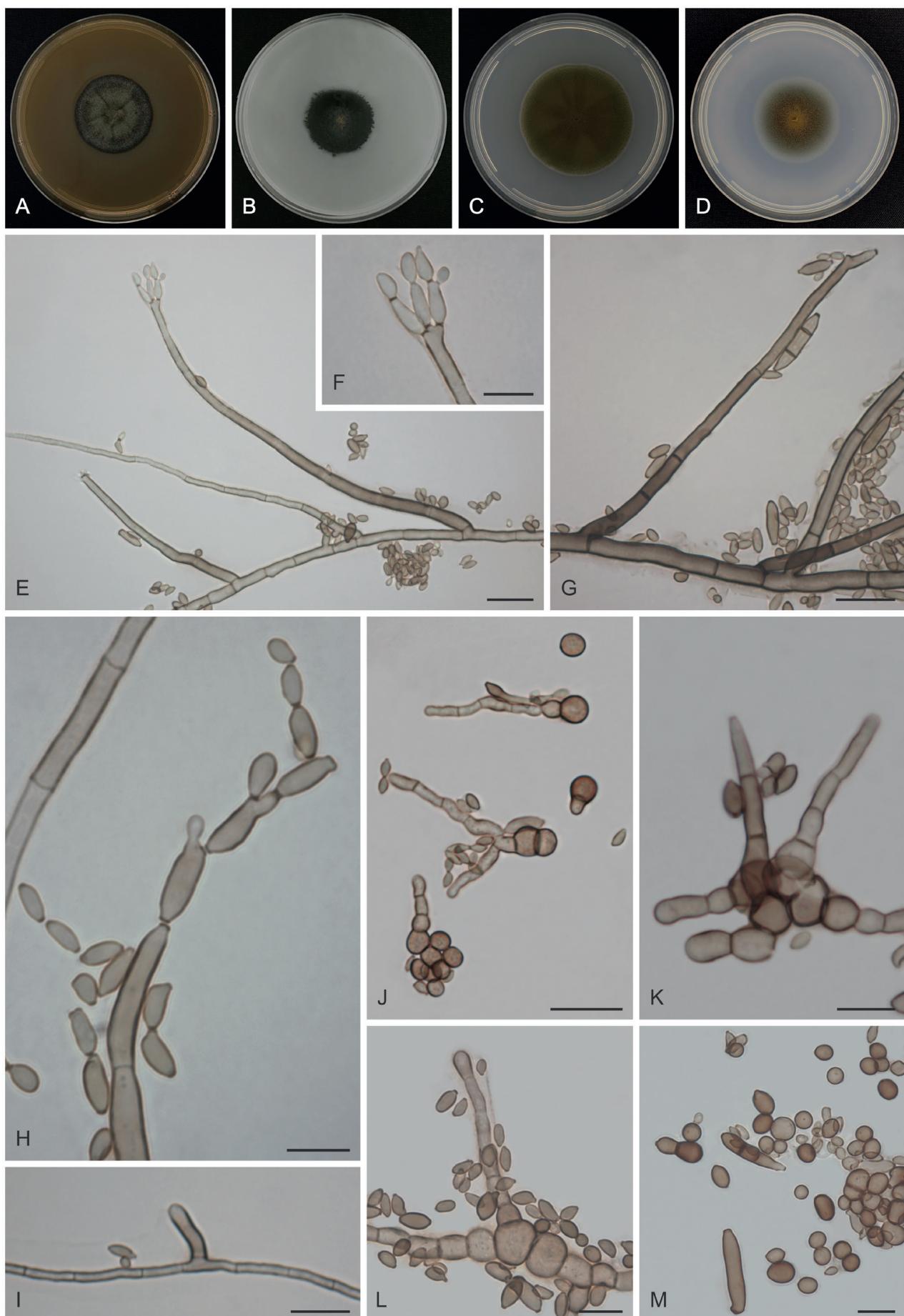
For a detailed description and illustrations, see Wang et al. (2022).

**Notes:** Only one, among all the *Cladosporium* isolates found on CLR during this study, clustered within the *C. guizhouense* clade and it has a similar morphology to that described by Wang et al. (2022). However, isolate COAD 3471 has significantly larger conidiophores (81.5–277 × 3.5–5.5 µm vs. 13–100 × 3–4.5 µm) and smaller secondary ramoconidia (5–13.5 × 2.5–4.5 µm vs. 6.5–23 × 3–5.5 µm). Like *C. eucommiae*, this species was originally described and isolated from leaves of *Eucommia ulmoides* in China (Wang et al. 2022). Subsequently, it was also isolated from leaves of *Prunus pseudocerasus* (Rosaceae) and *Ribes burejense* (Grossulariaceae) (Yang et al. 2023), in China. Recently, two isolates of *C. guizhouense* were obtained from uredinia of *A. psidii* on leaves of *P. guajava* and *Syzygium jambos* in Brazil (Silva et al. 2023). *Cladosporium guizhouense* would seem, therefore, to have a pantropical geographical distribution with a broad host range. This is the first report of *C. guizhouense* from Africa and from uredinia of *H. vastatrix*. It is possible that this species is a generalist mycoparasite of rust fungi, based on the records from Brazil and Ethiopia, and may be a contaminant on fruit trees in China.

