

Novel Brazilian *Meliolaceae*, including the new genera *Parappendiculella* and *Setoasteridiella*

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Abstract: The *Meliolaceae* is a large family that encompasses *ca* 2.400 spp. included in six to eight genera. These are biotrophic parasites of aerial parts of plants. Taxa in this family typically produce black colonies and are collectively known in the plant pathology literature as black mildews. Although the number of species included in the family is large, it is thought that the diversity of the *Meliolaceae* remains underestimated, particularly in tropical countries such as in Brazil. This manuscript represents an example of this undescribed mycodiversity as it combines findings from a few *ad hoc* collections made by RWB at two Brazilian municipalities: Nova Friburgo (state of Rio de Janeiro) and Viçosa (state of Minas Gerais). Black mildews on four different plant hosts were examined: *Acrocomia aculeata* (*Arecaceae*), *Austroeupatorium inulaefolium* (*Asteraceae*), *Cissus sicyoides* (*Vitaceae*) and *Struthanthus* sp. (*Loranthaceae*). A polyphasic study of these specimens, integrating host-association data, morphological characteristics and phylogenetic analyses justified the proposition of *Meliola acrocomiae* sp. nov. (on *A. aculeata*), *Appendiculella setosa* sp. nov. (on *A. inulaefolium*) as well as two novel genera, namely: *Parappendiculella struthanthi* gen. et sp. nov. (on *Struthanthus* sp.) and *Setoasteridiella valliformis* gen. et sp. nov. (on *C. sicyoides*). Those findings contribute to expand the known diversity of the Brazilian *Meliolaceae* and the list of genera in the family.

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INTRODUCTION

Although *Meliola*, the type species of the family *Meliolaceae*, was sanctioned by Fryes in 1825, the family *Meliolaceae* (*Ascomycota*, *Sordariomycetes*) was only erected much later (Hansford 1946). They include obligate (biotrophic) ectoparasites of plants, predominantly found in tropical or subtropical regions worldwide (Hongsanan *et al.* 2015, Rodríguez Justavino *et al.* 2015). *Meliolaceae* have hosts belonging to 194 plant families (Zeng *et al.* 2017). These fungi are recognized by their typical feature of producing black spiderweb-like colonies on the surface of leaves, stems, and other aerial parts of plants. This appearance has led to their treatment in the plant pathology literature as black mildews (Hongsanan *et al.* 2015, Rodríguez Justavino *et al.* 2015, Hyde *et al.* 2020).

From six (Marasinghe *et al.* 2020) to eight (Wijayawardene *et al.* 2020) genera are recognized as members of the *Meliolaceae*: *Amazonia*, *Appendiculella*, *Asteridiella*, *Cryptomeliola*, *Endomeliola*, *Irenopsis*, *Meliola*, and *Setameliola*. According to Zeng *et al.* (2017), 2400 spp. have been described in the family. *Meliola* is the largest genus in the *Meliolaceae*, with more than 1765 spp. (Khan *et al.* 2025b).

Here, a polyphasic approach, as discussed by Gannibal (2022), was applied to clarify the taxonomy of four meliolaceous fungi collected in Brazil.

MATERIAL AND METHODS

Sampling material and morphology

From 2020 to 2024, plants presenting black mildew colonies were serendipitously found at different locations in Viçosa, (state of Minas Gerais) and Nova Friburgo (state of Rio de Janeiro), Brazil. The following plant species bearing black mildew symptoms were collected: *Acrocomia aculeata* (*Arecaceae*), *Cissus sicyoides* (syn. *Cissus verticillata*) (*Vitaceae*), *Austroeupatorium inulaefolium* (*Asteraceae*), and *Struthanthus* sp. (*Loranthaceae*). Colonized leaves were collected, dried in a plant press, and brought to the laboratory for further analyses. Representative specimens were deposited in the fungarium of Universidade Federal de Viçosa (VIC) (Table 1).

Fresh material was examined under an Olympus SZX7 (Olympus, Tokyo, Japan) stereomicroscope, and fungal structures were scraped with a scalpel and mounted in lactoglycerol or lactofuchsin. The slides were observed under an Olympus BX51 (Olympus, Tokyo, Japan) light microscope equipped with an Olympus Q-Color 3 (Olympus, Canada) digital capture system. Biometric data were based on the observation of at least 30 representative fungal structures.

Selected fragments were examined under a scanning electron microscope (SEM) Carl-Zeiss Model LEO VP 1430 SEM (Jena,

Table 1. List of members of the *Meliolaceae* included in the phylogenetic analyses and their GenBank accession numbers.

