Mycological Progress (2023) 22:65
https://doi.org/10.1007/s11557-023-01913-5

ORIGINAL ARTICLE

Diversity of hyperparasitic fungi on *Meliolales* (*Sordariomycetes*, *Ascomycota*): new species, records, and molecular data from Benin and Panama

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Received: 18 April 2023 / Revised: 17 July 2023 / Accepted: 25 July 2023 / Published online: 23 August 2023

Abstract

Meliolales (black mildews) is an order of plant parasitic ascomycetous fungi in the tropics and subtropics. They are frequently overgrown and parasitized by other fungi, known as hyperparasites. During the last few years, species of hyperparasitic fungi on *Meliolales* have been collected in Benin and Panama. A new species of *Paranectria* and seven new reports of hyperparasites of different systematic groups are presented here with detailed descriptions and illustrations, together with new data concerning fungal hosts and host plants. The new species is called *Paranectria longiappendiculata*, characterized by exceptionally long appendages carried by the ascospores. New records for Benin and Panama are *Calloriopsis herpotricha*, *Dimerosporiella cephalosporii*, *Isthmospora glabra*, *Isthmospora trichophila*, *Malacaria meliolicola*, *Paranectriella hemileiae*, and *Paranectriella minuta*. *Calloriopsis herpotricha* is recorded for Africa and *D. cephalosporii* and *P. hemileiae* for America for the first time, suggesting an apparently pantropical distribution. Findings show a blatant lack of investigation on hyperparasitic fungi in the tropics. The phylogenetic positions of three of these newly reported species, *C. herpotricha*, *D. cephalosporii*, and *P. minuta*, are shown based on the analysis of internal transcribed spacer (ITS), large subunit (LSU), and small subunit (SSU) rDNA sequences. These sequences were generated in the context of the present study for the first time.

Keywords Black mildews · Hyperparasites · 1 new taxon · ITS/LSU/SSU rDNA · Pantropical distribution

Introduction

Meliolales (*Sordariomycetes*, *Ascomycota*), commonly known as "black mildews", form a large order of biotrophic, obligate plant parasitic fungi in the tropics and subtropics. Species of this order develop on leaves, petioles, twigs, and sometimes fruits of vascular plants (Piepenbring et al. 2011; Hongsanan et al. 2015; Zeng et al. 2017). Black mildews cause a reduction of chlorophyll, starch, sugar, proteins, and amino acids in the plant tissues they infect, as well as alterations in the photosynthetic and respiratory rates (Old et al. 2003).

Meliolales are frequently infected by hyperparasites (Hawksworth 1981; Gams et al. 2004). There are approximately 200 species of fungi reported to be hyperparasitic on colonies of *Meliolales* (Bermúdez-Cova et al. 2023), but we expect a much greater number of species to exist in the tropics. Fungal hyperparasites belong to diverse taxonomic groups, and therefore comprise species producing a high diversity of reproductive structures, such as apothecia, cathecia, perithecia, pycnidia, and synnemata, among others. They are generalists concerning genera of *Meliolales*, but many of these hyperparasites seem to be restricted only to meliolalean hosts (Bermúdez-Cova et al. 2022).

Knowledge of species diversity of black mildews in the tropics is still limited. Only three species are known for Benin and 105 for Panama (Piepenbring et al. 2011; Piepenbring et al.

Section Editor: Tanay Bose

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2020; Hofmann and Piepenbring 2021). Information on hyperparasitic fungi of *Meliolales* in Benin and Panama is inexistent (Bermúdez-Cova et al. 2022). For a better understanding of the diversity, evolution, and biology of hyperparasitic fungi, it is necessary to increase sampling efforts and to undertake further morphological, molecular, and ecological studies.

Materials and methods

Sample collection and morphological characterization

Samples of leaves infected with black mildews were opportunistically collected in Western Panama from January to March 2020 and in Benin in February as well as September 2022. For the present study, colonies of *Meliolales* parasitized by hyperparasites were considered. Infected leaves were dried in a plant press and deposited in the herbarium at the Universidad Autónoma de Chiriquí (UCH, specimens from Panama) and in the mycological herbarium of the University of Parakou (UNIPAR) in Benin. If a given sample was large enough, a duplicate was deposited in the Botanische Staatssammlung München (M).

Dried specimens were observed by stereomicroscopy and by light microscopy. Measurements of at least 20 ascospores, conidia, and other structures have been made for each specimen at a magnification of × 600 and × 1000. Measurements are presented as mean value ± standard deviation with extreme values in parentheses. Line drawings were made freehand on scaled paper. Images and drawings were edited with Photoshop (Adobe, San Jose, California).

Host plant identification

Host plants were identified by morphological characteristics and in some cases by molecular sequence data. Morphological identifications were made by comparison with herbarium specimens, literature (e.g., Akoègninou et al. 2006; Condit et al. 2011), and with the help of local botanists. Molecular sequence data for species identifications were obtained by polymerase chain reaction (PCR) for the amplification of the partial region of chloroplast rbcL with the primer pairs rbcLa-F (Levin et al. 2003) and rbcLa-R (Kress et al. 2009). DNA was extracted from approx. 0.05 g of leaf tissue dried with silica gel using the innuPREP plant DNA kit (Analytik Jena, Germany) and following the manufacturer's instructions. Protocols for PCR were carried out as described by Fazekas et al. (2012).

DNA extraction, PCR amplification, and sequencing of fungal DNA

DNA was isolated from the ascomata of dry specimens using the EZNA forensic DNA extraction kit, following the manufacturer's instructions. To extract total genomic DNA, a small amount of clean ascomata were transferred into a sterile Eppendorf tube with approx. 200 µL of distilled water using sterilized tweezers, and trying to avoid picking cells of any other organism associated with the leaves and the colonies of black mildews. The samples were frozen for 24 h at − 20 °C and later homogenized for 10–12 min. using a Retsch mixer mill MM301 with TL buffer and 2.5-mm zirconia beads. Isolated DNA was re-suspended in elution buffer and stored at − 20 °C.

Two partial nuclear gene regions (ribosomal loci) were amplified and sequenced: For the large subunit nuclear ribosomal DNA (nrLSU, 28S rDNA), the primers LSU1Fd and LSU3Rd (Crous et al. 2009), NL1 and NL4 (O'Donnell 1993), LR0R (Wagner and Ryvarden 2002), and LR5 (Vilgalys and Hester 1990) were used. For small subunit nuclear ribosomal DNA (nrSSU, 18S rDNA), the primers SSU1Fd and SSU3Rd (Crous et al. 2009) were used. For the internal transcribed spacer region of ribosomal DNA (ITS), the primers ITS5 and ITS4 (White et al. 1990) were used. The PCR mixtures consisted of 1 µL genomic DNA, 15 × $MgCl_2$ reaction buffer (Bioline, Luckenwalde, Germany), 25 mM $MgCl_2$, 25 µM of each dNTP, 10 µM of each primer, and 5 U Taq DNA polymerase (VWR) in a total volume of 30 µL. Cycling parameters of the PCR for ITS, LSU, and SSU were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of amplification [denaturation at 94 °C for 30 s, primer annealing at 52 °C for 30 s and primer extension at 72 °C for 45 s], and a final extension at 72 °C for 5 min, followed by storage at 8 °C. PCR products were checked on 1.5% agarose electrophoresis gels containing HDGreenPlus DNA stain. Amplified PCR products were purified with the Cycle Pure Kit (VWR-Omega, USA). Sequencing was performed at Seqlab GmbH, Germany.

Numerous attempts were made to obtain DNA sequence data of ITS, LSU, and SSU regions from all the specimens collected in the context of this study. Except for four specimens, these attempts failed.

Phylogenetic analyses

Consensus sequences of trace files were generated with Geneious 10.2.2 (https://www.geneious.com, Kearse et al. 2012) and searched against GenBank (https://www.ncbi.nlm.nih.gov/, Benson et al. 2014) with MegaBLAST. Ambiguous and miscalled bases were corrected, when possible, after examination of the corresponding chromatogram files. Sequences with a high similarity were aligned with MAFFT v. 7 using the L-INS-i algorithm (Nakamura et al. 2018). The alignments were manually checked by using MEGA v. 7 (Kumar et al. 2016). Gblocks v. 0.91b (Talavera and Castresana 2007) was used to remove poorly aligned positions and divergent regions from the DNA alignment. Phylogenetic analyses of this study were conducted by

applying maximum likelihood (ML) in RAxML-HPC2 v.8.2.12 (Stamatakis 2014) on XSEDE (Miller et al. 2010) and Bayesian phylogenetic inference with the program MrBayes 3.2.6. (Ronquist et al. 2012) on XSEDE (Miller et al. 2010), available on the CIPRES Science Gateway web portal (http://www.phylo.org/sub_sections/portal/). The alignments and trees were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S30529).

Results

Apothecioid hyperparasites

Calloriopsis herpotricha (Berk.) R. Sant., *Svensk bot. Tidskr.* 45(1): 300, 1951 (Figs. 1 and 2).

≡ *Peziza herpotricha* Berk., *Hooker's J. Bot. Kew Gard. Misc.* 3: 16, 1851.

≡ *Helotiella herpotricha* (Berk.) Sacc., *Syll. fung.* (Abellini) 8: 477, 1889.

= *Calloria meliolicola* P. Henn., *Botanisch. Jahrb.* 25: 509, 1898.

≡ *Coryne meliolicola* (P. Henn.) v. Höhnel, *Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt.* 1. 118: 106, 1909.

= *Peziza gelatinosa* Ell. & Mart., *Amer. Nat.* 17: 1283, 1883.

≡ *Orbilia gelatinosa* Sacc., *Syll. Fung.* 8: 624, 1889.

≡ *Coryne gelatinosa* (Sacc.) Rehm, *Ann. Mycol.* 5: 518, 1907.

≡ *Calloriopsis gelatinosa* (Sacc.) Sydow, *Ann. Mycol.* 15: 254, 1917.

Colonies composed of white hyphae covering the colonies of *Meliola* sp. Hyphae thin-walled, septate, 2–3 µm, hyaline. Apothecia 400–600 µm diam., disc pale orange to orange when old, margin slightly paler, translucent. Gelatinous material present throughout the hymenium, subhymenium, ectal excipulum and medullary excipulum. The subhymenium is composed of tightly interwoven hyphae. The ectal excipulum is composed of septate parallel hyphae which are swollen at the tips. Asci clavate, thick-walled especially in young asci, 40–52 µm, 8-spored. Paraphyses filamentous, unbranched, 1–3 µm, sometimes swollen at the tip. Ascospores ellipsoid to fusoid, sometimes curved, (10–) 13–16 × 3–6 µm, mostly 1-septate, hyaline, smooth.

Anamorph – Not known.