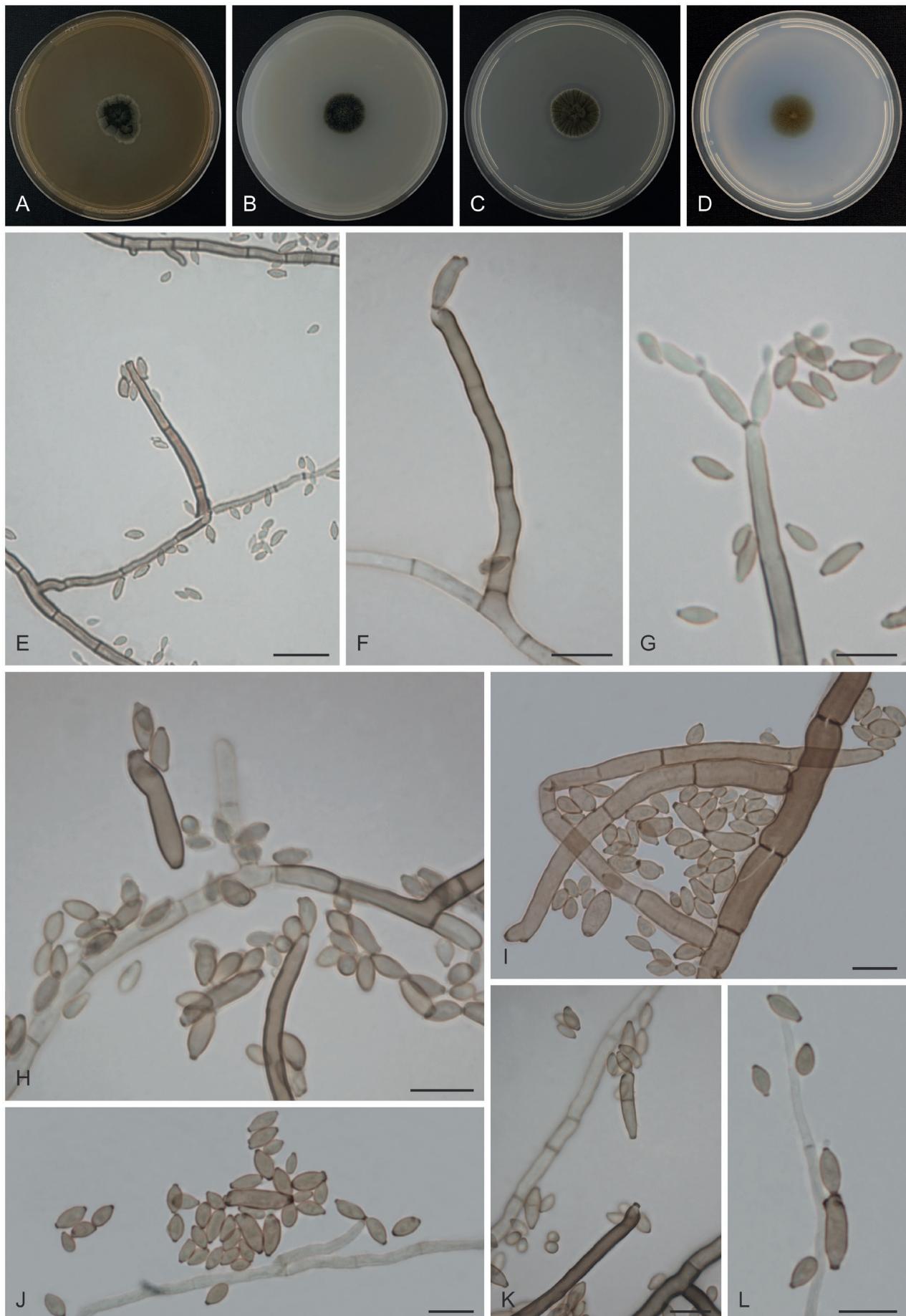
***Cladosporium hemileiicola*** C.M. Pereira & R.W. Barreto, **sp. nov.** MycoBank MB 850457. Fig. 3.

**Etymology:** Based on its host substrate, uredinia of *Hemileia vastatrix*.

**Typus:** Brazil, Minas Gerais, São Sebastião da Vitória, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, Sep. 2015, A.A. Colmán (**holotype** VIC 47526, dried metabolically inactive culture, ex-type culture COAD 2567).



**Fig. 2.** *Cladosporium chlamydosporiformans* sp. nov. (COAD 2571). **A.** Colony on MEA. **B.** Colony on OA. **C.** Colony on PDA. **D.** Colony on SNA. **E, G.** Conidiophores. **F, H.** Conidiogenous cells with conidia. **I.** Micronematous conidiophore. **J.** Chlamydospores, note chlamydospores germinating and originating from conidiophores. **K, L.** Conidiophores with bulbous base arising from chlamydospores. **M.** Chlamydospores and conidia. Scale bars: E, G, I, J = 20 µm; F, H, K–M = 10 µm.



**Fig. 3.** *Cladosporium hemileiicola* sp. nov. (COAD 2567). **A.** Colony on MEA. **B.** Colony on OA. **C.** Colony on PDA. **D.** Colony on SNA. **E–I.** Conidiophores. **G.** Conidiogenous cells with conidia. **H.** Primary ramoconidium, micronematous conidiophore and conidia. **J–L.** Conidia. Scale bars: E = 20 µm; F–L = 10 µm.



**Fig. 4.** *Cladosporium mucilaginosum* sp. nov. (COAD 2581). Arrows indicate the mucilaginous layer covering fungal structures. **A.** Colony on MEA. **B.** Colony on OA. **C.** Colony on PDA. **D.** Colony on SNA. **E, F.** Conidiophores. **G, I.** Conidiogenous cells with conidia. **H, J.** Conidiophores arising laterally from hyphae and covered by mucilaginous layer. **K.** Intercalary conidium attached to a primary ramoconidium. **L.** Conidia produced in chains. Scale bars: E = 20 µm; F–L = 10 µm.

GenBank: ITS = OP535376; act = OP598128; tef1 = OP676087.

**Mycelium** sparse, branched, 2–4.5 µm wide, septate, without swellings or constrictions, subhyaline to light brown, smooth, thin-walled. **Conidiophores** macronematous, solitary, 24.5–258 × 3–6 µm, arising terminal or laterally from hyphae, erect, sometimes sinuous, cylindrical attenuated towards the apex, subhyaline, pale brown to brown olivaceous, unbranched, 1–9-septate, smooth, thick-walled, micronematous, sparse, almost distinguishable from hyphae, lateral, 6.5–88.5 × 2–5 µm, subcylindrical, sometimes reduced to conidiogenous cell, subhyaline to light brown, one apical locus, up to 5-septate, smooth, thick-walled. **Conidiogenous cells** integrated, terminal, in micronematous conidiophores, cylindrical, 8.5–68.5 × 2–5 µm, with 1–5 loci at the apex, conidiogenous loci protuberant, convex to slightly truncated, 1.5–2.5 µm diam, sometimes thickened and darkened-refractive. **Ramoconidia** cylindrical, straight to slightly curved or distorted, 7–25.5 × 2.5–5.5 µm, aseptate to occasionally 1-septate, pale brown, truncate base, smooth, thin-walled. **Secondary ramoconidia** ellipsoid to oblong, truncate at base, 2–3 prominent distal coronate hila, 4.5–13.5 × 2.5–5 µm, aseptate, pale brown, smooth, thin-walled. **Conidia** numerous, catenulate, in acropetal and branched chains, aseptate or rarely 1-septate, pale brown, smooth, thin-walled; **intercalary conidia** limoniform to ellipsoid, 2.5–4.5 × 0.5–2 µm, with hila protuberant coronate and dark-refractive; **terminal conidia** globose to apiculate, 3.5–7 × 2.5–4.5 µm, aseptate, hila sometimes evident, coronate and darkened-refractive.

**Culture characteristics:** Colonies on MEA up to 21 mm diam, umbonate, with few furrows, margins undulate, aerial mycelium felty to silky, centre olivaceous grey becoming greenish olivaceous with a small dull green area and white margin, reverse olivaceous black. Colonies on OA up to 19 mm diam, umbonate and radially furrowed, bearing mycelial tufts centrally, margins entire, aerial mycelium scarce, felty, olivaceous black to grey olivaceous, reverse similar to surface. Colonies on PDA up to 22 mm diam, slightly umbonate, radially furrowed and folded, margins narrow, undulate, aerial mycelium velvety, with a central tuft, grey olivaceous to dull green towards the edge with white margins, reverse olivaceous black. Colonies on SNA up to 21 mm diam, flat and radially furrowed, margins thin, entire, aerial mycelium sparse, powdery, tufted centrally, grey olivaceous to greenish olivaceous centrally, white periphery, reverse similar to surface. Sporulation abundant on MEA, OA and SNA, moderate on PDA.