Genera	Species	Culture accession number(s)	Host/ substrate	GenBank Access Number		References
				LSU	ITS	
<i>Appendiculella</i>	<i>Appendiculella lozanellae</i>	MP 3432	<i>Lozanella enantiophylla</i>	DQ508302	n/a	Rodríguez Justavino <i>et al.</i> (2015)
	<i>Appendiculella sororcula</i>	VIC 32065	<i>Mikania</i> sp.	KC618640	n/a	Unpublished
	<i>Appendiculella viticis</i>	MFLU 19-1008*	<i>Vitex canescens</i>	MT108888	MT108889	Marasinghe <i>et al.</i> (2020)
	<i>Appendiculella setosa</i> sp. nov.	VIC 49606*	<i>Austro eupatorium inulaefolium</i>	n/a	PV983259	This study
<i>Asteridiella</i>	<i>Asteridiella atricha</i>	VIC 32064	<i>Myrciaria strigipes</i>	KC618650	n/a	Unpublished
	<i>Asteridiella callista</i>	MTCHT150	Unidentified	OM296103	n/a	Unpublished
	<i>Asteridiella combreticola</i>	MFLU 17-1041*	<i>Combretum</i> sp.	MN747481	NR_174812	Zang <i>et al.</i> (2020)
	<i>Asteridiella nitidae</i>	ppMP 796	<i>Buddleja nitida</i>	EF094839	n/a	Rodríguez Justavino <i>et al.</i> (2015)
	<i>Asteridiella obesa</i>	VIC 31239*	<i>Balfourodendron riedelianum</i>	NG057014	NR_120256	Pinho <i>et al.</i> (2012b)
	<i>Asteridiella pittieri</i>	DP114	<i>Duranta repens</i> var. <i>aurea</i>	KC618639	n/a	Unpublished
<i>Endomeliola</i>	<i>Endomeliola dingleyae</i>	PDD 98304	<i>Coprosma robusta</i>	GU138866	GU138865	Unpublished
	<i>Irenopsis cornuta</i>	VIC 32058	<i>Lygodium volubile</i>	KC618642	n/a	Unpublished
	<i>Irenopsis crotonicola</i>	MFLU 14-0078*	<i>Croton persimilis</i>	KY554798	n/a	Zang <i>et al.</i> (2018)
	<i>Irenopsis moelleriana</i>	VIC32059ad	<i>Pavonia</i> sp.	KC618646	n/a	Unpublished
<i>Irenopsis</i>	<i>Irenopsis vincensii</i>	VIC 31752*	<i>Hevea brasiliensis</i>	JX096807	NR_154061	Pinho <i>et al.</i> (2012a)
	<i>Irenopsis walsurae</i>	MFLU 13-0621*	<i>Walsura tubulata</i>	KT021648	NR_154075	Hongsanan <i>et al.</i> (2015)
<i>Meliola</i>	<i>Meliola acaciae</i>	MFLU 16-0077	<i>Acacia auriculiformis</i>	MN747479	NR_174811	Zang <i>et al.</i> (2020)
	<i>Meliola acrocomiae</i> sp. nov.	VIC 49604*	<i>Acrocomia aculeata</i>	PV983262	PV983258	This study
	<i>Meliola anacardii</i>	URM 90240	<i>Anacardium occidentale</i>	OP221711	n/a	Alves <i>et al.</i> (2023)
	<i>Meliola aristolochiae-tagalae</i>	MFLU 16-0088*	<i>Aristolochia tagala</i>	NG_079535	n/a	Zang <i>et al.</i> (2020)
	<i>Meliola brachyodonta</i>	VIC32066	<i>Anacardium occidentale</i>	KC618644	n/a	Unpublished
	<i>Meliola caesalpiniicola</i>	VIC 32061	<i>Fabaceae</i>	KC618641	n/a	Unpublished
	<i>Meliola camporesii</i>	MFLU 19 1005*	<i>Mangifera indica</i>	NG_228823	NR_186987	Bhunjun <i>et al.</i> (2022)
	<i>Meliola centellae</i>	VIC 31244*	<i>Centella asiatica</i>	JQ734545	NR_137799	Pinho <i>et al.</i> (2012a)
	<i>Meliola citri-maximae</i>	MFLU 14-0288	<i>Citrus maxima</i>	KX458474	NR_185523	Hyde <i>et al.</i> (2016)
	<i>Meliola clerodendricola</i>	MFLU 13-0620*	<i>Clerodendrum</i> sp.	KT021647	n/a	Hongsanan <i>et al.</i> (2015)
	<i>Meliola clerodendri-infortunati</i>	MFLU 13-0624*	<i>Clerodendrum infortunatum</i>	MF175626	NR_160479	Zang <i>et al.</i> (2020)
	<i>Meliola crescentiae</i>	VIC 32056	<i>Arrabidaea subincana</i>	KC618649	n/a	Unpublished
	<i>Meliola danielliae</i>	VIC 32057	<i>Apuleia leiocarpa</i>	KC618648	n/a	Unpublished
	<i>Meliola daviesii</i>	MTCHT170	Unidentified	OM296104	n/a	Unpublished
	<i>Meliola gorongosensis</i>	ILLS00173293*	<i>Philenoptera violacea</i>	n/a	MK802897	Crous <i>et al.</i> (2019)
	<i>Meliola jasmini-sambac</i>	MFLU 17-1044*	<i>Jasminum sambac</i>	MN747482	NR_174813	Zang <i>et al.</i> (2020)
	<i>Meliola lithocarpigena</i>	MFLU 13-0628*	<i>Lithocarpus</i> sp.	MN747474	NR_174809	Zang <i>et al.</i> (2020)
	<i>Meliola mangiferae</i>	URM 90236	<i>Mangifera indica</i>	OP221712	n/a	Alves <i>et al.</i> (2023)
	<i>Meliola monnieriae</i>	VIC 32062	<i>Dictyoloma vandellianum</i>	KC618647	n/a	Unpublished
	<i>Meliola mucunicola</i>	MFLU 15-0386*	<i>Mucuna pruriens</i>	KT157533	KT157534	Hongsanan <i>et al.</i> (2015)

<i>Meliola niessleana</i>	UBC F23799	<i>Gaultheria shallon</i>	KC833049	n/a	Unpublished
<i>Meliola panici</i>	VIC 32063	<i>Lasiacis ligulata</i>	KC618651	n/a	Unpublished
<i>Meliola peruiferae</i>	VIC 31249	<i>Myroxylon peruiferum</i>	KC618638	n/a	Unpublished
<i>Meliola pistaciicola</i>	MFLU 16-0070*	<i>Pistacia</i> sp.	MN747478	n/a	Zang <i>et al.</i> (2020)
<i>Meliola pottsiae</i>	MFLU 13-0631*	<i>Pottisia laxiflora</i>	MN747475	NR_174810	Zang <i>et al.</i> (2020)
<i>Meliola pseudosasaiae</i>	MFLU 16-2136*	<i>Sasa borealis</i>	KX845434	n/a	Hyde <i>et al.</i> (2016)
<i>Meliola puerariae</i>	MFLU 16-0087*	<i>Pueraria montana</i>	NG_079534	n/a	Zang <i>et al.</i> (2020)
<i>Meliola schimigena</i>	MFLU 17-1048*	<i>Schima</i> sp.	MN747484	n/a	Zang <i>et al.</i> (2020)
<i>Meliola tamarindi</i>	MFLU 14-0282*	<i>Tamarindus indica</i>	NG_228738	n/a	Liu <i>et al.</i> (2015)
<i>Meliola tamarindi</i>	MFLU 14-0080	<i>Tamarindus indica</i>	KY554799	n/a	Zang <i>et al.</i> (2017)
<i>Meliola telosmae</i>	MFLU 14-0003	Unidentified	MK103389	n/a	Unpublished
<i>Meliola thailandicum</i>	MFLU15-0044*	<i>Dimocarpus longan</i>	KR868697	KR868702	Hongsanan <i>et al.</i> (2015)
<i>Meliola thailandicum</i>	MFLUCC 15-0047	<i>Dimocarpus longan</i>	KR868698	KR868703	Hongsanan <i>et al.</i> (2015)
<i>Meliola trichostroma</i>	VIC 32068	<i>Psidium guajava</i>	KC618643	n/a	Unpublished
<i>Meliola variaseta</i>	DRJ 54	<i>Cupania</i> sp. (Sapindaceae)	EF094840	n/a	Unpublished
<i>Meliola vernaliae</i>	VIC 31240*	<i>Cupania vernalis</i>	JX096808	n/a	Pinho <i>et al.</i> (2012a)
<i>Parappendiculella</i>	<i>Parappendiculella struthanthi</i> sp. nov.	<i>Struthanthus</i> sp.	PV983263	PV983260	This study
<i>Setoasteridiella</i>	<i>Setoasteridiella valliformis</i> sp. nov.	<i>Cissus verticillata</i>	PV983264	PV983261	This study
<i>Chaetosphaeria</i>	<i>Chaetosphaeria innumera</i>	decaying wood, <i>Acer pseudoplatanus</i>	OP455464	OP455358	Reblova & Nekvindova (2022)

* Type specimens are indicated with an asterisk and sequences generated in this study are highlighted in bold.

Germany) at an operating voltage of 12 kV and a working distance between 10 and 30 mm. Prior to examination, such specimens were thoroughly dried, attached to copper stubs, and coated with a gold layer using a Quorum Q150RS rotary pumped coater (Laughton, England).