Specimens examined – On *Meliola* sp. on living leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 42″ N 2° 7′ 50″ E, 69 m, 28 February 2022, M.A. Bermúdez, A. Tabé, I. Agonglo, O.P. Agbani, N.S. Yorou, MB178; on *Meliola* sp. on living leaves of *Coffea arabica*,

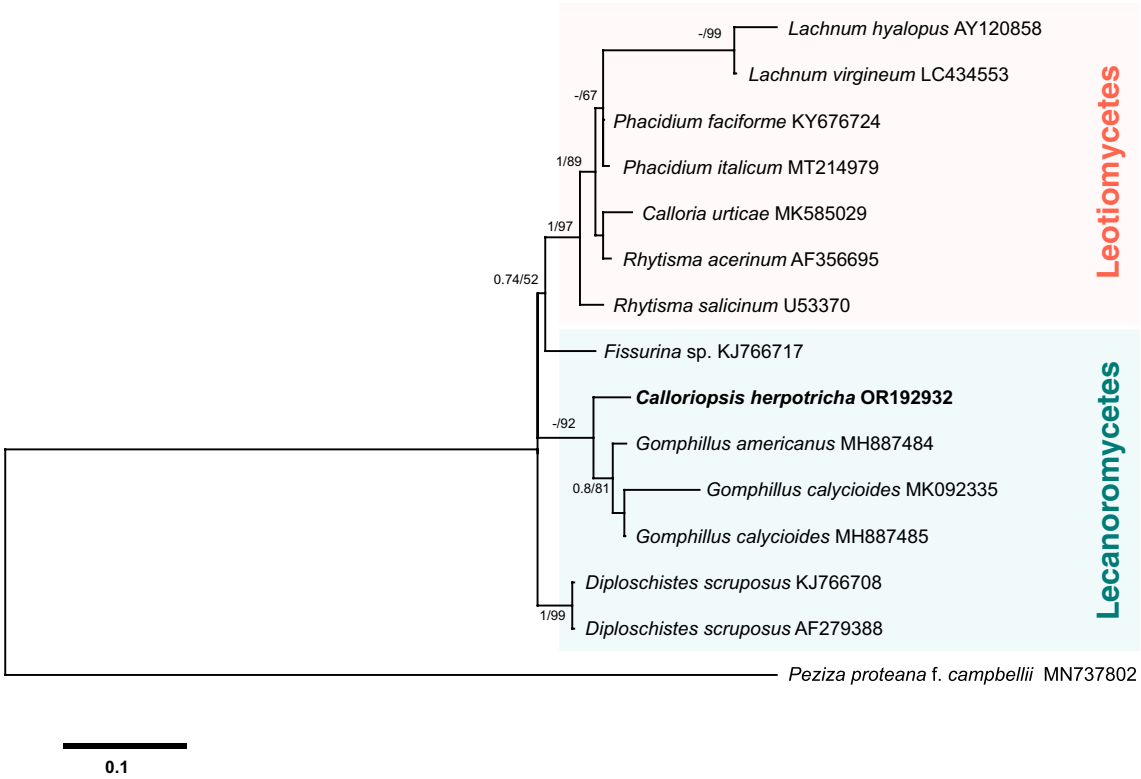

Fig. 1 Phylogenetic tree inferred from the maximum likelihood analysis of SSU sequences of members of the *Leotiomycetes* and *Lecanoromycetes*, including a new sequence for *Calloriopsis herpotricha.* The tree is rooted with *Peziza proteana* f. *campbellii* (*Pezizomycetes*). Bootstrap values and posterior probabilities are indicated above the branches. Sequences downloaded from GenBank are cited with accession numbers

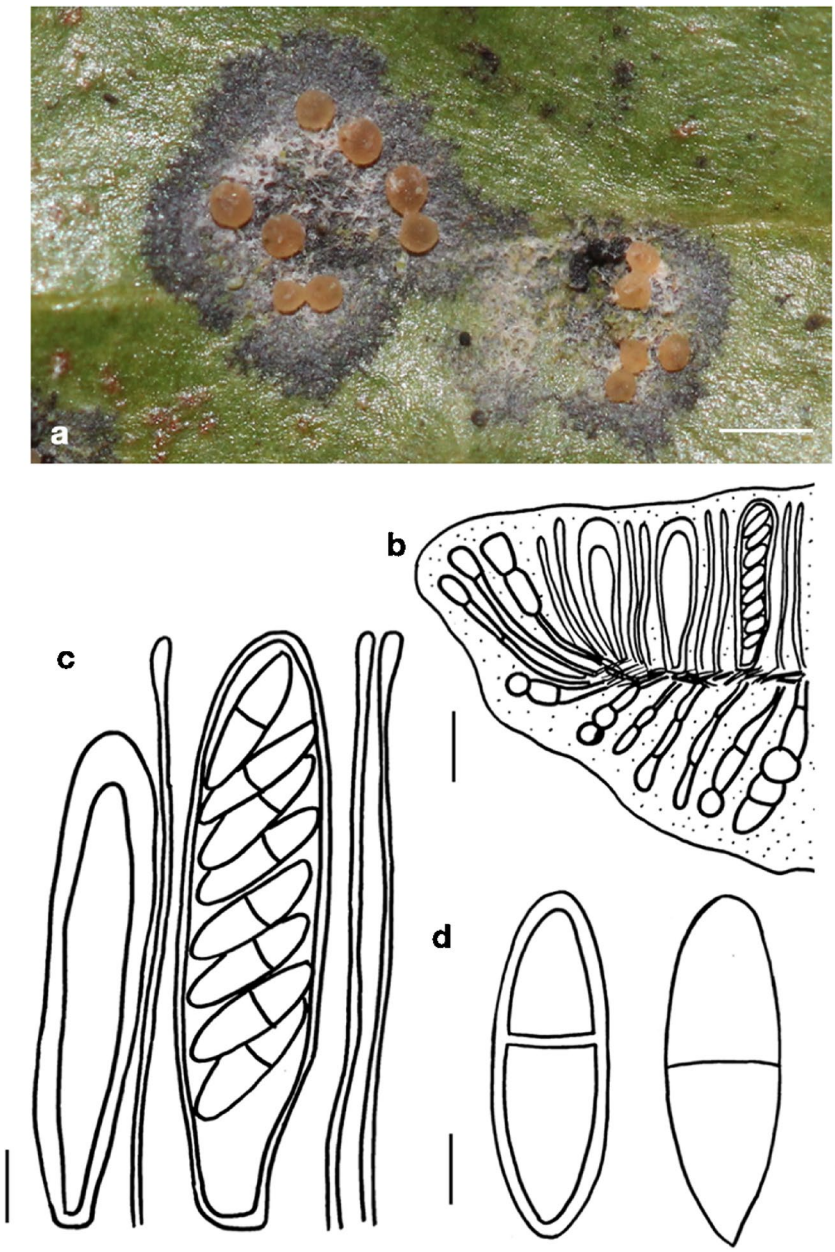

Fig. 2 ***Calloriopsis herpotricha*** **(AK4H)**. **a** Apothecia growing on colonies of *Meliola* sp. on living leaves of *Coffea arabica*; **b** part of a longitudinal section of an apothecium. Dots indicate the presence of gelatinous material; **c** young and mature asci as well as paraphyses; **e** ascospores, shown in optical section (the thickness of the wall is indicated only in the drawing on the left-hand side). Scale bars: 300 µm (**a**); 20 µm (**b**); 6 µm (**c**); 3 µm (**d**)

Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 23″ N 2° 8′ 26″ E, 119 m a.s.l., 19 September 2022, A. Krauß, A. Tabé, O. Koukol, N.S. Yorou, AK4H (UNIPAR, M, GenBank accession number: OQ800930).

Known hosts and distribution – On *Meliola* sp. on living leaves of *Persea palustris* (*Lauraceae*) in the USA (Ellis and Martin 1883); on living leaves of an unknown plant host in Pará, Brazil (Hooker 1851; Saccardo 1889); on *Meliola* sp. on living leaves of *Phragmites* sp. (*Poaceae*) in Papua New Guinea (Hennings 1898); on *Meliola ramosii* on living leaves of *Homonoia riparia* (*Euphorbiaceae*) in the Philippines; on *Perisporiaceae* on living leaves of *Scaevola* sp. (*Goodeniaceae*) in Hawaii (Cash 1938); on *Meliola* sp. on living leaves of an unknown tree in the Philippines (Santesson 1951); on *Meliola substenospora* on living leaves of *Phragmites* sp. (*Poaceae*) in Java, Indonesia (Pfister 1976); on *Meliola* sp. on living leaves of herbs in Puerto Rico (Pfister 1976); without host data in Guadeloupe, France (Pfister 1976); on *Meliola* sp. on living leaves of *Nyssa* sp. (*Nyssaceae*) in the USA (Pfister 1976); on *Meliola* sp. on living leaves of *Coffea arabica* (*Rubiaceae*) in Benin (this study). *C. arabica* is a new host of *C. herpotricha*, and the hyperparasite and the species of *Meliolales* are new records for Benin. This is also the first record of *C. herpotricha* in Africa.

Illustrations – This species was illustrated by Pfister (1976).

Notes – Two species of apothecioid hyperparasitic fungi have been reported to parasitize *Meliolales*, namely, *Calloriopsis herpotricha* and *Unguiculella meliolicola*, both

belonging to the *Leotiomycetes* (*Phacidiales* and *Helotiales*, respectively; Bermúdez-Cova et al. 2022). The monotypic genus *Calloriopsis* was proposed by Sydow and Sydow (1917) and is based on a parasitic discomycete which occurs on *Meliola* and related dark parasites. *Calloriopsis herpotricha* differs from other fungi of the *Leotiomycetes* by the parasitic habit and the gelatinous material that is present in all parts of the apothecium, including the hymenium (Pfister 1976; Baral and Marson 2001).

Sequence data – The SSU rDNA sequence obtained from fresh material of *C. herpotricha* (specimen AK4H) is 948 bp long. In the tree inferred from the analysis of SSU sequences of 14 specimens of *Leotiomycetes* and *Lecanoromycetes* (Fig. 1), the sequence of *C. herpotricha* is located within a strongly supported clade that comprises sequences of species of *Lecanoromycetes* which were obtained from lichenized fungi. Some lineages of non-lichenized *Ascomycota* are known to be derived from lichenized ancestors by the loss of the lichen symbiosis in favor of a saprotrophic, lichenicolous, or parasitic mode of nutrition (Lutzoni et al. 2001; Hawksworth 2015; Honegger 2022). Examples of these include non-lichenized members of *Arthoniales* (*Arthoniomycetes*) and *Ostropales* (*Lecanoromycetes*; Kendrick 2017). The foregoing and the fact that the sequences of the species of *Calloriopsis* clustered together with other sequences of species of *Ostropales* suggest that the genus *Calloriopsis* may belong to the *Lecanoromycetes* and not to the *Leotiomycetes* as previously assumed. A sequence for *C. herpotricha* is provided here for the first time.

Four sequences of an unidentified species of *Calloriopsis* are available in GenBank (accession numbers: MF322776, MF322774, OM103051, and OQ800930). The specimens that yielded these sequences were found on decayed twigs and branches of *Cornus sanguinea* L. (*Cornaceae*) and *Fraxinus excelsior* L. (*Oleaceae*) in Luxembourg (unpublished data provided by the herbarium LUX). These sequences also fall within the *Lecanoromycetes*. However, these sequences lack the SSU region; thus, it was not possible to compare them with the sequence of *Calloriopsis herpotricha*. We also obtained a DNA sequence of the ITS region of *C. herpotricha* (GenBank accession number: OR243608). This sequence presents 94% identity with the aforementioned sequences of *Calloriopsis* and other members of the *Lecanoromycetes*, confirming the systematic placement of *C. herpotricha* in the *Lecanoromycetes*.

Dematiaceous hyphomycetes

Isthmospora glabra F. Stevens, *Bot. Gaz.* 65(3): 244, 1918 (Fig. 3a–c).