**Additional material examined:** Ethiopia, SNNPR Sheka Zone Gamadiro Coffee Farm, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 3 Jan. 2018, K.B. Belachew (culture COAD 3350).

**Notes:** Although only two isolates of *C. hemileiicola* were obtained during this study, these records are from Brazil and Ethiopia and suggest that this is a widespread species that may have been overlooked during the surveys. *Cladosporium hemileiicola* belongs to the *C. cladosporioides* species complex and forms a basal branch within this complex, between *C. myrtacearum* and *C. scabrellum*. Those two species were isolated originally from symptomatic leaves: *C. myrtacearum* from leaf spots on *Corymbia polycarpa* (Myrtaceae) (Braun et al. 2005) and *C. scabrellum* from wilted leaves of *Ruscus hypoglossum* (Asparagaceae) (Bensch et al. 2010). *Cladosporium hemileiicola*

has significantly longer conidiophores (24.5–258 µm) and conidiogenous cells (8.5–68.5 µm) than those described for *C. myrtacearum* (conidiophores 9–85(–120) µm, conidiogenous cells 12–45 µm) (Braun et al. 2005) and for *C. scabrellum* (conidiophores 40–115(–185) µm, conidiogenous cells 25–53 µm) (Bensch et al. 2010).

***Cladosporium mucilaginosum* C.M. Pereira & R.W. Barreto, sp. nov.** MycoBank MB 850458. Fig. 4.

**Etymology:** In reference to the thin mucilaginous layer covering the mycelium in culture.

**Typus:** Ethiopia, SNNPR Kafa Zone Bita-Chaga kebele, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 5 Jan. 2018, K.B. Belachew (**holotype** VIC 47531, a dried metabolically inactive culture, culture ex-type COAD 2581).

GenBank: ITS = OP535386; act = OP598138; tef1 = OP676097.

**Mycelium** diffuse, sparingly branched, 4–6.5 µm wide, septate, often thickened and pigmented (black) at septa, with swellings and constrictions at septa, thick-walled, covered by a mucilaginous layer, light brown to brown olivaceous, slightly verruculose when young, becoming rough or smooth-walled. **Conidiophores** macronematous, solitary, 14.5–67.5 × 2.5–5 µm, arising terminally or laterally from hyphae, erect, occasionally sinuous, cylindrical, light brown to brown, occasionally branched, 2–6-septate, often thickened and pigmented (black) at septa, sometimes with swellings and constriction at the base, smooth, thick-walled; micronematous uncommon and almost indistinguishable from hyphae, lateral, 5–33.5 × 3–5.5 µm, conical to slightly cylindrical, subhyaline to light brown, with a single locus, 1–3-septate. **Conidiogenous cells** integrated, terminal, subcylindrical, occasionally with sympodial proliferations, 10–24 × 2.5–4 µm, with 1–3 loci at the apex, conidiogenous loci truncated, 0.5–1.5 µm diam, thickened and darkened-refractive. **Ramoconidia** cylindrical to clavate, 5.5–27.5 × 2.5–4 µm, 0–2-septate, often thickened and pigmented (black) at septa, light brown to pale brown, apex slightly convex, truncate base, smooth, thick-walled. **Secondary ramoconidia** cylindrical, ellipsoid to oblong, truncate at base, 2–3 discrete coronate hila, 4.5–13 × 2.5–5 µm, 0–1-septate, light brown to brown, apex obtuse, smooth, thin-walled. **Conidia** numerous, catenulate, in acropetal and branched chains, aseptate, occasionally 1-septate, light brown to pale brown, smooth, thin-walled; **intercalary conidia** globose to ellipsoid or fusiform, 4–10 × 2.5–4 µm, with hila coronate and dark refractive; **terminal conidia** subglobose, globose to apiculate, 3–6.5 × 2.5–4.5 µm, aseptate, hila coronate and darkened-refractive.

**Culture characteristics:** Colonies on MEA, OA, PDA, and SNA with limited growth, reaching only 19, 14, 12 and 12 mm diam, respectively after 14 d. On MEA, raised, corrugated, margins narrow, undulate, aerial mycelium felty, grey olivaceous to dark herbage green, white narrow periphery, reverse olivaceous black. On OA, umbonate, bearing central mycelium tufts, margin entire to slightly undulate, aerial mycelium felty, grey olivaceous centrally, becoming olivaceous black, margins narrow, white, reverse olivaceous black. On PDA, umbonate, elevated centrally and corrugated, radially furrowed, rugose, margins lobate, aerial mycelium velvety to felty, grey olivaceous with dark herbage

green periphery, narrow white margins, reverse olivaceous black. On SNA, flat, with mycelium aggregates at the centre, margins slightly undulate to entire, aerial mycelium powdery, grey olivaceous with an area in dull green and white margins, reverse olivaceous black. Sporulation abundant on MEA, OA and PDA, moderate on SNA.

**Additional material examined:** Ethiopia, SNNPR Kafa Zone Gimbo-Yeyebito kebele, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 5 Jan. 2018, K.B. Belachew (culture COAD 2580).

**Notes:** *Cladosporium mucilaginosum* belongs to the *C. sphaerospermum* species complex and is phylogenetically related to *C. langeronii* and *C. psychrotolerans* but forms a separate and well-supported clade (Fig. 1). In *C. mucilaginosum*, its mycelium is coated by a mucilaginous layer, similar to *C. langeronii* and *C. psychrotolerans* (Zalar et al. 2007, Bensch et al. 2018). This may be a common feature for *Cladosporium* species in this clade. However, some morphological features of *C. mucilaginosum* are clearly distinct from its closer relatives, namely: the presence of swellings and constrictions at the septa – absent in *C. langeronii* and *C. psychrotolerans* (Zalar et al. 2007, Bensch et al. 2018). Also, the biometric differences are significant, such as *C. mucilaginosum* having shorter conidiophores (14.5–67.5 µm) than those described for *C. langeronii* ((20–)50–235(–470) µm) (Bensch et al. 2018) and for *C. psychrotolerans* (20–220 µm) (Zalar et al. 2007). This species is known only from Ethiopia and was not recorded during the surveys in Cameroon and Brazil.

***Cladosporium pseudotenuissimum*** P.P. Costa et al., *Phytotaxa* 560: 21. 2022. MycoBank MB 840952.

**Material examined:** Brazil, Minas Gerais, Varginha, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 8 Jul. 2015, A.A. Colmán (culture COAD 2558).