DNA extraction and PCR amplification

Black mildew colonies from each sample were selected under a dissecting microscope, and only clean structures free of mycoparasites were collected with a sterile scalpel and separately transferred to each of four 1.5 mL microcentrifuge tubes. Genomic DNA was obtained using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) by following the manufacturer's instructions adjusted according to Pinho *et al.* (2013).

The internal transcribed spacer (ITS) and the 28S large subunit (LSU) of the rDNA were amplified using the primers ITS4/ITS1 (White *et al.* 1990) and LR0R/LR5 (Vilgalys & Hester 1990), respectively. Each 12.5 µL of PCR reaction contained 0.5 µL of each forward and reverse primer (10 µM), 0.5 µL of dimethyl sulfoxide (DMSO, Sigma-Aldrich), 1.25 µL of sterile ultrapure water, 1.5 µL of bovine serum albumin (BSA, Sigma-Aldrich), 2 µL genomic DNA (30 ng/µL), and 6.25 µL of DreamTaq DNA polymerase (Thermo Fisher Scientific, Vilnius, Lithuania). PCR was performed under the conditions described by Pinho *et al.* (2013). PCR products were analysed on 1 % agarose electrophoresis gels stained with GelRed™ (Biotium Inc., Hayward, CA, USA) and visualized under UV light. The remaining amplified reactions were purified using an ExoSAP IT purification kit (Amersham Biosciences, Arlington Heights, IL, USA) and sequenced by

MacroGen (Seoul, South Korea, <http://www.macrogen.com>).

Nucleotide sequences were examined using SeqAssem software (SequentiX Digital DNA Processing, Klein Raden, Germany). Nucleotides with ambiguous positions were manually adjusted, and consensus sequences were generated. The sequences which were obtained were deposited in GenBank (Table 1).

Phylogenetic analysis

In order to clarify the relationship between our isolates and members of the *Meliolaceae*, sequences were retrieved from GenBank and recent articles on this family were consulted (Hyde *et al.* 2020, Marasinghe *et al.* 2020, Zeng *et al.* 2020, Bhunjun *et al.* 2022). *Chaetosphaeria innumera* CBS 145639 was selected as the outgroup (Reblova & Nekvindova 2023) (Table 1). Sequences were aligned using the MUSCLE algorithm in MEGA X v. 10.2.2 software (Kumar *et al.* 2018), and concatenated alignment was obtained using SequenceMatrix v. 1.8 software (Vaidya *et al.* 2011).

Phylogenetic studies were conducted using two methods: maximum likelihood (ML) and Bayesian inference (BI), based on tools available through the CIPRES portal (Miller *et al.* 2010). The tool RAXML-HP v. 8.2.12 (Stamatakis 2014) was used to obtain ML trees. Maximum likelihood was performed with 1000 bootstrap replicates, and bootstrap support (BS) values were determined.

Prior to applying BI, the best nucleotide substitution model for each gene region was determined using jModeltest v. 2.1.7 (Darriba *et al.* 2012), which was also available within the CIPRES portal. For the analysis, MrBayes v. 3.2.7a (Ronquist *et al.* 2012) was used, employing the Markov

Chain Monte Carlo method (MCMC). Two simultaneous and independent analyses were run, each with four MCMC chains. In each analysis, trees were randomly generated for 5 M generations with samples occurring every 500 generations, yielding 10000 sampled trees. The first 2500 trees (25 %) were discarded as burn-in, and the remaining 7500 trees from each analysis were used to produce the consensus tree, from which the posterior probability (PP) values were determined. The trees which were obtained were visualized using FigTree v. 1.4.4. software (Rambaut 2018).

RESULTS

Phylogenetic analysis

Consensus sequences obtained in this study varied between 544 and 582 bp (ITS) and 790–848 bp (LSU). BLASTn searches were performed for all sequences and compared with sequences available in GenBank for members of the *Meliolaceae*, which were included in the phylogenetic study.

The final alignments consisted of 54 taxa (Table 1). The combination of the ITS and LSU regions was represented by 2144 characters, including gaps (ITS: 1056, LSU: 1088 sites), 711 of which were conserved (ITS: 222, LSU: 489 sites), and 723 were potentially parsimony informative (ITS: 420, LSU: 303 sites).

The results generated by jModeltest led to the adoption of the GTR+I+G model for ITS and LSU. The ML and BI trees had equivalent topologies. Our phylogenetic tree (Fig. 1) indicated that among the four black mildew fungi collected in the study, two belonged to known genera, *Meliola* (VIC 49604) and *Appendiculella* (VIC 49606), whereas the other two (VIC 49607 and VIC 49410) did not group with any known genera in the *Meliolaceae*. These were regarded as potential new genera in the family.

VIC 49604 grouped with high support (100 % / 1.00, ML / BI) with *M. panici* and *M. pseudosasa* in the *Meliola*-like clade. Nevertheless, it formed a branch that was separated from these two species.

VIC 49606 grouped with high support (100 % / 1.00, ML / BI) within the *Appendiculella* clade but was separated from other species in the clade.

VIC 49607 was close to the *Appendiculella* clade but formed a long branch separated from the other taxa in this genus, indicating a significant phylogenetic distance from *Appendiculella*. Additional features were, therefore considered, leading to the proposal of a new genus to accommodate this fungus.

VIC 49410 was closely related to *Asteridiella* but formed a distinct long branch, indicating a significant phylogenetic divergence. This prompted us to search for additional distinguishing features that justified the proposal of a new genus to accommodate this fungus.

Taxonomy

Appendiculella Höhn, 1919, *Sber. Akad. Wiss. Wien, Math. -naturw. Kl., Abt. 1* **128**(7-8): 556. 1919.

Type species: Appendiculella calostroma (Desm.) Höhn., *Sber. Akad. Wiss. Wien, Math. -naturw. Kl., Abt. 1* **128**(7-8): 556. 1919.

Emended diagnosis: Genus of *Meliolaceae* with perithecia bearing vermiform appendages, either having no setae or having setae emerging from mycelium.

Appendiculella setosa N.M.P. Silva, C.M. Pereira & R.W. Barreto, *sp. nov.* MB 860962. Fig. 2.

Etymology: Based on the formation of mycelial setae, unusual for *Appendiculella*.

Diagnosis: Differs from *A. eupatorii* by the presence of mycelial setae and larger appressoria, perithecia, and ascospores.