Hyphae not evident, conidia in small pulverulent brownish heaps scattered over the colonies of *Meliola* sp. Conidiophores not found. Conidia are isthmospores, composed of 11–12 cells. Two pairs of subglobose thick-walled cells, each cell with rounded horns that are directed upwardly and inwardly, 5–6 × 4–5 µm, brown, smooth. These cells are connected by a central isthmus made of two cells. Connecting cells oblong with wedge-shaped ends, 3–4 µm diam., pale brown, smooth. On each side of the central cells, two to three flask-shaped cells extend upwardly and outwardly into a continuous cylindrical appendage, (15–)20–21(–24) × 1–2 µm, hyaline, smooth.

Teleomorph – Trichothyrium-like (according to Hughes 1953).

Specimen examined – On *Appendiculella sororcula* on living leaves of *Calea pittieri*, Panama, Chiriquí Province, David, Dolega district, Los Algarrobos, Majagua river trail, 8° 29′ 28″ N 82° 25′ 59″ W, approx. 150 m a.s.l., 29 December 2016, M. Piepenbring, A. Villarreal, E. Romero, V. Samudio, MP5326 (UCH10000).

Known hosts and distribution – On *Meliola melastomacearum* on living leaves of *Clidemia hirta* (*Melastomataceae*) in Puerto Rico; on *Meliola bicornis* on living leaves of *Meibomia supina* (*Leguminosae*) in Puerto Rico; on *Meliola glabroides* on living leaves of *Nectandra patens* (*Lauraceae*) and *Simarouba tulae* (*Simaroubaceae*) in Puerto Rico; on *Meliola glabra* on living leaves of unknown host in Puerto Rico (Stevens 1918); on *Appendiculella sororcula* on living leaves of *Calea pittieri* (*Asteraceae*) in Panama (this study). *A. sororcula* and *C. pittieri* are new hosts of *I. glabra*, and the hyperparasite is a new record for Panama.

Illustrations – This species was illustrated by Hughes (1953).

Notes – The genus *Isthmospora* (*Microthyriaceae*, *Microthyriales*) was proposed by Stevens (1918) and comprises two species of dematiaceous hyphomycetes with dark conidia consisting of two approximately equal halves connected by an isthmus. Both species of the genus, *I. glabra* and *I. spinosa*, are associated with colonies of black mildews (Damon 1953).

Isthmospora glabra is characterized by the presence of dark smooth central cells with large hyaline appendages (Stevens 1918; Hughes 1953). However, according to Damon (1953), this species is congeneric with *Spegazzinia chandleri*.

Isthmospora glabra has always been found on the hyphal mat of *Trichothyrium reptans*, a catathecioid hyperparasite of *Meliolales*. Therefore, *I. glabra* is considered to be the anamorphic stage of *T. reptans* (Hughes 1953). The specimen examined (MP5326) was also found together with a species of *Trichothyrium* (Fig. 3a, b); however, the size of the ascospores (10–12 × 4–6 µm) does not match with the size of ascospores of *T. reptans* (15–20 × 5–6.5 µm; Hughes 1953). There is no molecular evidence that supports the anamorph-teleomorph connection between these fungi.

Isthmospora trichophila (Atkinson) Damon, *Bull. Torrey bot. Club* 80: 160, 1953 (Fig. 3d–e).

≡ *Spegazzinia trichophila* G.F. Atk., *Bull. Cornell Univ.* 3(1): 49, 1897.

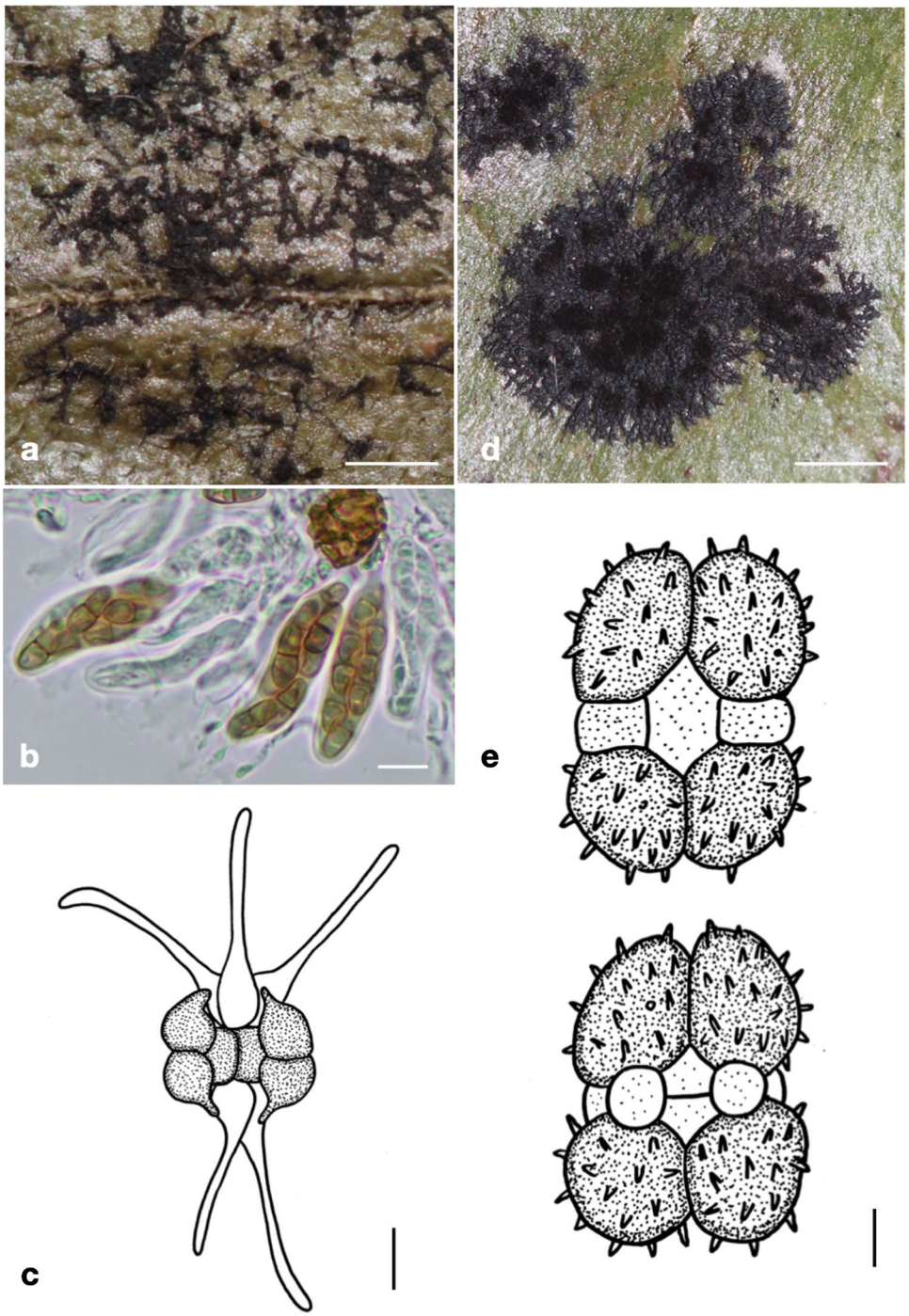

Fig. 3 ***Isthmospora*** **spp. on** ***Meliolales***. **a**–**c** (MP5326). **a** Isthmospores of *Isthmospora glabra* growing together with the hyphal mat and catathecia of *Trichothyrium* sp.; **b** asci and ascospores of *Trichothyrium* sp.; **c** isthmospore of *Isthmospora glabra*; **d**, **e** *Isthmospora trichophila* (AK4H). **d** Isthmospores growing in scattered heaps (see darker spots) on the hyphal mat of *Trichothyrium* sp.; **e** isthmospores drawn at diverse optical levels. Scale bars: 1 mm (**a**, **d**); 10 µm (**b**); 5 µm (**c**); 7 µm (**e**)

= *Isthmospora spinosa* F. Stevens, *Bot. Gaz.* 65(3): 244, 1918.

= *Spegazzinia coffeae* Henn., apud De Wildeman, *Mission E. Laurent* 3: 318, 1906.

= *Spegazzinia meliolae* Zimm., *Cent. f. Bakt. II*: 8: 221, 1902.

= *Spegazzinia meliolicola* Henn., *Hedwigia* 43: 398, 1904.

Hyphae not evident, conidia in small pulverulent brownish heaps scattered over the colonies of *Meliola* sp. Conidiophores erect, parallel, short, pale brown to brown. Conidia are isthmospores, composed of 11 cells: two pairs of subglobose cells, (14–)16–17(–23) × 11– 14(–19) µm, dark brown, echinulate, with spines up to 2 µm long. The cells are connected by a central isthmus made of three central cells that are oblong with wedge-shaped ends, 3–4 µm wide; there is a single central cell at the upper level and two cells resulting from a septation of another cell at the lower level. Two outer cells more or less oblong, 3–4 µm diam., hyaline, are attached on both sides of the central cells (one cell on each side). At the

base of each outer cell a basal cell, 2–3 µm wide, hyaline, is attached.

Teleomorph – Trichothyrium-like (according to Hughes 1953).

Specimens examined – On *Meliola* sp. on living leaves of *Xylopia frutescens*, Panama, Chiriquí Province, Cochea, trail to Cochea river, 8° 32′ 36″ N 82° 23′ 03″ W, 181 m a.s.l., 26 February 2020, M.A. Bermúdez, A. Sanjur, A. Villarreal, MB109 (UCH); on *Meliola* sp. on living leaves of an unknown host plant, Panama, Chiriquí Province, David, Cuesta de Piedra, 8° 41′ 13″ N 82° 36′ 33″ W, 903 m a.s.l., 6 March 2020, M.A. Bermúdez, S. Samaniego, MB118 (UCH); on *Meliola* sp. on living leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 42″ N 2° 7′ 50″ E, 69 m a.s.l., 28 February 2022, M.A. Bermúdez, A. Tabé, I. Agonglo, O.P. Agbani, N.S. Yorou, MB178; on *Meliola* sp. on living leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 23″ N 2° 8′ 26″ E, 119 m a.s.l., 19 September 2022, A. Krauß, A. Tabé, N.S. Yorou, O. Koukol, AK4H (UNIPAR, M).

Known hosts and distribution – On *Meliola anacardii* on living leaves of *Anacardium occidentale* (*Anacardiaceae*) in Indonesia; on *Meliola psidii* on living leaves of *Psidium guajava* (*Myrtaceae*) in Brazil (Saccardo and Saccardo 1906); on *Meliola* sp. on living leaves of *Coffea* sp. (*Rubiaceae*) in Ubangi, tropical Africa (Saccardo and Trotter 1913); on *Meliola psidii* on living leaves of *Psidium guajava* (*Myrtaceae*) in Puerto Rico; on *Meliola chiococcae* on living leaves of *Chiococca alba* (*Rubiaceae*) in Puerto Rico; on *Meliola byrsonimae* on living leaves of *Byrsonima lucida* (*Malpighiaceae*) in Puerto Rico; on *Meliola smilacis* on living leaves of *Smilax coriaceae* (*Smilacaceae*) in Puerto Rico; on *Meliola helleri* on living leaves of *Myrcia splendens* (*Myrtaceae*) in Puerto Rico; on *Meliola praetervisa* on living leaves of *Coccolobus sintenisii* and *Coccolobus pyrifolia* (*Polygonaceae*) in Puerto Rico; on *Meliola philodendri* on living leaves of *Philodendron krebsii* (*Araceae*) in Puerto Rico (Stevens 1918); on *Meliola* sp. on living leaves of *Xylopia frutescens* (*Annonaceae*) in Panama (this study); on *Meliola* sp. on leaves of *Coffea arabica* (*Rubiaceae*) in Benin (this study). *Coffea arabica* and *X. frutescens* are new hosts of *I. spinosa*, and the hyperparasite is recorded here for Benin and Panama for the first time.