For a detailed description and illustrations, see Costa et al. (2022).

**Notes:** This isolate grouped within the *C. pseudotenuissimum* clade (Fig. 1) and is morphologically similar to *C. pseudotenuissimum* as described by Costa et al. (2022). Some morphological differences were noted, however: COAD 2558 has longer conidiophores, conidiogenous cells and conidia than those in the original description (conidiophores 109.5–453 µm vs. 43.3–299.4 µm; conidiogenous cells up to 70 µm long vs. up to 51 µm; conidia up to 15 µm vs. up to 6.9 µm). Conversely, ramoconidia of COAD 2558 are significantly smaller (8–17 × 2–4 µm) than those described by Costa et al. (2022) (33.3–53.2 × 4.5–5.1 µm). *Cladosporium pseudotenuissimum* was proposed as a new species close to but distinct from *C. tenuissimum* (Costa et al. 2022). It was originally isolated from decayed leaves of *Chusquea anelytroides* (Poaceae) and *C. urelytra* in the coffee-growing area of Araponga, (Minas Gerais, Brazil). Although still restricted in its known distribution to Minas Gerais, it appears to have – as for other species of *Cladosporium* – considerable plasticity in terms of niche and habitats.

***Cladosporium puris*** M.L.R. Freitas & O.L. Pereira, *Phytotaxa* 482: 232. 2021. MycoBank MB 826908.

**Materials examined:** Brazil, Minas Gerais, Florestal, campus of the Universidade Federal de Viçosa, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 19 Jun. 2015, A.A. Colmán (cultures COAD 2566 and COAD 2572).

For a detailed description and illustrations, see Freitas et al. (2021).

**Notes:** Both isolates (COAD 2566 and COAD 2572) were obtained in Minas Gerais and as for *C. pseudotenuissimum*, *C. puris* was also isolated from the same region on submerged litter in a stream showing a similar habitat and substrate plasticity to *C. pseudotenuissimum*. In general, the morphological features of COAD 2566 and COAD 2572 match those described for *C. puris* (Freitas et al. 2021).

***Cladosporium ribis*** Y.Q. Yang & Yong Wang bis (as 'ribus'), *J. Fungi* 9(no. 250): 13. 2023. MycoBank MB 662567.

**Material examined:** Brazil, Minas Gerais, Três Corações, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 4 Jul. 2015, A.A. Colmán (COAD 2550).

For a detailed description and illustrations, see Yang et al. (2023).

**Notes:** *Cladosporium ribis* was isolated originally from leaves of *Ribes burejense* and *Prunus pseudocerasus* in China (Yang et al. 2023) and, therefore, as for *C. pseudotenuissimum* and *C. puris*, *C. ribis* appears to have significant ecological plasticity and adapts to a diverse range of ecological niches and habitats with a wide geographical range. Morphologically, COAD 2550 fits the description of Yang et al. (2023), although the conidiophores and secondary ramoconidia are longer (up to 209 µm and 13.5 µm respectively) than in the type (up to 103.5 µm and 8.5 µm, respectively).

***Cladosporium setoides*** C.M. Pereira & R.W. Barreto, *sp. nov.* MycoBank MB 850459. Fig. 5.

**Etymology:** Based on the shape of the young macronematous conidiophores, which are long and setae-like.

**Typus:** Ethiopia, Oromia Region, Jimma Zone Gera Agri Center, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 25 Dec. 2017, K.B. Belachew (**holotype** VIC 49376, a dried metabolically inactive culture, culture ex-type COAD 3470).

**GenBank:** ITS = OP535379; act = OP598131; tef1 = OP676090.

**Mycelium** dense, branched, 3–6 µm wide, septate, without swellings, with some constrictions at septa, subhyaline to greyish, smooth to slightly rough, thin-walled. **Conidiophores** macronematous, solitary, 22.5–326 × 2.5–5.5 µm, arising terminal or laterally from hyphae, erect, rarely sinuous, cylindrical attenuating towards the apex, greyish, unbranched, 2–12 septate, smooth, rough-walled especially towards the base; micronematous almost distinguishable from hyphae, often reduced to conidiogenous cells, lateral, 10–19 × 4–6 µm, cylindrical to subcylindrical, subhyaline to greyish, with an apical locus, 0–1-septate, smooth, thick-walled. **Conidiogenous cells** integrated, terminal, cylindrical, obtuse to subtruncate, proliferation sympodial, 12–81.5 × 2–5 µm, with 1–5 coronate



**Fig. 5.** *Cladosporium setoides* sp. nov. (COAD 3470). **A.** Colony on MEA. **B.** Colony on OA. **C.** Colony on PDA. **D.** Colony on SNA. **E.** Long and seta-like macronematous young conidiophores. **F, G.** Conidiogenous cells with conidia. **H.** Conidia abundantly produced. **I.** Micronematous conidiophore and conidia attached to conidiogenous loci. **J, K.** Ramoconidia and conidia. Scale bars: E, F, H, I = 20 µm; G, J, K = 10 µm.

and protuberant loci at the apex, 1.5–3 µm diam, thickened and darkened. *Ramoconidia* cylindrical, 12–30 × 2.5–4 µm, 0–1-septate (mostly aseptate), greyish, subtruncate base and apex, smooth, thin-walled. *Secondary ramoconidia* ellipsoid-ovoid to oblong, obconic to subtruncate ending in 2–3 prominent distal coronate hila, 5.5–14.5 × 2.5–4 µm, aseptate, greyish, smooth, thin-walled. *Conidia* numerous, catenulate, in acropetal and branched chains, aseptate, greyish, smooth, thin-walled; *intercalary conidia* cylindrical to ellipsoid, 4.5–16 × 2–3.5 µm, hila coronate, protuberant and darkened-refractive; *terminal conidia* globose to apiculate, 2.5–6.5 × 2–4 µm, hila, coronate, somewhat protuberant and darkened-refractive.

**Culture characteristics:** Colonies on MEA up to 32 mm diam, convex, margins wide, slightly undulate, aerial mycelium cottony-fluffy, smoke grey to olivaceous grey becoming dark herbage green towards the white margin, reverse olivaceous black with white edges. Colonies on OA up to 43 mm, flat and radially furrowed, bearing mycelial tufts dispersed throughout the colony, margins entire, aerial mycelium felty, olivaceous black to greenish glaucous, with white edges, reverse grey olivaceous. Colonies on PDA up to 28 mm diam, convex, radially furrowed, periphery slightly crenate, aerial mycelium woolly-fluffy to felty, olivaceous grey centrally, becoming smoke grey to glaucous grey towards the edge, narrow white margins, reverse olivaceous black. Colonies on SNA up to 40 mm diam, flat with a convex centre, margins entire to slightly undulate, aerial mycelium sparse, powdery, with a small central tuft, yellow layer subadjacent to a layer of white mycelium at the centre, becoming olivaceous buff, reverse greenish glaucous to yellow towards the edge. Sporulation moderate on MEA, PDA and SNA, rare on OA.