Colonies adaxial on living leaves of *Austroeupatorium inulaefolium*, up to 3.5 mm diam., epiphyllous, epigenous, subdense, circular to irregular, dark brown to black. *Hyphae* superficial, cells 9.5–19 × 2.5–5 µm, straight to somewhat undulated, branched, septate, brown to dark brown, darkened at septa, smooth, bearing appressoria, conidiogenous cells and setae. *Setae* arising from hyphae, subdense to dense, straight to arcuate, up to 195.5 µm long, apex obtuse, 3–9 septate, brown to dark brown, smooth. *Appressoria* alternate, antrorse, clavate, straight or occasionally curved, 10–15.5 × 5.5–10 µm, 2-celled; stalk cells cylindrical, straight to somewhat curved, 6.5–10 × 3.5–6 µm, brown to dark brown, smooth; head cells irregular, cylindrical or oval and lobate, 2.5–6 × 2–4.5 µm, brown to dark brown, smooth. Sexual morph: *Perithecia* abundant, scattered over the colonies, globose to subglobose, 35.5–103 µm diam., chestnut brown to very dark brown, verrucose, appendiculate. *Perithecial appendixes* cylindrical to corniform, larviform, subuncinate to uncinatate on upper half, transversally striate, 23–67 × 3.5–11 µm, pale brown, smooth. *Asci* thin-walled, evanescent. *Ascospores* subcylindrical to ellipsoidal, straight to slightly curved, 18.5–23.5 × 6.5–10.5 µm, apices rounded, 4-septate, constricted at septa, hyaline becoming chestnut brown at maturity, smooth. Asexual morph: *Phialides* unicellular, opposed or unilateral, intermixed with appressoria, ampulliform, slightly curved, 5.5–14 × 2–5 µm, brown, smooth. *Conidia* not observed.

Typus: Brazil, Rio de Janeiro, Nova Friburgo, on leaves of *Austroeupatorium inulaefolium*, Apr. 2024, R.W. Barreto (**holotype** VIC 49606). GenBank: ITS = PV983259.

Distribution: South America (so far known from Brazil).

Notes: Sixteen *Meliolaceae* species have been previously reported from hosts in the *Asteraceae*, including two species of the genus *Appendiculella* – *A. sororcula* and *A. eupatorii*. *Appendiculella sororcula* was originally described from *Austroeupatorium inulaefolium* (as *Eupatorium inulaefolium*) (Hansford 1961) and is the species most frequently reported in association with plants of the *Asteraceae* (Zeng et al. 2017). *Appendiculella setosa* clusters with high support with *A. viticis*, *A. lozaneellae*, and *A. sororcula* in the *Appendiculella* clade, but forms a distinct branch, demonstrating that it is a new species. In addition to being phylogenetically distinct from these species, our specimen from *A. inulaefolium* also differs morphologically from them as well as from *A.*

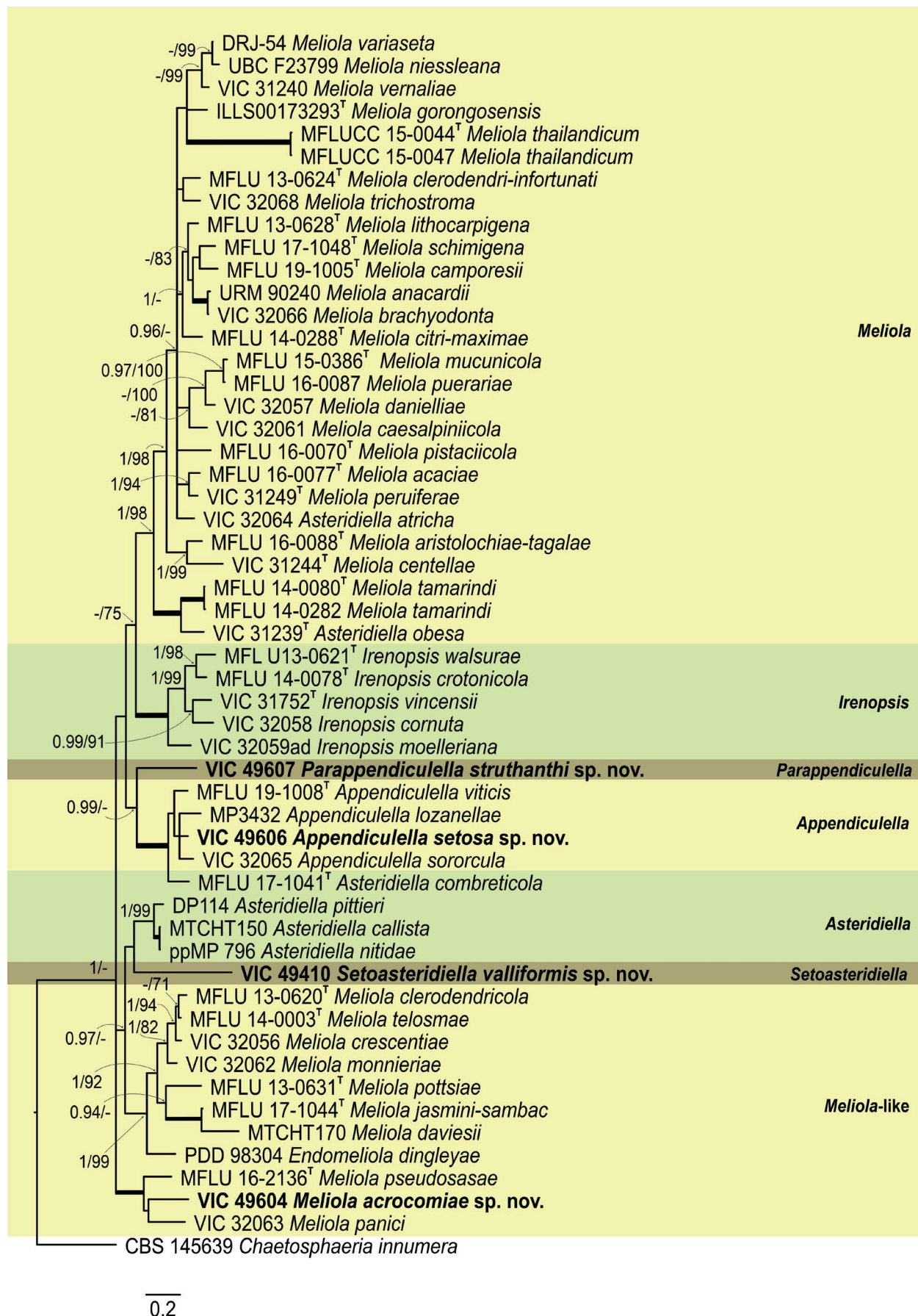


Fig. 1. Phylogenetic tree of the family Meliolaceae based on Bayesian Inference (BI) analysis from the combined dataset of ITS and LSU sequence alignment. Numbers above the nodes indicate Posterior Probabilities (PP) from BI (right, PP ≥ 0.9) and Maximum Likelihood bootstrap values (BS) (left, BS ≥ 70 %), lower values are indicated as “-”. Thickened branches indicate maximum support in both analyses (1.00 PP/100% BS). Novel taxa described in the present study are indicated in bold. Ex-type strains are indicated in “T” after the collection number. The tree was rooted with *Chaetosphaeria innumera* (CBS 145639).