Illustrations – This species was illustrated by Hughes (1953), Damon (1953) and Tubaki (1963).

Notes – *Isthmospora trichophila* (*Microthyriaceae*, *Microthyriales*) is morphologically similar to species of *Spegazzinia* (*Apiosporaceae*, *Sordariomycetes*), and several known species are easily confused (Damon 1953). However, the complex morphology of the isthmospores and the association with species of *Meliolales* are strong features to distinguish *I. trichophila* from other species of dematiaceous hyphomycetes (Damon 1953; Hughes 1953).

Isthmospora trichophila has always been recorded growing on the hyphal mat of *Trichothyrium asterophorum* (*Microthyriaceae*, *Microthyriales*), a catathecioid hyperparasite of *Meliolales*; thus, it is considered to be the anamorphic stage of *T. asterophorum* (Hughes 1953). The specimens examined were also found together with a species of *Trichothyrium*, which could not be identified because it was not fertile. There is no molecular evidence that supports the anamorph-teleomorph connection between these two species of fungi.

Perithecioid hyperparasites

Dimerosporiella cephalosporii (Hansf.) Rossman & Samuels, *Stud. Mycol.* 42: 23, 1999 (Figs. 4, 5, and 6).

≡ *Calonectria cephalosporii* Hansf., *Mycol. Pap.* 15: 117, 1946.

≡ *Nectriopsis cephalosporii* (Hansf.) Samuels, *Mem. New York Bot. Gard.* 48: 38, 1988.

Colonies white, cottony, growing on *Meliola* spp. Hyphae septate, 1.7 µm wide, hyaline. Perithecia superficial, globose, (100–)110–150(–220) µm diam., yellow to orange, slightly translucent, not changing color in KOH, smooth; perithecial hairs arising from perithecial apex, septate, unbranched, (10–)17–25(–35) × 3–5.5 µm, wall 0.5–1 µm thick. Perithecial wall 7–9 µm wide, composed of small cells; perithecial apex formed by hyphae that grow outwardly to form perithecial hairs, and inwardly to form periphyses. Asci clavate, apex simple, (25–)32–45(–53) × (6–)7–9(–10) µm, 8-spored. Ascospores completely filling each ascus, ellipsoidal to fusiform, biguttulate, (8.5–)10–15.5(–18) × 1.7–4 µm, 1-septate, hyaline, smooth.

Anamorph – Acremonium-like anamorph with conidiophores arising from aerial mycelium, mononematous, macronematous, septate, monophialidic. Phialides thick-walled, with a distinctive collarette, (30–)40–50 µm long × 3–5 µm wide at the base, tapering to 1 µm width at the tip, hyaline. Conidia oblong to ellipsoidal, unicellular, (5–)7.5–9(– 12) × (1.5–)2–3(–3.5) µm, hyaline, smooth.

Specimens examined – On *Meliola* sp. on leaves of *Olyra latifolia*, Panama, Chiriquí Province, David, Botanical Garden of the Universidad Autónoma de Chiriquí (UNACHI), 8° 25′ 55″ N 82° 27′ 4″ W, 34 m a.s.l., 23 January 2020, M.A. Bermúdez, MB86 (UCH13408); on *Meliola* sp. on leaves of *Olyra latifolia*, Panama, Chiriquí Province, David, Los Algarrobos, Majagua river trail, 8° 28′ 47″ N 82° 24′ 46″ W, 80 m a.s.l., 26 February 2020, M.A. Bermúdez, MB113 (UCH13407); on *Meliola pinnatae* on leaves of *Paullinia pinnata*, Benin, Atlantique, Allada, Sékou, 6° 38′ 59″ N 2° 11′ 46″ E, 48 m a.s.l., 15 February 2022, M.A. Bermúdez, A. Tabé, D. Dongnima, M. Piepenbring, N.S. Yorou, MB139 (UNIPAR, M, GenBank accession number: OQ787065); on *Meliola pinnatae* on leaves of *Paullinia pinnata*, Benin, Atlantique,

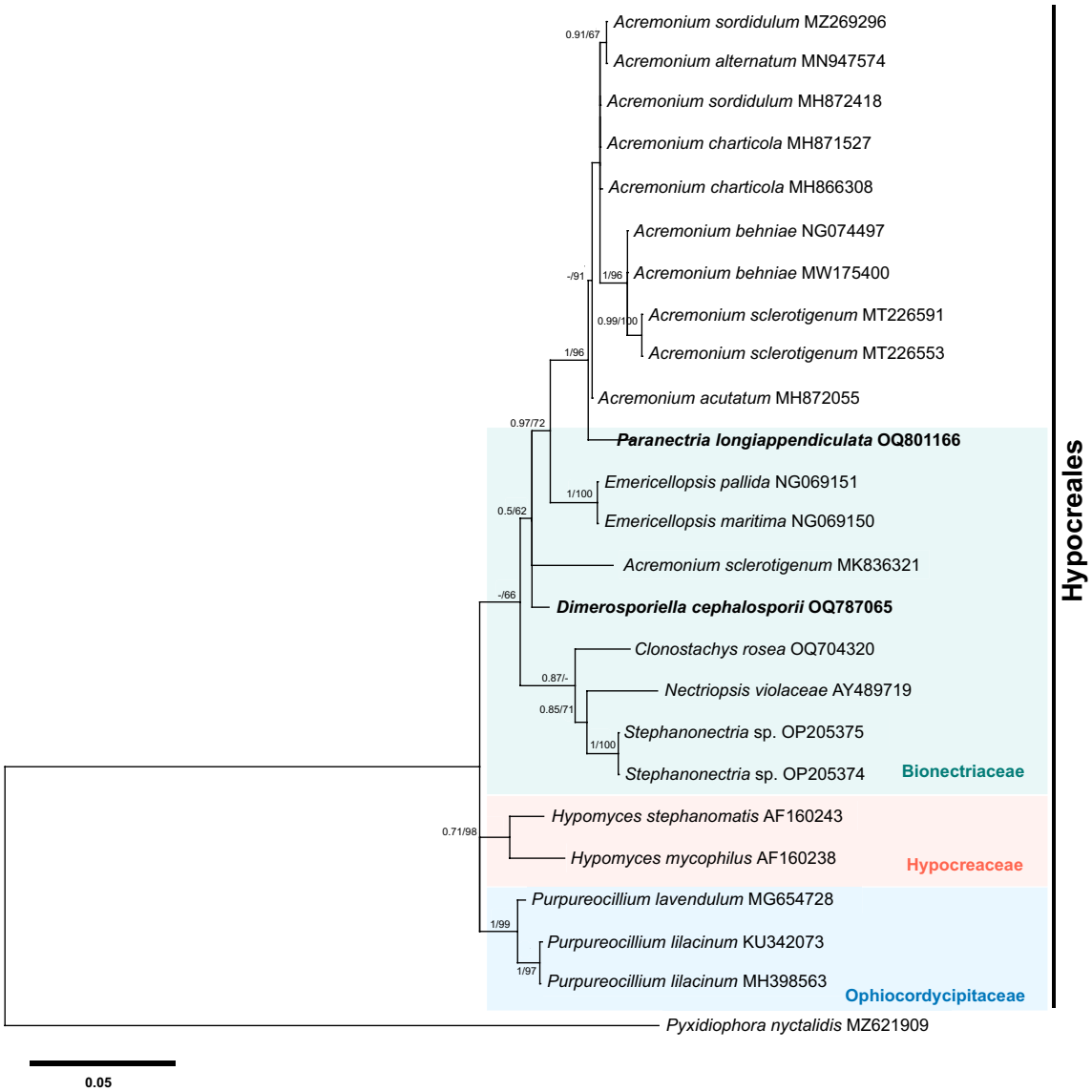

Fig. 4 Phylogenetic tree inferred from a maximum likelihood analysis of nuc LSU sequences of members of the *Bionectriaceae*, *Hypocreaceae*, and *Ophiocordycipitaceae* (*Hypocreales*), including a new sequence of *D. cephalosporii* and a new sequence of *Paranectria longiappendiculata*. The tree is rooted with *Pyxidiophora arvernensis* (*Pyxidiophoraceae*). Bootstrap values and posterior probabilities are indicated above the branches. Sequences downloaded from GenBank are given with accession numbers

Zalimey, Lama Forest, 6° 58′ 15″ N 2° 11′ 26″ E, 43 m a.s.l., 20 September 2022, A. Krauß, A. Tabé, N.S. Yorou, O. Koukol, AK20H (UNIPAR, M).

Known hosts and distribution – On *Meliola markhamiae* on living leaves of *Markhamia platycalyx* (*Bignoniaceae*) in Uganda (Hansford 1946); on *Meliola* sp. on living leaves of *Olyra latifolia* (*Poaceae*) in Panama (this study); on *Meliola pinnatae* on leaves of *Paullinia pinnata* (*Sapindaceae*) in Benin (this study). *M. pinnatae*, *O. latifolia*, and *P. pinnata* are new hosts of *D. cephalosporii*, and the hyperparasite is recorded here for mainland America (Panama) and Benin for the first time.

Illustrations – This species was illustrated by Gams (1971, anamorph only), Pirozynski (1977), and Samuels (1988).

Notes – Approximately 70 species of perithecioid fungi are reported as hyperparasites of *Meliolales* (Bermúdez-Cova et al. 2022). Among these species, *Dimerosporiella cephalosporii* (*Bionectriaceae*, *Hypocreales*) is one of the most common parasites in Uganda (Hansford 1946; Gams 1971; Gams et al. 2004; Bermúdez-Cova et al. 2022). The genus *Dimerosporiella* was proposed by Spegazzini (1908) and now comprises species that were previously placed in the *Nectria leucorrhodina* group or treated within *Nectriopsis* (Samuels 1976, 1988; Rossman 1983). Species of the genus are fungicolous (i.e., growing on other fungi) and grow on colonies of species of *Asterina*, *Meliolales*, or *Schiffnerula* (Rossman et al. 1999). Species of *Dimerosporiella* are differentiated