**Additional material examined:** Ethiopia, Oromia Region, Illubabor Metu Agri Research Center, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 28 Dec. 2017, K.B. Belachew (culture COAD 2576).

**Notes:** *Cladosporium setoides* belongs to the *C. cladosporioides* species complex and forms a sister clade to a larger group containing *C. anthropophilum*, *C. compactisporum*, and *C. devikae*. This clade contains species obtained from an eclectic range of hosts / substrates and localities: *C. anthropophilum* is a human opportunistic pathogen isolated from human bronchoalveolar lavage fluid in the USA (Sandoval-Denis *et al.* 2016); *C. compactisporum* was isolated from air samples in Egypt (Moharram *et al.* 2022), and *C. devikae* is implicated as a plant pathogen since it was isolated from flower blight of *Macadamia integrifolia* in Australia (Prasannath *et al.* 2021). Compared to the aforementioned species, *C. setoides* has shorter conidiophores (22.5–326 µm) than *C. anthropophilum* and *C. devikae* (up to 550 µm and 200–700 µm, respectively) (Sandoval-Denis *et al.* 2016, Prasannath *et al.* 2021), but is similar in size to *C. compactisporum* (Moharram *et al.* 2022). In addition, the conidia are larger in *C. setoides* (4.5–16 µm long) compared to those of the other three species (4.5–11 µm long in *C. anthropophilum*, 4–6 µm long in *C. compactisporum*, and 3.5–7 µm long in *C. devikae*) (Sandoval-Denis *et al.* 2016, Prasannath *et al.* 2021, Moharram *et al.* 2022).

**Cladosporium sphaerospermum** Penzig, *Michelia* 2: 473. 1882.  
MycoBank MB 119529.

**Material examined:** Ethiopia, Oromia Region, Illubabor Gore, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, 30 Dec. 2017, K.B. Bekele (culture COAD 2578).

For a detailed description and illustrations, see Zalar *et al.* (2007) and Bensch *et al.* (2018).

**Notes:** The Ethiopian isolate (COAD 2578) matches the description in Bensch *et al.* (2018) for *C. sphaerospermum*, although both the conidiogenous cells are longer in COAD 2578 (8.5–27 µm vs. 6–18 µm) and the secondary ramoconidia are shorter (6.5–11.5 µm vs. 8–24(–38) µm). Originally, this species was isolated from *Citrus* in Jakarta (as Batavia) Indonesia (Penzig, 1882), and has a cosmopolitan distribution on a broad diversity of substrates (Bensch *et al.* 2018). In an assay with detached leaves, Srivastava *et al.* (1985) documented that an isolate of *C. sphaerospermum* could penetrate and parasitize teliospores of four microcyclic rusts, namely: *Puccinia horiana*, *P. dianthi*, *P. malvacearum* and *P. glomerata*. There are no previous reports of its association with CLR.

**Cladosporium xanthochromaticum** Sandoval-Denis *et al.* (as 'xanthochromaticum'), *Persoonia* 36: 295. 2016. MycoBank MB 817340.

**Material examined:** Brazil, Minas Gerais, São Sebastião da Vitória, on uredinia of *Hemileia vastatrix* on leaves of *Coffea arabica*, Sep. 2015, A.A. Colmán (culture COAD 2559).

For a detailed description and illustrations, see Sandoval-Denis *et al.* (2016).

**Notes:** The Brazilian isolate (COAD 2559), grouped within the *C. xanthochromaticum* clade and its morphology is very similar to that described by Sandoval-Denis *et al.* (2016); including the production of a yellow diffusible pigment on PDA. *Cladosporium xanthochromaticum* was originally isolated from human bronchoalveolar lavage fluid (Sandoval-Denis *et al.* 2016). Subsequently, the fungus was isolated from air and soil samples, as well as from plants; including *Eucalyptus* and *Musa* in India, and *Strelitzia* and *Triticum aestivum* in South Africa (Bensch *et al.* 2018). This appears to be a new record for Brazil and for South America, in general, and the first report of *C. xanthochromaticum* as a mycoparasite of rust fungi.

## DISCUSSION

The genus *Cladosporium*, originally established by Link (1816) and recently classified in the family *Cladosporiaceae*, order *Cladosporiales* in the *Dothideomycetes* (Abdollahzadeh *et al.* 2020), is one of the largest and most heterogeneous genera of hyphomycetes (Crous *et al.* 2007, 2019). The genus is divided in three species complexes, namely: *Cladosporium cladosporioides*, *C. herbarum*, and *C. sphaerospermum* (Bensch *et al.* 2018). The largest is the *C. cladosporioides* complex which includes more than half of the known species (Costa *et al.* 2022).

The genus encompasses over 230 species (Iturrieta-González *et al.* 2021) that are widespread and able to colonize a broad diversity of substrates. *Cladosporium* has been commonly isolated from the air and from soil samples, but the majority of species are considered to be saprobes (Bensch *et al.* 2018,

Pereira et al. 2022). Nevertheless, some species have also been reported as human and animal pathogens, endophytes and phytopathogens, as well as occupying fungicolous niches (Heuchert et al. 2005, Sandoval-Denis et al. 2015, Prasannath et al. 2021, Chen et al. 2022).

There are various reports of fungi, and even of Oomycetes, serving as substrates for *Cladosporium* species: such as, the oomycetes *Peronospora arborescens* and *P. gaeumannii* – hosts of *C. uredinicola* (Heuchert et al. 2005); the gall-causing fungus, *Taphrina pruni* – colonized by *C. delicatulum* (Baharvandi & Zafari 2005) and *C. phyllophilum* (Heuchert et al. 2005); and, the powdery mildew *Phyllactinia guttata* – host of *C. phyllactiniicola* and *C. uredinicola* (Dugan & Glawe 2006, Bensch et al. 2010).

The rust fungi (*Pucciniales*) are parasitized by several species of *Cladosporium*, including: *C. cladosporioides*, *C. pseudocladosporioides*, *C. sphaerospermum* and *C. uredinicola* which have all been reported on *Puccinia horiana* (Srivastava et al. 1985, Torres et al. 2017); *C. tenuissimum* on *Cronartium flaccidum*, *C. pini*, *Melampsora laricis-populina*, and *Uromyces appendiculatus* (Sharma & Heather 1988, Moricca et al. 2001, Assante et al. 2004); *C. cladosporioides* on *Puccinia striiformis f. sp. tritici* (Zhang et al. 2022); and, *C. angulosum*, *C. anthropophilum*, *C. bambusicola*, *C. benschiae*, *C. guizhouense* and *C. macadamiae* on *Austropuccinia psidii* (Silva et al. 2023). Nevertheless, *Cladosporium* and mycoparasites of rusts, in general, have largely been neglected – particularly, in the context of their potential for management of plant diseases, even for those of major economic importance, such as *Hemileia vastatrix*. Here, and in the related series of papers (Crous et al. 2018, Colmán et al. 2021, Guterres et al. 2021, Rodríguez et al. 2021, Salcedo-Sarmiento et al. 2021, Kapeua-Ndacnou et al. 2023a, b), we have attempted to redress the situation.