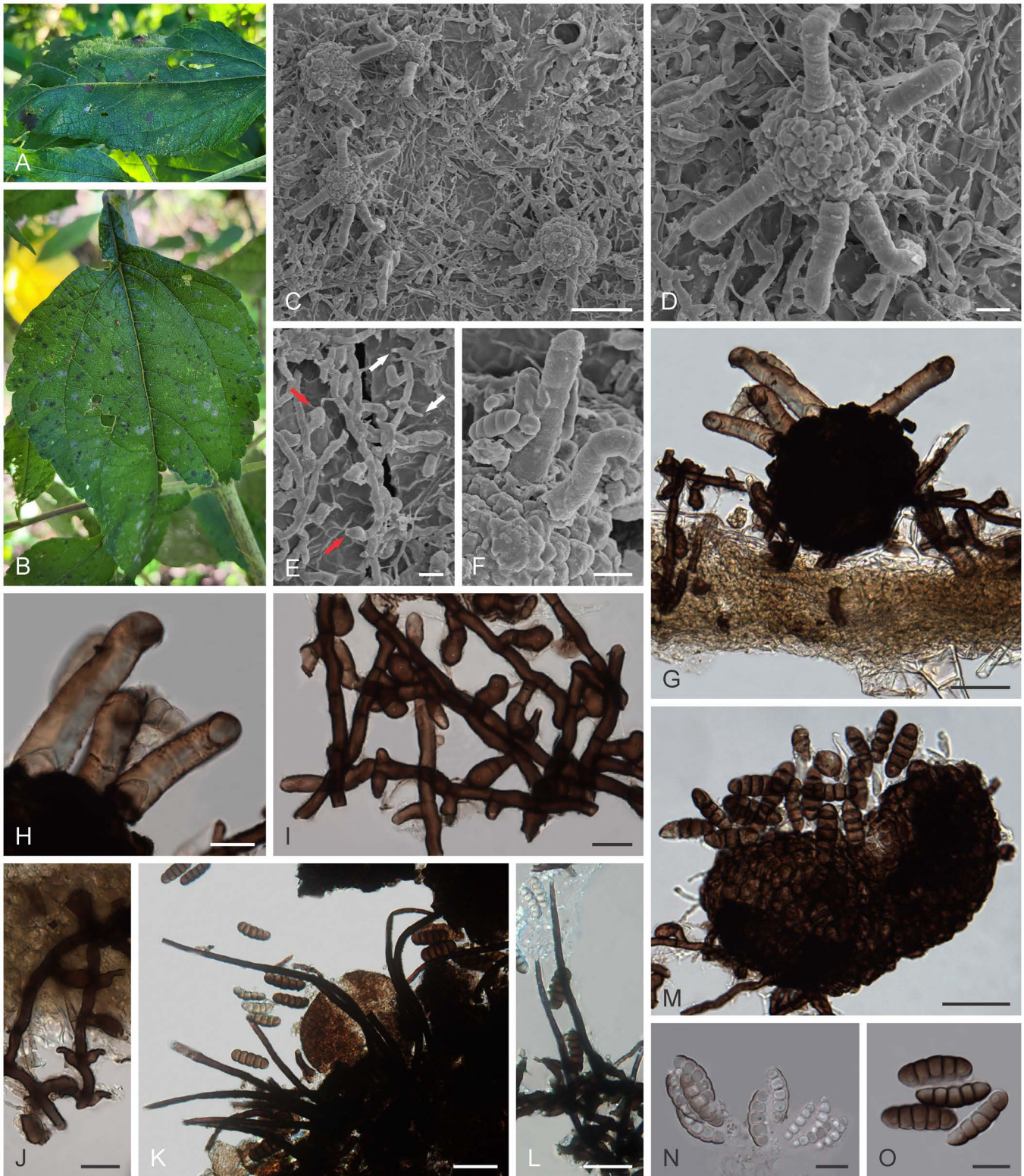


Fig. 2. *Appendiculella setosa* sp. nov. (VIC 49606) on *Austro eupatorium inulaefolium*. **A, B.** Leaves of *A. inulaefolium* colonized by *A. setosa*. Note on B white colonies of an unidentified mycoparasite. **C–F.** Scanning electron micrographs of *A. setosa*. **C.** Dense mycelium layer showing hyphae bearing appressoria and conidiogenous cells, and also three ascomata with larviform appendages. **D.** Single perithecium bearing five larviform appendages. **E.** Superficial hyphae. Note conidiogenous cells (white arrows) and appressoria (red arrows). **F.** Close-up of corniform/larviform appendages and ascospores. **G.** Section through leaf or *A. inulaefolium* showing single perithecium bearing corniform/larviform appendages. **H.** Close-up of corniform/larviform appendages. **I.** Superficial reticulated mycelium with hyphae bearing both appressoria and conidiogenous cells. **J.** Detail of fragment of hypha bearing conidiogenous cells with curved neck. **K.** Dense setae arising from hyphae. **L.** Detail of setae with obtuse apex. **M.** Ruptured ascoma releasing mature ascospores. **N.** Immature subhyaline ascospores. **O.** Mature darkened ascospores. Scale bars: C = 100 μ m; G, J–M = 50 μ m; D–F, H, I, N, O = 20 μ m.

eupatorii, a species not represented in the phylogeny due to the absence of deposited sequences. *Appendiculella setosa* differs from all known species of *Appendiculella* by presenting hyphal setae, a characteristic not yet recorded for the genus. In addition to simple hyphal setae up to 195.5 µm, *A. setosa* has appressoria, perithecia, and ascospores which are smaller than those of species *A. eupatorii*, *A. lozanellae*, *A. sororcula* and *A. viticis*. Since the phylogenetic study clearly indicates that *A. setosa* belongs to the genus *Appendiculella*, an emendation of the generic description was then required.

This fungus was found during a brief survey for fungal pathogens of *A. inulifolium* for later evaluation as potential biological control agents of this native neotropical plant, which became an invasive weed in Indian Ocean countries (Padmanaba *et al.* 2017, Pethiyagoda & Nanayakkara 2011). Nevertheless, it would not be considered for use in biocontrol, as although often regarded to be host specific, the black mildews in general are known to cause limited damage to their hosts.

Meliola acrocomiae C.M. Pereira, N.M.P. Silva, M.F. Bracale & R.W. Barreto, *sp. nov.* MB 860963. Fig. 3.

Etymology: In reference to the host genus, *Acrocomia*.

Diagnosis: Differs from *M. acristae* by forming larger colonies, larger perithecia and longer setae which are branched 2 to 3 times and present 2 to 5 teeth per branch.