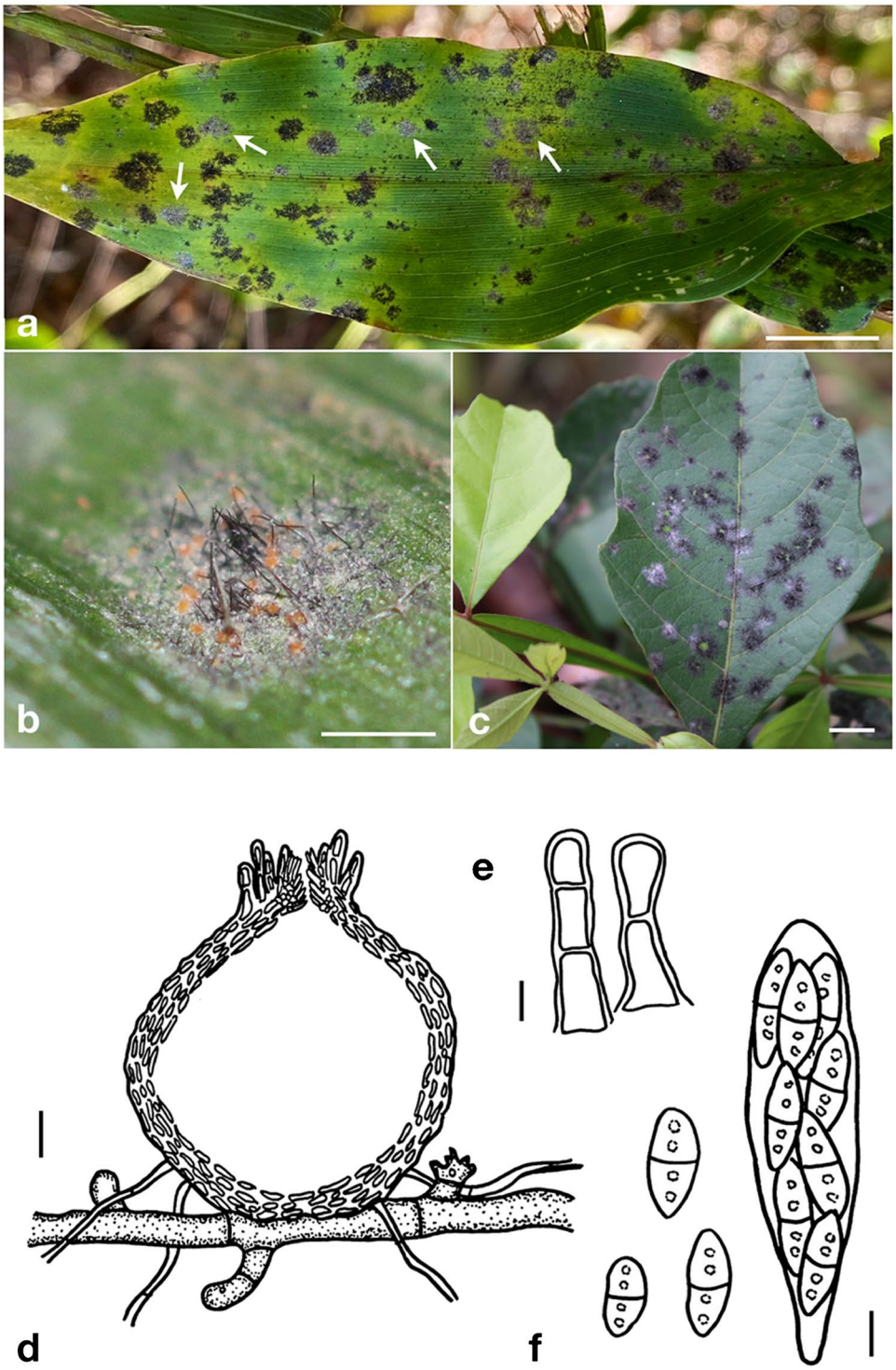

Fig. 5 ***Dimerosporiella cephalosporii*** **(MB86, MB139)**. **a** A leaf of *Olyra latifolia* parasitized by *Meliola* sp. Note that some of the black colonies are whitish/greyish (arrows) due to the presence of the hyperparasite; **b** orange perithecia between the setae of *Meliola* sp.; **c** a leaf of *Paullinia pinnata* infected by *Meliola pinnatae* (MB139). Note that some of the black colonies are whitish/greyish due to the presence of *D. cephalosporii*; **d** perithecium on a hypha of *Meliola* sp.; **e** perithecial hairs; **f** ascus and ascospores. Scale bars: 1 cm (**a**, **c**); 1 mm (**b**); 13 µm (**d**); 5 µm; 4.5 µm (**f**)

primarily by features of the surface of ascomatal walls and characteristics of the ascospores. For a detailed key to species of the genus, see Rossman et al. (1999).

Dimerosporiella cephalosporii is similar to *D. sensitiva*, from which it differs by a simple ascal apex and perithecial hairs (Samuels 1988). Both species are commonly associated with an *Acremonium*-like anamorph with thick-walled conidiophores and phialides (Pirozynski 1977). The conidial form is always found together with the perithecia, but there is no molecular evidence that supports this anamorph-teleomorph connection.

Sequence data – The LSU rDNA sequence obtained from fresh material of *D. cephalosporii* (specimen MB139) is 498 bp long and presented 32 ambiguous bases. In the tree

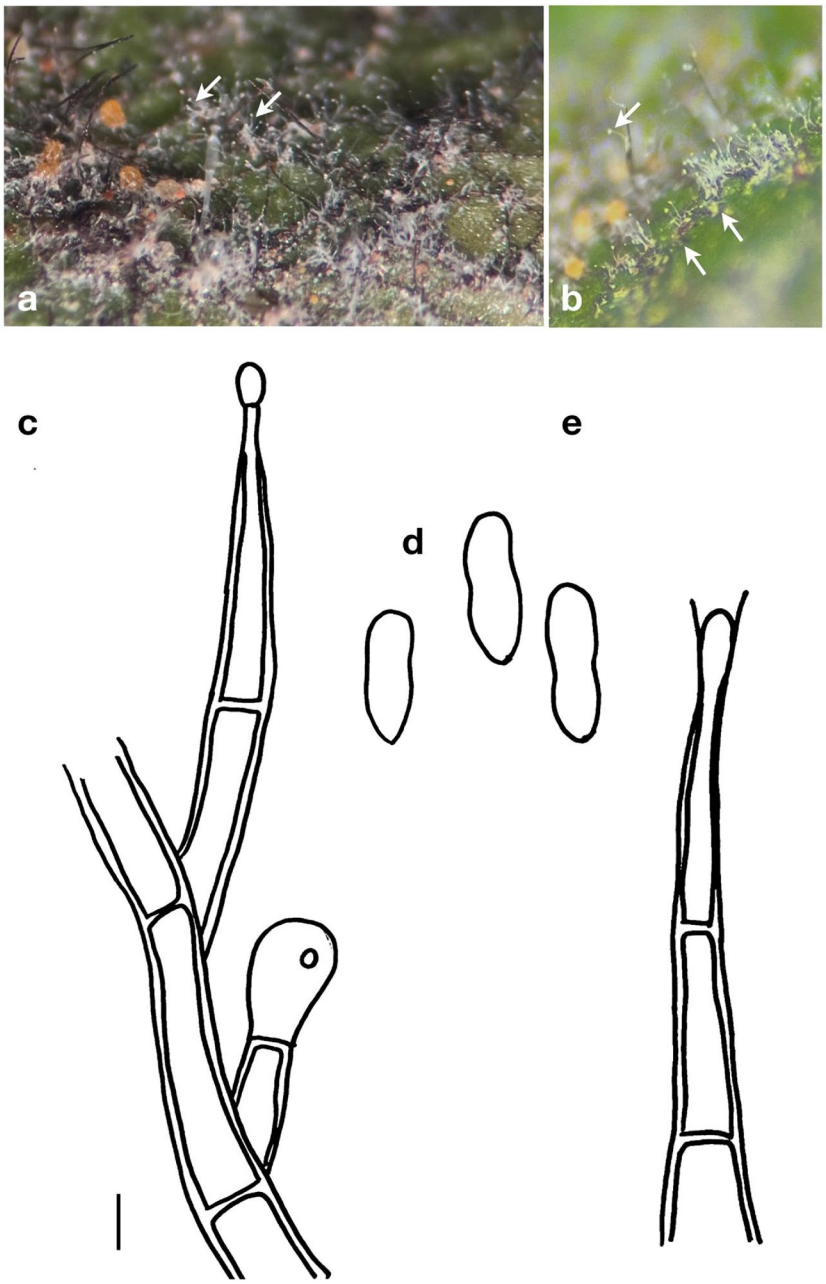

Fig. 6 **The *Acremonium*-like anamorph of *Dimerosporiella cephalosporii*** on ***Meliola pinnatae*** **(MB139)**. **a**, **b** Conidiophores (arrows) with orange perithecia of *D. cephalosporii* on colonies of *Meliola pinnatae*; **c** conidiophore on a hypha of *Meliola* sp. and a young conidium; **d** conidia; **e** tip of a conidiophore with a young conidium. Scale bar: 3 µm (**c–e**)

inferred from the analysis of LSU sequences of 24 specimens (Fig. 4), *D. cephalosporii* is located within a strongly supported clade that comprises sequences of *Acremonium* spp. and other species within the *Bionectriaceae*. It does not cluster with any sequence of *Dimerosporiella*, because no sequences are available for this genus up to now.

Malacaria meliolicola Syd., *Annls Mycol.* 28(1/2): 69, 1930 (Fig. 7).

= *Malacaria flagellata* (Hansf.) Hansf., *Mycol. Pap.* 15: 128, 1946.

≡ *Paranectria flagellata* Hansf., *Proc. Linn. Soc. London* 153(1): 28, 1941.

Colonies white, hyphae growing closely appressed to the dark hyphae of *Meliolales*, 1–2 µm wide, hyaline, thin-walled. Pseudothecia superficial, growing between the synnemata of *Atractilina parasitica*, ovate to elongate ovate with rounded apex, 150–200 × 100–140 µm, dark vinaceous when seen macroscopically, dark cinnamon or brick when seen by light microscopy, not changing color in KOH, smooth. Pseudothecial wall 12–17 µm thick, composed of angular cells with 6–15 µm diam. (surface view). Asci bitunicate, narrowly clavate to cylindric, apex rounded, (40–)52–56(–64) × (9.5–)10–12(–16) µm, 8-spored. Pseudoparaphyses unbranched, abundant, up to 120 µm long, 1–2 µm wide, septate, hyaline, rounded at the ends, with

a gelatinous external layer. Ascospores completely filling each ascus, mostly 3-septate, narrowly clavate, with an elongated base and rounded tips, (37–)44–54(–64) × 3–4.5(–5) µm, pale smoke-grey, smooth.

Specimens examined – On *Meliola* sp. on leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 42″ N 2° 7′ 50″ E, 69 m a.s.l., 28 February 2022, M.A. Bermúdez, A. Tabé, D. Dongnima, M. Piepenbring, N.S. Yorou, MB178; on *Meliola* sp. on living leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 23″ N 2° 8′ 26″ E, 119 m a.s.l., 19 September 2022, A. Krauß, A. Tabé, N.S. Yorou, O. Koukol, AK4H (UNIPAR, M).

Known hosts and distribution – On *Irenina glabra* on leaves of *Coffea robusta* (*Rubiaceae*) in Uganda (Hansford 1941). On *Meliola* sp. on leaves of *Hamelia erecta* (*Rubiaceae*) in Venezuela (Rossman 1987). On *Meliola* sp. on leaves of *Coffea arabica* (*Rubiaceae*) in Benin (this study). *C. arabica* is a new host of *M. meliolicola*, and the hyperparasite is recorded here for Benin for the first time.

Illustrations – This species was illustrated by Rossman (1987).