The present study has revealed a wealth of *Cladosporium* species associated with uredinia of *H. vastatrix* in both Africa and Brazil, and amongst the 12 species identified, four are new to science: *C. chlamydosporiformans*, *C. hemileiicola*, *C. setoides* and *C. mucilaginosum*. In Silva et al. (2023), a limited survey in Brazil yielded six species of *Cladosporium* on pustules of *Austropuccinia psidii*. None of these had previously been reported from this rust. Two of these species – *C. bambusicola* and *C. ghuizouense* – were also found on *H. vastatrix* during the present surveys.

In the genus *Cladosporium*, species identification and delimitation based solely on morphological features is an arduous task since many species have overlapping characteristics (Marin-Felix et al. 2017). Thus, a polyphasic approach has become a critical and mandatory requirement for identification. Although the ITS region has been recognized as a universal DNA fungal barcode marker (Schoch et al. 2012), for *Cladosporium* this region has limited resolution for differentiating species, especially within the same complex. Therefore, complementary regions are necessary and the protein-encoding genes *ACT* and *TEF1* are needed for species delimitation (Marin-Felix et al. 2017). Particularly for our dataset, the partial *ACT* gene proved to be a very useful region for obtaining trees with higher resolutions.

In the earlier literature, a report was made of a cladosporioid fungus, initially named *C. hemileiicola*, occurring as a parasite of *H. vastatrix* in central Africa (Steyaert et al. 1930). Later, in an extensive monograph on *Cladosporium*, using light and scanning electron microscopy, Heuchert et al. (2005) re-examined all those species considered to be mycoparasitic or

fungicolous. *Cladosporium hemileiicola* was recognized as having morphological differences from true *Cladosporium* species and the new genus *Digitopodium* was proposed to accommodate this fungus. Later, Colmán et al. (2021) also found *D. tectonae* on uredinia of *H. vastatrix* on *C. arabica* in Ethiopia. Although morphologically similar to *Cladosporium*, *Digitopodium* is phylogenetically distinct. Colmán et al. (2021) concluded, based on circumstantial evidence, that *Digitopodium* is a specialist genus adapted to a mycoparasitic lifestyle on rust fungi. In a recent paper, however, a new species *Digitopodium citri* has been reported as an endophyte in healthy citrus fruit in China, with no indication that it is associated with a rust host (Jie-Rong et al. 2023). During our surveys, in addition to isolating directly from the uredinia of CLR, samples of healthy *Coffea* tissues (stem, leaf, fruit) were collected for endophyte isolation – as described in Rodríguez et al. (2021) – but no endophytic isolates of *Cladosporium* were obtained. Isolates of *Cladosporium*, however, have been reported to be endophytic, as for example *C. tenuissimum* (Chen et al. 2022) and *C. cladosporioides* (Wang et al. 2013) in China; both producing secondary metabolites of potential use in biological control.

Preliminary *in vitro* assays (Pereira 2021) have demonstrated that some of the isolates – notably, COAD 2558 (*C. pseudotenuissimum*) and COAD 2565 (*C. bambusicola*) – are capable of inhibiting the germination of urediniospores of *H. vastatrix*. However, these results need to be expanded upon and replicated in greenhouse and field trials before the mycoparasitic status of all the *Cladosporium* isolates can be confirmed and the mechanisms involved can be elucidated. Such studies have been published for other *Cladosporium*/rust associations, namely: *C. gallicola* on western gall rust (*Endocronartium harknessii*) (Tsuneda & Hiratsuka 1979); *C. tenuissimum* on rust fungi on a range of host plants, including pine and willow (Moricca et al. 2001, Assante et al. 2004, Moricca et al. 2005); and, *C. oxysporum* on poplar rust, *Melampsora medusae f. sp. deltoidae* (Tyagi et al. 2023), with hyperparasitism and antibiosis amongst the mechanisms involved.

Silva et al. (2023) has speculated that pustules of the *Pucciniales* may provide an especially favorable micro-habitat for the establishment of *Cladosporium* spp. The large diversity of species of *Cladosporium* found inhabiting the pustules of *H. vastatrix*, described herein, combined with the results of the study of *Cladosporium* spp. on *Austropuccinia psidii*, have expanded the list of known purported rust mycoparasites of *Cladosporium* spp. from only six, in the earlier literature, to a total of 22. This is a clear indication that a wealth of mycoparasitic *Cladosporium* spp. still await discovery and description from rust pustules.

The present paper, as well as others in the series involving different genera (Crous et al. 2018, Colmán et al. 2021, Guterres et al. 2021, Rodríguez et al. 2021, Salcedo-Sarmiento et al. 2021, Kapeua-Ndacnou et al. 2023a, b), provide only a snapshot of the fungi associated with the uredinia of CLR. Indeed, an additional paper in the series which will cover “the white colony-forming mycoparasites” describes several new genera and a host of new species (Colmán et al., in prep.) further demonstrating the mega-diversity in this micro-habitat whilst offering a rich source of isolates with the potential to be exploited for the biological control of this socio-economically devastating disease of the world’s most popular beverage crop.