Colonies on living leaves of *Acrocomia aculeata*, up to 4.5 mm diam., epiphyllous, epigenous, subdense to dense, irregular, often coalescing, black. *Hyphae* superficial, 11–46.5 × 6.5–11 µm, cells cylindrical, straight to slightly flexuous, branched, septate, brown to dark brown, darkened at septa, smooth, bearing setae, appressoria and conidiogenous cells. *Setae* arising from hyphae over the colony and grouped at the base of perithecia, dense, straight to slightly curved, up to 288 µm long, branching apically – bi or trifurcate, each branch tip 2–5 dentate, indistinctly septate, dark brown to black, smooth. *Appressoria* abundant, alternate, antrorse, clavate, straight or curved, 18.5–25.5 × 17.5–27 µm, 2-celled; stalk-cells cylindrical, straight to slightly curved, 11–16 × 12–17 µm, dark brown, smooth; head cells globose to oval, 5–11 × 5.5–10.5 µm, brown to dark brown, smooth. Sexual morph: *Perithecia* scattered, globose to subglobose, 128–298 µm diam., glabrous, brown to dark brown, verrucose. *Asci* thin-walled, evanescent. *Ascospores* oblong, 26.5–54 × 8.5–20 µm, 3–4 septate, constricted at septa, hyaline to pale brown becoming dark brown at maturity, smooth. Asexual morph: *Phialides* unicellular, alternate, occasionally opposed, intermixed with appressoria, ampulliform, straight to slightly curved, 7–23 × 3.5–9.5 µm, brown, smooth. *Conidia* not observed.

Typus: Brazil, Minas Gerais, Viçosa, on leaves of *Acrocomia aculeata*, May 2020, R.W. Barreto (**holotype** VIC 49604). GenBank: ITS = PV983258; LSU = PV983262.

Distribution: South America (so far known from Brazil).

Notes: Eighteen species of *Meliola* have been recorded in association with members of the *Arecaceae*. Zeng *et al.* (2017) reported that *Meliola* spp. on palms are restricted geographically and have a narrow host-range, with most species known only from a single host-species or genus. None of those species has ever been reported on *Acrocomia aculeata*. There is, nevertheless one notable exception of a polyphagous *Meliola* attacking palms, *Meliola acristae*. Although it has not been reported from *A. aculeata*, this species appears to differ from other *Meliola* spp. on palms. It has a seemingly broad and disparate geographic distribution, including records in Brazil, China and Puerto Rico (Castlebury & Dominick 2025) and it has been reported on hosts including *Amphilophium lactiflorum* (syn. *Distictis lactiflora* and *Macrodiscus lactiflorus*), *Cocos nucifera*, *Coccothrinax* (2 spp.), *Leucothrinax* (2 spp.) (syn. *Thrinax*), *Licuala* (1 sp.), *Orbignya* (no sp. id.), *Phyllostachys* (no sp. id.), *Prestoea* (1 sp.), and *Sabal* (1 sp.) (Hansford 1961, Zeng *et al.* 2017). *Meliola acrocomiae* is clearly morphologically distinct from *Meliola acristae*. It forms larger colonies (3 mm in *M. acristae* vs 4.5 mm in *M. acrocomiae*), larger appressorial stalk cells (3–10 µm in *M. acristae* vs 11–16 µm in *M. acrocomiae*), smaller appressorial head cells (11–17 µm in *M. acristae* vs 5–11 µm in *M. acrocomiae*), and larger perithecia (up to 200 µm in *M. acristae* vs up to 298 µm in *M. acrocomiae*). Furthermore, it has longer hyphal setae (200 µm in *M. acristae* vs 288 µm in *M. acrocomiae*) that are branched 2 to 3 times, presenting 2 to 5 teeth per branch, differently from *M. acristae* (Hansford 1961). Despite the lack of molecular information for *Meliola* spp. on palms, morphological, host and geographical distribution differences indicate that *M. acrocomiae* is distinct from the species of *Meliola* on the *Arecaceae*. *Meliola acrocomiae* groups with a well-supported clade including *Meliola panici* and *Meliola pseudosasa*. There are, nevertheless, clear morphological differences between the new species and those two species. *Meliola panici* has much longer setae (up to 600 µm vs up to 288 µm in *M. acrocomiae*) ending in an obtuse apex, differently from the apically branched and dentate format typical of the new species and has smaller appressoria (20 µm wide vs 27 µm in *M. acrocomiae*) (Hansford 1961). *Meliola acrocomiae* is morphologically close to *M. pseudosasa* but *M. pseudosasa* has smaller setae (up to 210 µm vs up to 288 µm in *M. acrocomiae*) which emerge exclusively from the hyphae and not from the base of the perithecia as seen in the new species. Perithecia of *M. pseudosasa* are also significantly smaller (up to 160 µm diam.) (Hyde *et al.* 2016) than those of *M. acrocomiae* 128–298 µm diam.). *Meliola panici*, *M. pseudosasa* and *M. acrocomiae*, together with seven other species belong into a clade which is informally treated by Zeng *et al.* (2020) as “*Meliola*-like”, which includes species that are phylogenetically distinct but morphologically equivalent to species traditionally placed in *Meliola* (Zeng *et al.* 2020, Bhunjun *et al.* 2022). *Meliola acrocomiae* is not phylogenetically close to the type species of *Meliola* (*M. evansii*). Nevertheless, it is acknowledged that the present consensus is that the genus *Meliola* is not monophyletic, and a taxonomic revision of the entire genus is pending. For the moment, it was decided that treating the fungus associated with *A. aculeata* as a new species of *Meliola* would be the best option.