Notes – *Malacaria meliolicola* (*Tubeufiaceae*, *Tubeufiales*) resembles other perithecioid species such as *Nematothecium vinosum* and *Hyalosphaera miconiae*, but it differs from these species by the presence of unbranched

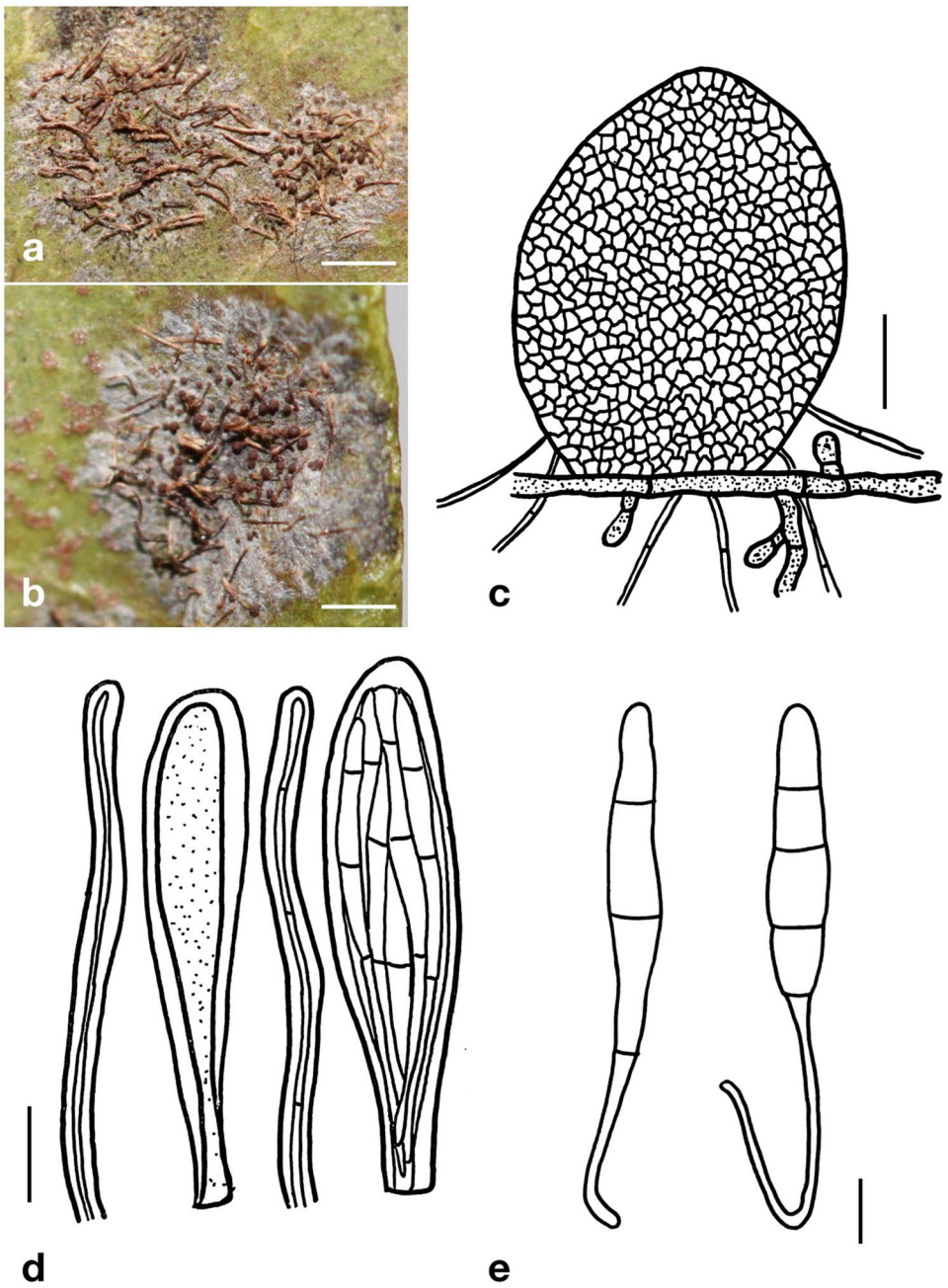

Fig. 7 ***Malacaria meliolicola*** **(AK4H, MB178)**. **a**, **b** Pseudothecia on black hyphae of *Meliola* sp. on living leaves of *Coffea arabica*; **c** pseudothecium on a hypha of *Meliola* sp.; **d** young and mature asci with pseudoparaphyses; **e** ascospores. Scale bars: approx. 500 µm (**a**); approx. 300 µm (**b**); 40 µm (**c**); 10 µm (**d**); 5 µm (**e**)

pseudoparaphyses (Rossman 1987). Hansford (1941, 1946) described *M. meliolicola* as the probable teleomorph of *Atractilina parasitica* (cited as *Arthrobotryum parasiticum*), a common hyperparasite of *Meliolales*. Apparently, the pseudothecia are only found when *A. parasitica* is present. However, there is no molecular evidence of this anamorph-teleomorph connection. According to Deighton and Pirozynski (1972), the connection is doubtful.

Paranectria longiappendiculata Berm.-Cova & M. Piepenbr., sp. nov. (Figs. 4 and 8).

MycoBank: MB#848317.

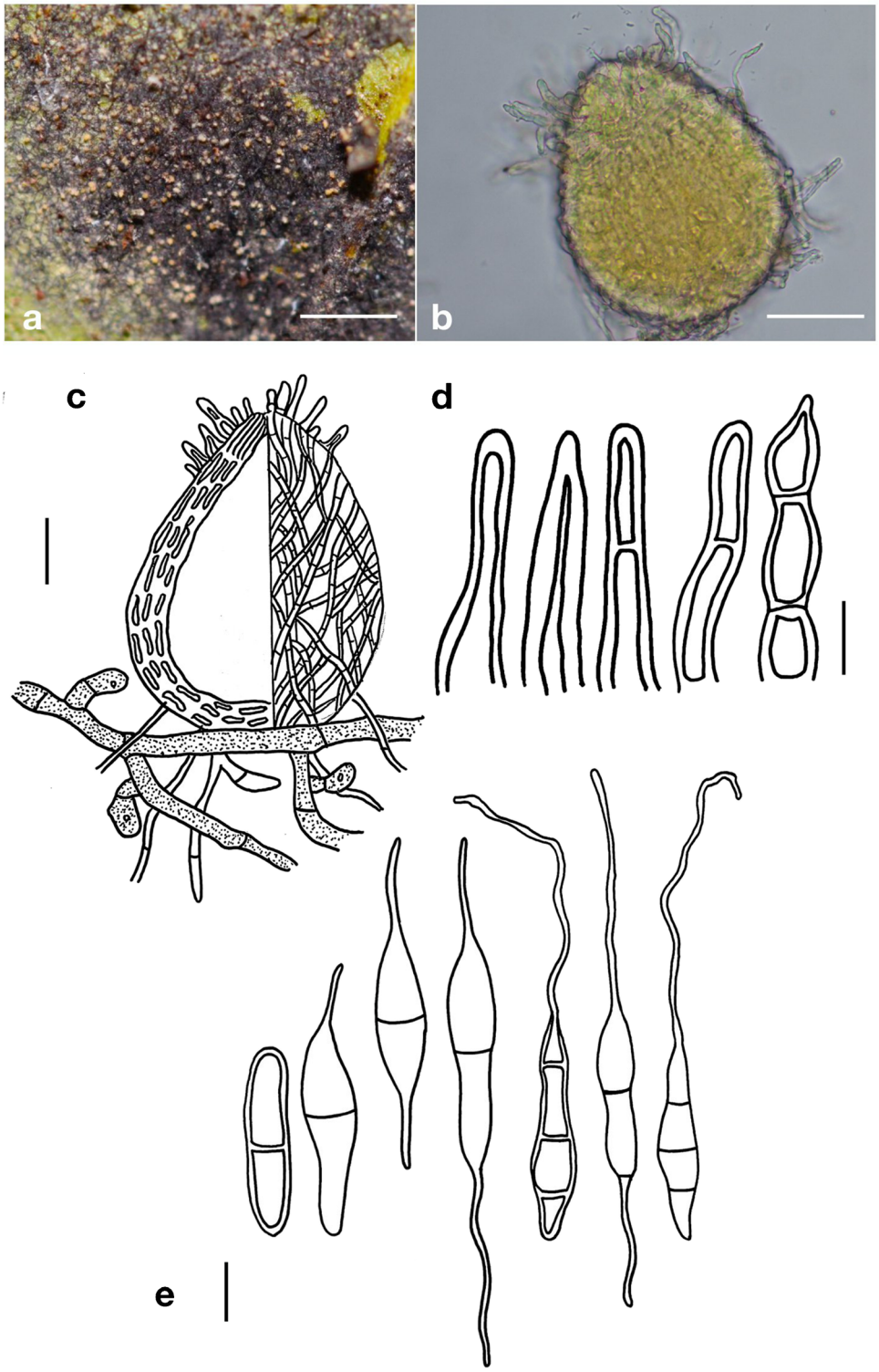

Fig. 8 ***Paranectria longiappendiculata*** **(MB175)**. **a** Perithecia on black hyphae of *Meliola* sp.; **b** perithecium, as seen by light microscopy; **c** A perithecium on hyphae of *Meliola* sp. Left side: cross section view, right side: surface view; **d** perithecial hairs; **e** ascospores. The thickness of the walls is shown for two spores. Scale bars: 3 mm (**a**); 36 µm (**b**); 20 µm (**c**); 5 µm (**d**, **e**)

Holotype – On *Meliola* sp. on living leaves of *Angylocalyx oligophyllus*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 42″ N 2° 7′ 50″ E, 69 m a.s.l., 28 February 2022, M.A. Bermúdez, A. Tabé, D. Dongnima, I. Agonglo, O.P. Agbani, M. Piepenbring, N.S. Yorou, MB175 (M, GenBank accession number: OQ801166).

Paratype – Same locality, collection date, fungal and plant hosts, MB169 (UNIPAR).

Etymology – Named for the long appendages of the ascospores.

Colonies of white thin hyphae covering the colonies of *Meliolales*. Hyphae thin-walled, septate, 1–2 µm wide, hyaline. Perithecia solitary or in small groups, scattered, superficial, ovate to elongate ovate with rounded apex, (70–)90–104(–113) µm diam., pale orange to orange, not changing color in KOH, with ascomatal hairs mostly around the apex. Hairs straight to crooked, non-septate or septate, unbranched, apex obtuse or pointed, 14–20 × 2–4 µm, hyaline. Ascomatal wall 10–13 µm wide, composed of elongated cells parallel to the inner surface of the perithecium as seen in longitudinal section, and of loosely interwoven septate hyphae (surface view). Asci not found. Ascospores fusiform to ellipsoid, (12–)16–21(–32) × 2–4 µm (measurements without appendages), 1–3-septate, hyaline, smooth, with straight or curved appendages at one or both tips (rarely without appendages), up to 40 µm long. Ascospores tend to stick together when liberated from the perithecia. Ascospores tend to separate from each other when KOH is added.

Anamorph – Not known.

Known distribution – On colonies of *Meliola* sp. on living leaves of *Angylocalyx oligophyllus* (*Fabaceae*) in Benin.