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## REFERENCES

- Abdollahzadeh J, Groenewald JZ, Coetze MPA, et al. (2020). Evolution of lifestyles in *Capnodiales*. *Studies in Mycology* **95**: 381–414.
- Assante G, Maffi D, Saracchi M, et al. (2004). Histological studies on the mycoparasitism of *Cladosporium tenuissimum* on urediniospores of *Uromyces appendiculatus*. *Mycological Research* **108**: 170–182.
- Avelino J, Cristancho M, Georgiou S, et al. (2015). The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Security* **7**: 303–321.
- Baharvandi HA, Zafari D (2015). Identification of *Cladosporium delicatulum* as a mycoparasite of *Taphrina pruni*. *Archives of Phytopathology and Plant Protection* **48**: 688–697.
- Bensch K, Groenewald JZ, Dijksterhuis J, et al. (2010). Species and ecological diversity within the *Cladosporium cladosporioides* complex (*Davidiellaceae*, *Capnodiales*). *Studies in Mycology* **67**: 1–94.
- Bensch K, Braun U, Groenewald JZ, et al. (2012). The genus *Cladosporium*. *Studies in Mycology* **72**: 1–401.
- Bensch K, Groenewald JZ, Braun U, et al. (2015). Common but different: The expanding realm of *Cladosporium*. *Studies in Mycology* **82**: 23–74.
- Bensch K, Groenewald JZ, Meijer M, et al. (2018). *Cladosporium* species in indoor environments. *Studies in Mycology* **89**: 177–301.
- Braun U, Cunningham J, Priest MJ, et al. (2005). An annotated check list of *Ramularia* species in Australia. *Australasian Plant Pathology* **34**: 509–515.
- Carbone I, Kohn LM (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.
- Chen H, Chen J, Qi Y, et al. (2022). Endophytic fungus *Cladosporium tenuissimum* DF11, an efficient inducer of tanshinone biosynthesis in *Salvia miltiorrhiza* roots. *Phytochemistry* **194**: 113021.
- Colmán AA (2018). *Hidden diversity behind the white colony-forming mycoparasites on Hemileia vastatrix (coffee leaf rust)*, PhD dissertation, Departamento de Fitopatología, Universidade Federal de Viçosa, Minas Gerais, Brazil.
- Colmán AA, Evans HC, Salcedo-Sarmiento SS, et al. (2021). A fungus-eat-fungus world: *Digitopodium*, with particular reference to mycoparasites of the coffee leaf rust, *Hemileia vastatrix*. *IMA Fungus* **12**: 1.
- Costa PP, Rosado AWC, Pereira OL (2022). Six new species of *Cladosporium* associated with decayed leaves of native bamboo (*Bambusoideae*) in a fragment of Brazilian Atlantic Forest. *Phytotaxa* **560**: 1–29.
- Crous PW, Braun U, Schubert K, et al. (2007). Delimiting *Cladosporium* from morphologically similar genera. *Studies in Mycology* **58**: 33–56.
- Crous PW, Luangsa-Ard JJ, Wingfield MJ, et al. (2018). Fungal Planet description sheets: 785–867. *Persoonia* **41**: 238–417.
- Crous PW, Verkley GJM, Groenewald JZ, et al. (2019). *Fungal Biodiversity*. 2<sup>nd</sup> edn. Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands.
- Darriba D, Taboada GL, Doallo R, et al. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772–772.
- Dhingra OD, Sinclair JB (1995). *Basic Plant Pathology Methods*. 2<sup>nd</sup> edn. CRC Press, New York.
- Dugan FM, Glawe DA (2006). *Phyllactinia guttata* is a host for *Cladosporium uredinicola* in Washington State. *Pacific Northwest Fungi* **1**: 1–5.
- Freitas MLR, Gomes AAM, Rosado AWC, et al. (2021). *Cladosporium* species from submerged decayed leaves in Brazil, including a new species and new records. *Phytotaxa* **482**: 223–239.
- Gonçalves RC, Alfenas AC, Mafia RG (2016). Armazenamento de microrganismos em cultura com ênfase em fungos fitopatogênicos. In: *Métodos em Fitopatologia* (Alfenas AC, Mafia RG, eds). Editora UFV, Viçosa, Brazil: 93–105.
- Guterres DC, Ndacnou MK, Saavedra-Tobar LM, et al. (2021). *Cryptococcus depauperatus*, a close relative of the human-pathogen *C. neoformans*, associated with coffee leaf rust (*Hemileia vastatrix*) in Cameroon. *Brazilian Journal of Microbiology* **52**: 2205–2214.
- Hepperle D (2021). SeqAssem - Contig Sequence Assembly Software. <https://www.sequentix.de/>
- Heuchert B, Braun U, Schubert K (2005). Morphotaxonomic revision of fungicolous *Cladosporium* species (hyphomycetes). *Schlechtendalia* **13**: 1–78.
- Iturrieta-González I, García D, Gené J (2021). Novel species of *Cladosporium* from environmental sources in Spain. *MycoKeys* **77**: 1–25.
- Jie-Rong L, Senanayake IC, Dong Z-Y, et al. (2023). *Digitopodium citri* sp. nov.; an endophytic species associated with *Citrus medica* L. var. *sarcodactylis* from Guangdong Province, China. *Phytotaxa* **616**: 69–78.
- Kapeua-Ndacnou M, de Abreu LM, de Macedo DM, et al. (2023a). Assessing the biocontrol potential of *Clonostachys* species isolated as endophytes from *Coffea* species and as mycoparasites of *Hemileia* rusts of coffee in Africa. *Journal of Fungi* **9**: 248.
- Kapeua-Ndacnou M, Nóbrega TF, Batista LR, et al. (2023b). *Aspergillus flavus* from coffee in Cameroon: a non-aflatoxigenic endophytic isolate antagonistic to coffee leaf rust (*Hemileia vastatrix*). *Journal of Applied Microbiology* **134**: Ixad076.
- Koutouleas A, Jørgensen HJL, Jensen B, et al. (2019). On the hunt for the alternate host of *Hemileia vastatrix*. *Ecology and Evolution* **9**: 13619–13631.
- Kumar S, Stecher G, Li M, et al. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547–1549.
- Link HF (1816). Observationes in ordines plantarum naturales. Dissertation II, sistens nuperas de *Mucedinum* et *Gastromycorum* ordinibus observationes. *Magazin der Gesellschaft naturforschender Freunde zu Berlin* **7**: 25–45.
- Lv W, Yu Y, Zhong X, et al. (2023). Identification and characterization of *Cladosporium* species associated with tea plants (*Camellia sinensis*) in China. *Plant Pathology* **72**: 868–880.
- Mafia RG, Alfenas AC (2016). Preparações e observações microscópicas de espécimes fúngicos. In: *Métodos em Fitopatologia* (Alfenas AC, Mafia RG, eds). Editora UFV, Viçosa, Brazil: 207–222.
- Marin-Felix Y, Groenewald JZ, Cai L, et al. (2017). Genera of phytopathogenic fungi: GOPHY 1. *Studies in Mycology* **86**: 99–216.