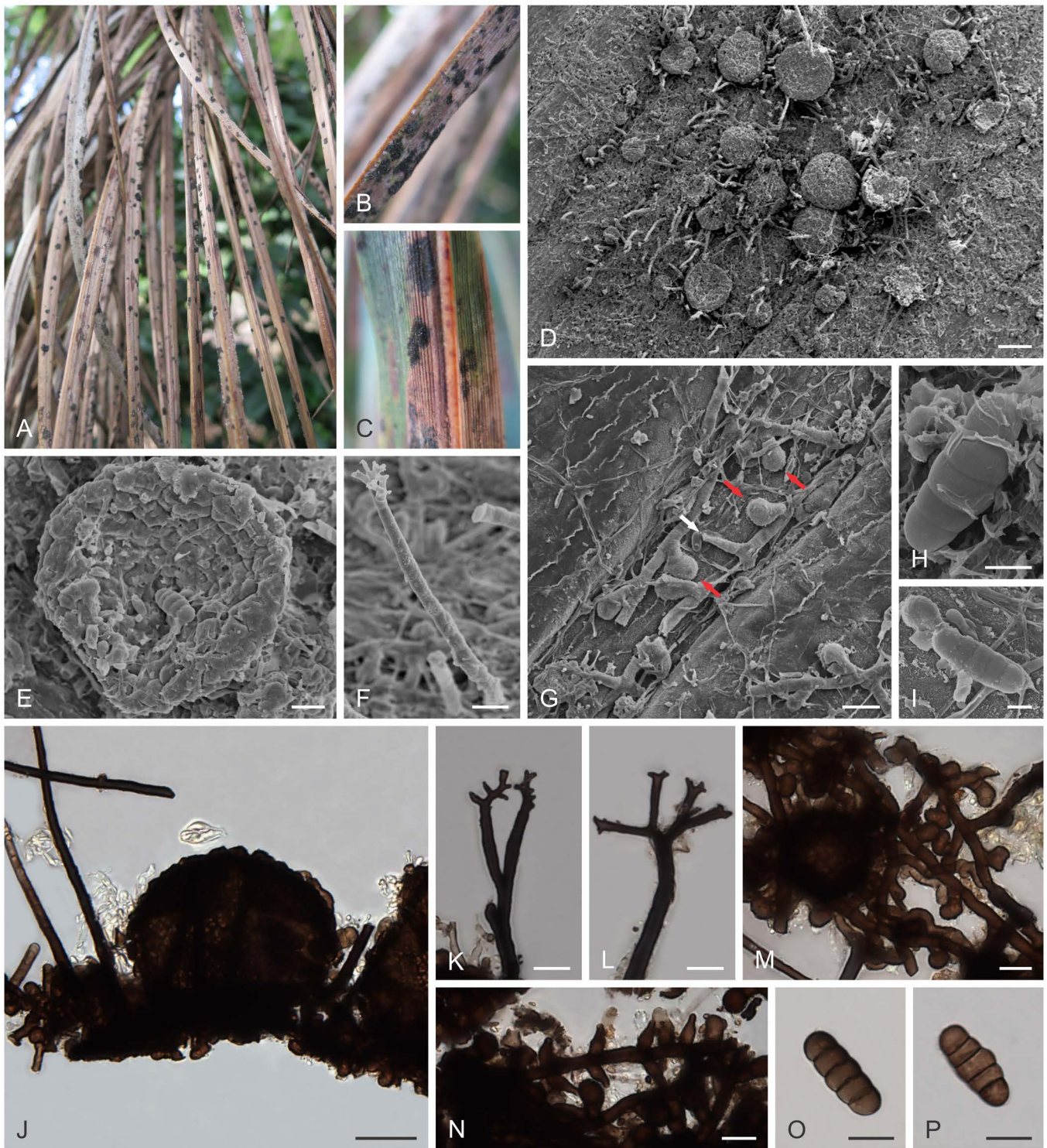


Fig. 3. *Meliola acrocomiae* sp. nov. (VIC 49604) on *Acrocomia aculeata*. **A–C.** Black colonies of *M. acrocomiae* on leaves of *A. aculeata* in the field. **D–I.** Scanning electron micrographs of *M. acrocomiae*. **D.** General view of colony including external layer of setose mycelium with several glabrous perithecia. **E.** Single glabrous perithecium (collapsed). Note ascospore centrally on perithecium. **F.** Single seta. Notice apical branching pattern with dentate apex. **G.** Fragments of superficial hyphae bearing conidiogenous cell (white arrow) and appressoria (red arrows). **H, I.** Mature ascospores. **J.** Single glabrous perithecium attached to setose mycelium. **K, L.** Close-up of apical portion of setae showing branching patterns. **M–N.** Reticulate mycelium bearing appressoria (**M**) and conidiogenous cells (**N**). **O, P.** Conidia. Scale bars: D = 100 µm; J = 50 µm; E–G, K–P = 20 µm; H, I = 10 µm.

This fungus was found during a broad survey for fungal pathogens of *A. aculeata* in a study aimed at describing the fungi found attacking the macaw palm in its native habitats in Brazil, in order to detect the potential threats for future broad scale plantations for biofuel production (Motoike, 2024). Nevertheless, the black mildews are broadly recognized as causing little damage to their hosts. *Meliola acrocomiae* appears to be of no agronomical concern to this newly domesticated crop plant, based on the observations that were made in the field.

Parappendiculella N.M.P. Silva, C.M. Pereira & R.W. Barreto, **gen. nov.** MB 860964.

Etymology: In reference to the phylogenetic proximity to *Appendiculella*.

Diagnosis: Genus of *Meliolaceae* without vermiform perithecial appendages and without conoid cells on perithecial surface, morphologically similar to *Meliola* but phylogenetically closer to *Appendiculella*.

Type species: *Parappendiculella struthanthi* N.M.P. Silva, C.M. Pereira & R.W. Barreto

Parappendiculella struthanthi N.M.P. Silva, C.M. Pereira & R.W. Barreto, **sp. nov.** MB 860965. Fig. 4.

Etymology: In reference to the host genus, *Struthanthus*.

Diagnosis: *Meliola*-like but phylogenetically closer, but molecularly distinct, to species of *Appendiculella*.

Colonies on living leaves of *Struthanthus* sp., up to 3 mm diam., adaxial, subdense, circular, dark brown to black. *Hyphae* superficial, cells 19–50 × 6–10.5 µm, straight to slightly flexuous, branched, septate, brown to dark brown, darkened at septa, smooth, bearing setae, appressoria and conidiogenous cells. *Setae* arising from hyphae, densely produced, straight to slightly curved, up to 340 µm long, apex obtuse, 1–7 septate, dark brown, smooth. *Appressoria* alternate, unilateral, occasionally opposed, antrorse to subantrorse, cylindrical, globose or oval, occasionally subclavate, straight or curved, 13–22.5 × 15.5–24.5 µm, 2-celled; stalk cells cylindrical, straight to slightly curved, 2–10 × 5–10 µm, dark brown, smooth; head cells irregular, cylindrical, globose or oval, 8–17 × 6.5–14.5 µm, brown to dark brown, smooth. Sexual morph: *Perithecia* superficial, numerous, scattered, glabrous, globose to subglobose, 47–200 µm diam., brown to dark brown, verrucose. *Asci* thin-walled, evanescent. *Ascospores* oblong to ellipsoidal, 32–53 × 8.5–18 µm, 4-septate, constricted at septa, hyaline becoming chestnut brown with age, thick walled, smooth. Asexual morph: *Phialides* scarce, unicellular, opposed or unilateral, intermixed with appressoria, conical to ampulliform, straight or curved, 12–73.5 × 5–17 µm, brown, smooth. *Conidia* not observed.

Typus: Brazil, Minas Gerais, Viçosa, on leaves of *Struthanthus* sp., Apr. 2024, R.W. Barreto (**holotype** VIC 49607). GenBank: ITS = PV983260; LSU = PV983263.

Distribution: South America (so far known from Brazil).

Notes: Although 13 spp. of *Meliolaceae* have been recorded on members of the *Loranthaceae*, none is known to be associated with *Struthanthus* spp. All species of *Meliolaceae* previously reported on this host family belong either to *Meliola* or *Irenopsis* (Zeng *et al.* 2017). The fungus in our specimens is phylogenetically closest to the *Appendiculella* clade but forming a distinct branch. Morphologically, the fungus found on *Struthanthus* is clearly distinct from *Appendiculella* by not bearing the typical vermiform appendages on its perithecia. It is also different from the majority of the members of *Appendiculella* (except our newly described *A. setosa*) by having mycelial setae. The combination of unique molecular and morphological features justifies the proposal of a new genus to accommodate the species *P. struthanthi*.

Struthanthus spp. is a genus including hemi-parasitic plants, known in Brazil as “erva-de-passarinho” (bird-weed). It is generally regarded as a nuisance for cultivated woody plants, such as in public gardens and domestic orchards (Leal *et al.* 2006). Little is known about the fungal pathogens of the plants belonging to this genus. *Parappendiculella struthanthi* was found serendipitously on a small group of unmanaged lime plants overtaken by “erva-de-passarinho”.