Notes – The genus *Paranectria* was proposed by Saccardo (1878), with *P. affinis* as type species, a wood-inhabiting species of the *Hypocreales* (*Sordariomycetes*). The genus initially comprised species with hyaline, 3-septate ascospores that carry appendages at both tips (Rossman 1987). Based on this description, Stevens (1918), Hansford (1941, 1946) and other authors proposed new species in this genus, all with fungicolous lifestyle. However, none of these authors seemed to notice that many of these fungi have bitunicate asci, a feature that is present in the *Dothideomycetes* and not in the *Sordariomycetes*. Therefore, Pirozynski (1977) transferred many of these species to the genus *Paranectriella* (P. Henn.) Piroz., a genus that comprises eight species of tropical hyperparasites of plant parasitic fungi that resemble *Paranectria*, but differ fundamentally in possessing bitunicate asci. In addition to this, cells of perithecial walls of species of *Bionectriaceae* and *Nectriaceae* (*Hypocreales*) typically are thin-walled and elongated parallel to the surface of the perithecia as seen in longitudinal sections (Rossman et al. 1999), while corresponding cells of species of *Paranectriella* are isodiametric (see the examples of *Paranectriella hemileiae* and *Paranectriella minuta* below).

Paranectria longiappendiculata (specimens MB169, MB175) resembles species of the genera *Paranectria* and *Paranectriella* by the fungicolous lifestyle and partly 3-septate ascospores with appendages at the tips. In comparison to *Paranectria affinis* (spores 24–34 µm long; Saccardo 1878), the ascospores of *P. longiappendiculata* are shorter (up to 21 µm long). *Paranectriella hemileiae* and *Paranectriella minuta* produce hairs on the surface of the ascomata like *P. elongata*, but *P. elongata* differs by ascospores with long terminal appendages that can reach a length of up to 40 µm. Appendages of all the other known species of *Paranectria* and *Paranectriella* only reach up to 20 µm (Saccardo 1878; Rossman 1987). Asci were not found in the examined specimens, so it is not possible to assign them to *Sordariomycetes* or *Dothideomycetes* based on details of the walls of asci. The cells of the asci of *P. longiappendiculata*, however, resemble those of species of hypocrealean fungi within the Bionectriaceae and Nectriaceae (Rossman et al. 1999).

Sequence data – The LSU rDNA sequence obtained from fresh material of *P. longiappendiculata* (specimen MB175) is 811 bp long. Based on a MegaBLAST search in the NCBI GenBank nucleotide database using the LSU sequence data of *P. longiappendiculata*, the closest match was *Acremonium acutatum* (GenBank MH872055; identities 726/799, i.e., 90.86%), as well as other species of hypocrealean fungi. The morphological features discussed above, together with the results of the MegaBLAST search, confirm the placement of *P. longiappendiculata* in the *Hypocreales* and in the genus *Paranectria*. In the tree inferred from the analysis of LSU sequences of 24 specimens (Fig. 4), *P. longiappendiculata* is located within a strongly supported clade that comprises sequences of *Acremonium* spp. and other species within the *Bionectriaceae*.

Paranectriella hemileiae (Hansf.) Piroz., *Kew Bull.* 31: 598, 1977 (Fig. 9).

≡ *Paranectria hemileiae* Hansf., *Proc. Linn. Soc. Lond.* 153: 28, 1941.

Colonies of white hyphae spreading over the colonies of *Meliola* sp. Pseudothecia solitary, scattered, superficial, globose to subglobose, 130–180 µm diam., pale luteous to white, not changing color in KOH, with sparse to abundant ascomatal hairs, scattered all over the ascomatal surface. Hairs straight to slightly sigmoid, septate or non-septate, unbranched, thick-walled, 14–30 × 4–6 µm, hyaline. Pseudothecial wall composed of isodiametric cells, 5–9 µm, thin-walled (surface view). Asci bitunicate, clavate to broadly cylindric, apex rounded, 50–68 × 9–14 µm, 8-spored. Pseudoparaphyses not seen. Ascospores fusiform, mostly 3-septate, slightly constricted at the septa, with straight appendages mostly at both tips, (14–)16–18(–20) × 5–7 µm, hyaline, smooth.

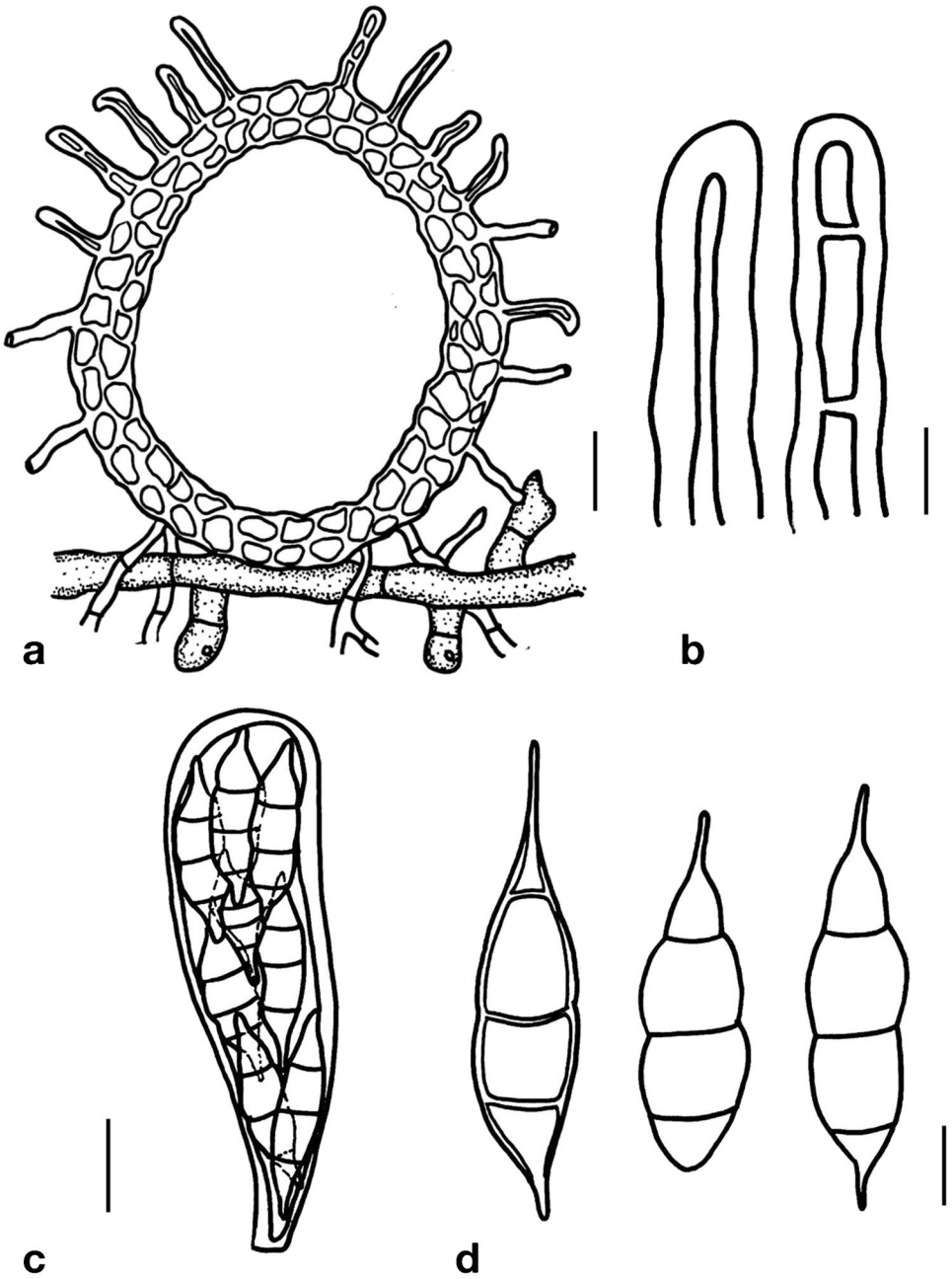

Fig. 9 ***Paranectriella hemileiae*** **(MB108)**. **a** Pseudothecium on a hypha of *Meliola* sp. (content not drawn); **b** perithecial hairs; **c** ascus with ascospores; **d** ascospores. Scale bars: 25 µm (**a**); 5 µm (**b**); 10 µm (**c**); 3 µm (**d**)

Anamorph – Not observed (*Titaea hemileiae* Hansf. according to Rossman 1987).

Specimen examined – On *Meliola* sp. on living leaves of *Xylopia frutescens*, Panama, Chiriquí Province, Cochea, Cochea river trail, 8° 32′ 37″ N 82° 23′ 03″ W, 181 m a.s.l., 26 February 2020, M.A. Bermúdez, A. Sanjur, A. Villarreal, MB108 (UCH13409).

Known hosts and distribution – On sori of *Hemileia vastatrix* (*Pucciniales*) on leaves of *Coffea robusta* (*Rubiaceae*) in Uganda (Rossman 1987); on *Meliola* sp. on leaves of *Xylopia frutescens* (*Annonaceae*) in Panama (this study). *Meliola* sp. and *X. frutescens* are new hosts of *P. hemileiae*, and the hyperparasite is recorded here for mainland America (Panama) for the first time.

Illustrations – This species was illustrated by Pirozynski (1977) and Rossman (1987), as well as by Carmichael et al. (1980, anamorph only) and Hansford (1946, anamorph only).

Notes – Up to now, the sexual form *Paranectriella hemileiae* is only known from the type specimen, growing on sori of *Hemileia vastatrix.* Despite its occurrence on a rust, the species is retained in the genus *Paranectriella* due to the presence of 3-septate ascospores with terminal appendages (Rossman 1987). There is a possible associated

anamorph to this species, namely, *Titaea hemileiae*. It produces staurospores, like some other species (e.g., *P. miconiae*; Pirozynski 1977, Rossman 1987). However, no conidia were found in the examined specimen (MB108).

Paranectriella minuta (Hansf.) Piroz., *Kew Bull.* 31(3): 600, 1977 (Fig. 10).

≡ *Paranectria minuta* Hansf., *Proc. Linn. Soc. London* 153(1): 30, 1941.

Colonies of white hyphae covering colonies of *Meliolales*. Hyphae thin-walled, septate, 2–3 µm wide, hyaline. Pseudothecia solitary or in small groups, scattered, superficial, globose, (80–)90–115(–150) µm diam., pale luteous, pale orange to white, translucent, not changing color in KOH, with ascomatal hairs more or less close to the apex. Hairs straight to crooked, unbranched, apex obtuse, non-septate, 24–40 × 3–6 µm, hyaline. Pseudothecial wall 6–10 µm thick, composed of isodiametric cells 7–15 µm wide, thin-walled (surface view). Asci bitunicate, broadly cylindric to obovate, apex rounded, (37–)40–50(–61) × 12–18 µm, 8-spored. Pseudoparaphyses not seen. Ascospores fusiform to ellipsoid, 3-septate, slightly constricted at the septa, with a straight or curved appendage of 3–11 µm length at each tip, (14–)16–18 × 5–6 µm, hyaline, smooth.

Specimens examined – On *Meliola* sp. on living leaves of *Coffea arabica*, Benin, Atlantique, Attogon, Niaouli Forest, 6° 44′ 23″ N 2° 8′ 26″ E, 119 m a.s.l., 19 September 2022, A. Krauß, A. Tabé, N.S. Yorou, O. Koukol, AK4H (M); on *Meliola* sp. on living leaves of *Opilia celtidifolia*, Benin, Donga, Bassila, 8° 59′ 58″ N 1° 38′ 45″ E, 360 m a.s.l., 27 September 2022, A. Krauß, A. Tabé, N.S. Yorou, O. Koukol, AK38H (UNIPAR, GenBank accession number: OQ801179).