- McCook S (2006). Global rust belt: *Hemileia vastatrix* and the ecological integration of world coffee production since 1850. *Journal of Global History* **1**: 177–195.
- Miller MA, Pfeiffer W, Schwartz T (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010. New Orleans, Louisiana: 1–8.
- Moharram AM, Zohri ANA, Hesham AEL, et al. (2022). Production of cold-active pectinases by three novel *Cladosporium* species isolated from Egypt and application of the most active enzyme. *Scientific Reports* **12**: 15599.
- Moricca S, Ragazzi A, Assante G (2005). Biological control of rust fungi by *Cladosporium tenuissimum*. In: *Rust diseases of willow and poplar* (Pei MH, McCracken AR, eds). CABI Publishing, Wallingford, UK: 213–229.
- Moricca S, Ragazzi A, Mitchelson KR, et al. (2001). Antagonism of the two-needle pine stem rust fungi *Cronartium flaccidum* and *Peridermium pini* by *Cladosporium tenuissimum* in vitro and in planta. *Phytopathology* **91**: 457–468.
- Nóbrega TF (2021). *Fusarium spp. antagonists to Hemileia vastatrix from Brazil, Paraguay and Africa: Taxonomy and biocontrol potential*, PhD. dissertation, Departamento de Fitopatologia, Universidade Federal de Viçosa, Minas Gerais, Brazil.
- Penzig OAJ (1882). Funghi agrumicoli: contribuzione allo studio dei funghi parassiti degli agrumi. *Michelia* **2**: 385–508.
- Pereira CM (2021). *Taxonomia de fungos dos gêneros Cladosporium e Cordyceps com potencial para o biocontrole de Hemileia vastatrix*, MSc. dissertation, Departamento de Fitopatologia, Universidade Federal de Viçosa, Minas Gerais, Brazil.
- Pereira MLS, Carvalho JLVR, Lima JMS, et al. (2022). Richness of *Cladosporium* in a tropical bat cave with the description of two new species. *Mycological Progress* **21**: 345–357.
- Prasannath K, Shivas RG, Galea VJ, et al. (2021). Novel *Botrytis* and *Cladosporium* species associated with flower diseases of Macadamia in Australia. *Journal of Fungi* **7**: 898.
- Rayner RW (1970). *A Mycological Colour Chart*. Commonwealth Mycological Institute & British Mycological Society, Kew, Surrey, UK.
- Rodríguez MCH, Evans HC, de Abreu LM, et al. (2021). New species and records of *Trichoderma* isolated as mycoparasites and endophytes from cultivated and wild coffee in Africa. *Scientific Reports* **11**: 5671.
- Ronquist F, Huelsenbeck JP (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Rosado AWC, Custódio FA, Pinho DB, et al. (2019). *Cladosporium* species associated with disease symptoms on *Passiflora edulis* and other crops in Brazil, with descriptions of two new species. *Phytotaxa* **409**: 239–260.
- Salcedo-Sarmiento S (2018). *New mycoparasitic fungi with potential for the biocontrol of Hemileia vastatrix (coffee leaf rust)*, PhD. dissertation, Departamento de Fitopatologia, Universidade Federal de Viçosa, Minas Gerais, Brazil.
- Salcedo-Sarmiento S, Aucique-Pérez CE, Silveira PR, et al. (2021). Elucidating the interactions between the rust *Hemileia vastatrix* and a *Calonectria* mycoparasite and the coffee plant. *iScience* **24**: 102352.
- Sandoval-Denis M, Gené J, Sutton DA, et al. (2016). New species of *Cladosporium* associated with human and animal infections. *Persoonia* **36**: 281–298.
- Sandoval-Denis M, Sutton DA, Martin-Vicente A, et al. (2015). *Cladosporium* species recovered from clinical samples in the United States. *Journal of Clinical Microbiology* **53**: 2990–3000.
- Schoch CL, Seifert KA, Huhndorf S, et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences, USA* **109**: 6241–6246.
- Schubert K, Groenewald JZ, Braun U, et al. (2007). Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae, Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. *Studies in Mycology* **58**: 105–156.
- Sharma IK, Heather WA (1988). Light and electron microscope studies on *Cladosporium tenuissimum*, mycoparasitic on poplar leaf rust, *Melampsora larici-populina*. *Transactions of the British Mycological Society* **90**: 125–131.
- Silva NMP, Guterres DC, Borges LS, et al. (2023). Surveying potentially antagonistic fungi to myrtle rust (*Austropuccinia psidii*) in Brazil: fungicolous *Cladosporium* spp. *Brazilian Journal of Microbiology* **54**: 1899–1914.
- Srivastava AK, Défago G, Kern H (1985). Hyperparasitism of *Puccinia horiana* and other microcyclic rusts. *Journal of Phytopathology* **114**: 73–78.
- Steyaert RL (1930). *Cladosporium hemileiicola* n. spec. un parasite de l'*Hemileia vastatrix* Berk. et Br. *Bulletin de la Société Royale de Botanique de Belgique* **63**: 46–48.
- Stöver BC, Müller KF (2010). TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7.
- Talhinhas P, Batista D, Diniz I, et al. (2017). The coffee leaf rust pathogen *Hemileia vastatrix*: one and a half centuries around the tropics. *Molecular Plant Pathology* **18**: 1039–1051.
- Torres DE, Rojas-Martínez RI, Zavaleta-Mejía E, et al. (2017). *Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* as potential new fungal antagonists of *Puccinia horiana* Henn., the causal agent of chrysanthemum white rust. *PLoS ONE* **12**: 1–16.
- Tsuneda A, Hiratsuka Y (1979). Mode of parasitism of a mycoparasite, *Cladosporium gallicola*, on western gall rust, *Endocronartium harknessii*. *Canadian Journal of Plant Pathology* **1**: 31–36.
- Tyagi K, Kumar P, Pandey A, et al. (2023). First record of *Cladosporium oxysporum* as a potential novel fungal hyperparasite of *Melampsora medusae* f. sp. *deltoidae* and screening of *Populus deltoides* clones against leaf rust. *3 Biotech* **13**: 213.
- Vaidya G, Lohman DJ, Meier R (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27**: 171–180.
- Wang X, Radwan MM, Taráwneh AH, et al. (2013). Antifungal activity against plant pathogens of metabolites from the endophytic fungus *Cladosporium cladosporioides*. *Journal of Agricultural and Food Chemistry* **61**: 4551–4555.
- Wang SY, Wang Y, Li Y (2022). *Cladosporium* spp. (*Cladosporiaceae*) isolated from *Eucommia ulmoides* in China. *MycoKeys* **91**: 151–168.
- Ward R, Gonthier D, Nicholls C (2017). Ecological resilience to coffee rust: varietal adaptations of coffee farmers in Copán, Honduras. *Agroecology and Sustainable Food Systems* **41**: 1081–1098.
- White TJ, Bruns T, Lee S, et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds.). Academic Press, USA: 315–322.
- Xu YX, Shen HW, Bao DF, et al. (2021). Two new species of *Cladosporium* from leaf spots of *Paris polyphylla* in north-western Yunnan Province, China. *Biodiversity Data Journal* **9**: e77224.
- Yang Y, Luo W, Zhang W, et al. (2023). *Cladosporium* species associated with fruit trees in Guizhou Province, China. *Journal of Fungi* **9**: 250.

- Zalar P, de Hoog GS, Schroers HJ, et al. (2007). Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. *Studies in Mycology* **58**: 157–183.
- Zambolim L (2016). Current status and management of coffee leaf rust in Brazil. *Tropical Plant Pathology* **41**: 1–8.
- Zhang H, He M, Fan X, et al. (2022). Isolation, identification and hyperparasitism of a novel *Cladosporium cladosporioides* isolate hyperparasitic to *Puccinia striiformis f. sp. tritici*, the wheat stripe rust pathogen. *Biology* **11**: 892.
- Zimowska B, Becchimanzi A, Krol ED, et al. (2021). New *Cladosporium* species from normal and galled flowers of *Lamiaceae*. *Pathogens* **10**: 369.