Known hosts and distribution – On *Meliola paulliniae* on leaves of *Paullinia pinnata* (*Sapindaceae*) in Uganda (Hansford 1941); on *Meliola* sp. on leaves of *Coffea arabica* (*Rubiaceae*) in Benin (this study); on *Meliola* sp. on leaves of *Opilia celtidifolia* (*Opiliaceae*) in Benin (this study). *C. arabica* and *O. celtidifolia* are new hosts of *P. minuta*, and the hyperparasite is recorded here for Benin for the first time.

Anamorph – Not known.

Illustrations – This species was illustrated by Hansford (1941), Pirozynski (1977) and Rossman (1987).

Notes – *Paranectriella minuta* is similar to *P. hemileiae*, but the ascomatal hairs of *P. minuta* are located mostly close to the apex of the pseudothecium. The presence of appendages on the ascospores and small, translucent ascomata can also occur in some species of the genus *Hyalocrea*, but *Hyalocrea* spp. are characterized by the absence of pseudoparaphyses (Rossman 1987). Pseudoparaphyses, however, were not found in the specimen examined (AK38H). Nevertheless, we identify the specimens from Benin as *P. minuta*, because the ascospores of these specimens are smaller than those of hyperparasitic species of *Hyalocrea* (e.g., *H. meliolicola*, 26–35 × 7–9 µm; Rossman 1987).

Sequence data – The LSU rDNA sequence obtained from fresh material of *P. minuta* (specimen AK38H) is 494 bp long. Based on a MegaBLAST search in the NCBI GenBank nucleotide database using the LSU sequence data of *P. minuta*, the closest match was *Quixadomyces hongheensis* (GenBank MW264194; identities 460/491, i.e., 93.69%), as well as other species of *Pleosporales*. Hyde et al. (2013) designated the family *Paranectriellaceae* to accommodate hyperparasitic species of *Dothideomycetes* with bright colored ascomata, ascospores with transverse septa and prominent appendages. However, there is no molecular DNA sequence data that supports this designation. Ours represent the first DNA sequence of a fungus of the genus *Paranectriella*, and more sequences are necessary to evaluate this hypothesis.

Key to species of perithecioid hyperparasites on *Meliolales* known for Benin and Panama

1 Ascomata dark vinaceous to dark brick; ascospores smoke-gray..... ***Malacaria meliolicola***
1* Ascomata white, pale luteous to orange; ascospores hyaline..... 2
2 Ascospores 1-septate, biguttulate; asci unitunicate..... ***Dimerosporiella cephalosporii***
2* Ascospores (up to) 3-septate, with appendages at their tips; asci bitunicate..... 3
3 Appendages 20–40 µm long..... ***Paranectria longiappendiculata***
3* Appendages up to 20 µm long..... 4
4 Pseudothecia with sparse to abundant thick-walled ascomatal hairs, scattered all over the ascomatal surface..... ***Paranectriella hemileiae***
4* Pseudothecia with ascomatal hairs mostly close to the apex ***Paranectriella minuta.***

Discussion

Hyperparasitic fungi on *Meliolales* have been collected in the past mainly in Brazil, Dominican Republic, and Puerto Rico in America, as well as in Ghana, Sierra Leone, and Uganda in Africa (Bermúdez-Cova et al. 2022). In the context of the present study, we analyzed 16 specimens of *Meliolales* associated with hyperparasites, corresponding to eight species of hyperparasitic fungi. Seven species represent new records: five for Benin and four for Panama. One species is new to science. *Calloriopsis herpotricha* is recorded for the first time for Africa and *Dimerosporiella cephalosporii*

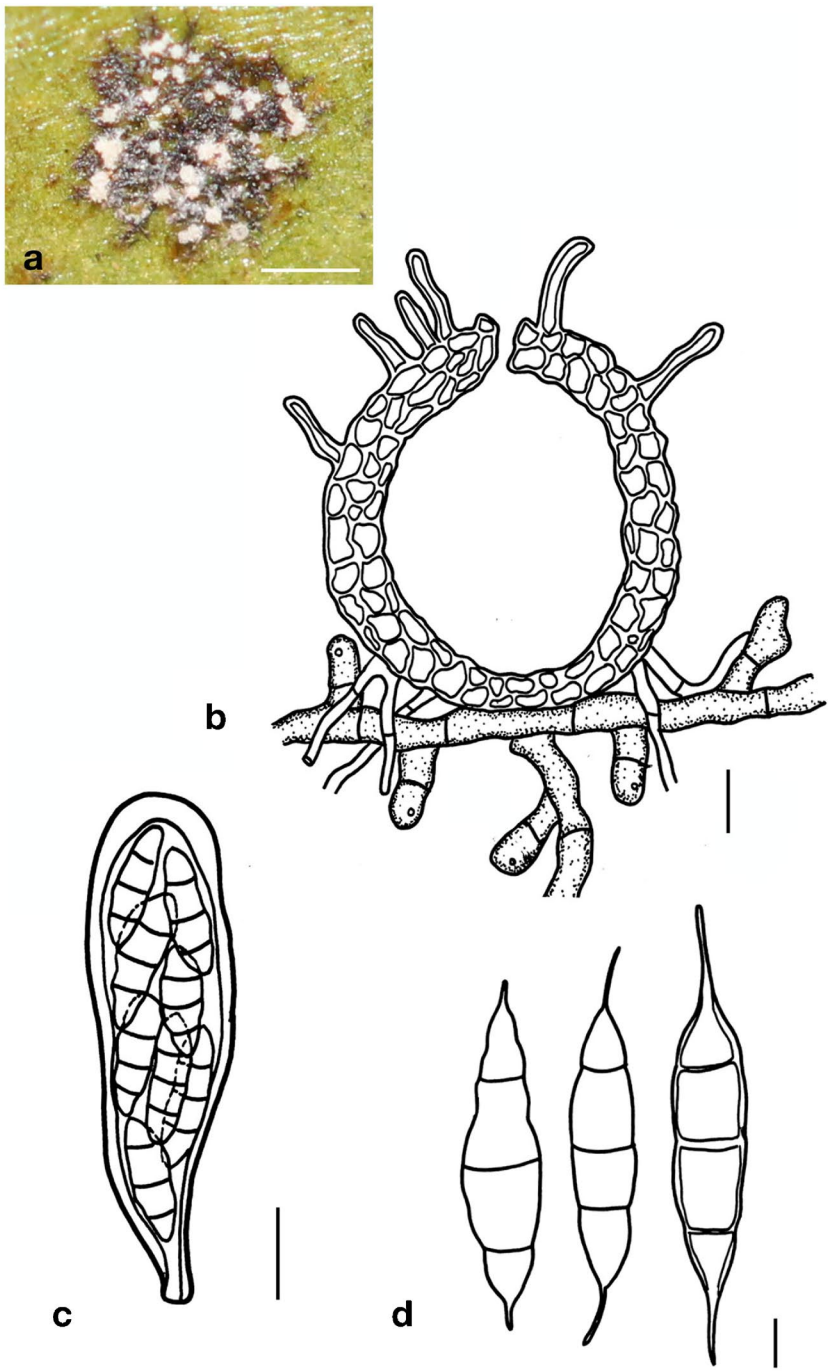

Fig. 10 ***Paranectriella minuta (AK4H, AK38H)***. **a** Pseudothecia on black hyphae of *Meliola* sp. on a living leaf of *Coffea arabica*; **b** pseudothecium on hyphae of *Meliola* sp.; **c** ascus with ascospores; **d** ascospores. Scale bars: 500 µm (**a**); 15 µm (**b**); 10 µm (**c**); 3 µm (**d**)

and *Paranectriella hemileiae* for mainland America. These findings are based on only three months of fieldwork and show a blatant lack of investigation on hyperparasitic fungi in the tropics.

Patterns of distribution of hyperparasitic fungi have been studied mainly for hyperparasites of rusts and powdery mildews (Zewdie et al. 2021), but never for those infecting black mildews. The distribution of hyperparasitic fungi is restricted to that of their host (Sun et al. 2019). As *Meliolales* are restricted to tropical and subtropical areas (Piepenbring 2015), hyperparasites are expected to be found in these regions as well. We also expect wide distribution areas of hyperparasitic fungi on *Meliolales* because of their broad spectra of host species (Bermúdez-Cova et al. 2022). In fact, the data presented in this study suggest that at least part of the species of hyperparasitic fungi of *Meliolales* have a pantropical distribution, as they have been recorded both in paleotropical and neotropical regions. This is consistent with the assumptions made by Samuels et al. (2002) regarding the pantropical distribution

of tropical perithecioid fungi. Extensive additional fieldwork is needed in order to unravel distribution patterns of hyperparasitic fungi on meliolalean hosts.

It is difficult to obtain molecular sequence data from hyperparasites especially because of their incapability of growing in artificial media and the fact that they develop intermingled with the primary parasite and many other organisms (Bermúdez-Cova et al. 2022). As a consequence, isolating and sequencing hyperparasitic fungi is a challenging task. There is also a lack of sequences of hyperparasitic fungi in public databases. Therefore, the sequences obtained in the context of the present work can be related to existing species concepts only based on morphology, and issues such as anamorph-teleomorph connections cannot be confirmed. Nevertheless, in this study for the first time ever, DNA sequences of hyperparasitic fungi on *Meliolales* are published. This example emphasizes that field work paired with molecular analysis still plays a crucial role for modern mycology, especially for challenging fungal groups, such as hyperparasites.

Acknowledgements We are grateful to the University of Parakou and the University of Abomey-Calavi, Benin, for the support and facilities made available for this study. We acknowledge help by Dr. Pierre Agbani (Botanical Garden of the Université d'Abomey-Calavi) for his assistance with the identification of host plants and help by Daouda Dongnima during fieldwork. We acknowledge the support and facilities made available by Orlando Cáceres and the Universidad Autónoma de Chiriquí (UNACHI) in Panama. The Environmental Ministry of Panama (MiAmbiente) is thanked for issuing the collection and export permits (SE/APHO-1-2019, SEX/H-5-2020, PA-01-ARG-049-2021). We are grateful to the Ministry of Environment of the Benin Republic for issuing the collecting permits and for the elaboration of the ABS Nagoya Protocol documents n° 636/DGEFC/ANC-APA/DCPRN/PF-APA.

Author contribution All authors contributed to the study conception and design. Affousatou Tabé, Alicia Sanjur, Anna Krauß, Meike Piepenbring, Miguel Bermúdez-Cova, and Nourou S. Yorou collected specimens. Samples and collection permits preparation were performed by Tina A. Hofmann and Nourou S. Yorou. The first draft of the manuscript was written by Miguel Bermúdez-Cova, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was supported by the Federal Ministry of Education and Research (BMBF, Germany) as part of the project "Diversity and Uses of Tropical African Fungi: Edible mushrooms of Benin" (01DG20015FunTrAf). A special gratitude goes to the German Academic Exchange Service (DAAD), for supporting the first author within the framework of the scholarship program for doctoral studies in Germany (Ref. no.: 91726217). AT is supported by the Franz Adickes Foundation Fund, within the framework of the scholarship program for international doctoral candidates offered by the Goethe Research Academy for Early Career Researchers (GRADE).

Data Availability Specimens are deposited in the herbarium at the Universidad Autónoma de Chiriquí (UCH), in the mycological herbarium of the University of Parakou (UNIPAR) and/or in the Botanische Staatssammlung München (M). Sequence data are submitted to GenBank.

Declarations

Competing interests The authors declare no competing interests.

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