Setoasteridiella C.M. Pereira, N. M. P. Silva & R.W. Barreto, **gen. nov.** MB 860966.

Etymology: In reference to the genetic affinities with *Asteridiella* but contrastingly with abundant setae.

Diagnosis: Genus of *Meliolaceae* with mycelial setae but phylogenetically closer to *Asteridilella*.

Type species: *Setoasteridiella valliformis* C.M. Pereira, N. M. P. Silva & R.W. Barreto

Setoasteridiella valliformis C.M. Pereira, N. M. P. Silva & R.W. Barreto. **sp. nov.** MB 860967. Fig. 5.

Etymology: Based on the robust erect palisade-defense-like aggregates of setae surrounding groups of perithecia.

Diagnosis: Differs from *Asteridiella perspicua*, by the absence of depressed perithecia with an incised lobed margin, the presence of abundant mycelial setae and molecular features.

Colonies on living leaves and branches of *Cissus sicyoides* (syn. *Cissus verticillata*), up to 2.5 mm diam. amphigenous and circular on leaves, irregular on stems, dense, black. *Hyphae* superficial, cells 12–32.5 × 4.5–9.5 µm, straight to slightly flexuous, branched, septate, brown to dark brown, darkened at septa, smooth, bearing setae, appressoria and conidiogenous cells. *Setae* densely aggregated around the perithecia, or sparsely distributed over colony, mostly straight, up to 247.5 µm long, apex bifurcate and terminating in 2–4 acute denticles, sometimes obtuse, 3–5 septate, brown to dark brown, smooth. *Appressoria* alternate, antrorse, clavate, straight or curved, 18–36.5 × 16.5–26 µm, 2-celled; stalk cells cylindrical, straight to slightly curved, 7–17.5 × 5.5–10.5 µm, brown, smooth; head

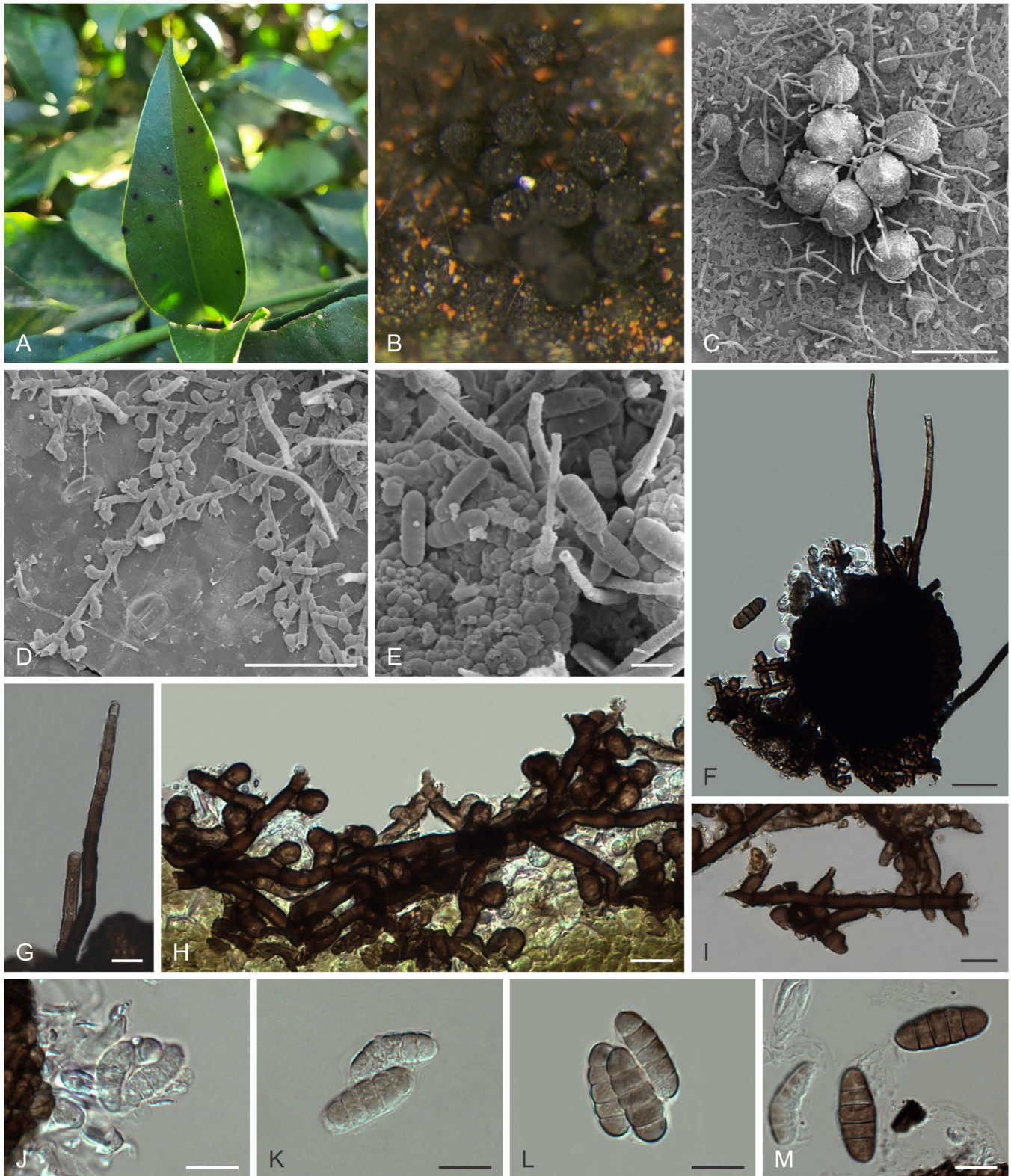


Fig. 4. *Parappendiculella struthanthi* (VIC 49607) on *Struthanthus* sp. **A.** Single leaf of *Struthanthus* sp. colonized by *P. struthanthi*. **B.** Cluster of dark perithecia. **C–E.** Scanning electron micrographs of *M. acrocomiae*. **C.** Group of glabrous perithecia over dense layer of setose mycelium. **D.** Superficial hyphae bearing abundant appressoria and some setae. **E.** Group of ascospores and unbranched setae. **F.** Squashed perithecium releasing some ascospores. Note mycelial setae flattened in squash-mount appearing as if arising from perithecium. **G.** Two mycelial setae (one broken – left and one intact – right). **H.** Section through leaf of *Struthanthus* sp. bearing superficial layer of mycelium with abundant appressoria. **I.** Fragment of superficial hyphae bearing conidiogenous cells. **J–M.** Mature (darkened) and immature (subhyaline) ascospores. Note in **J** and **K** thin-walled evanescent asci. Scale bars: **C** = 200 µm; **D** = 100 µm; **F** = 50 µm; **E, G–M** = 20 µm.



Fig. 5. *Setoasteridiella valliformis* (VIC 49410) on *Cissus sicyoides*. **A, B.** Colonies of *S. valliformis* in the field: on a leaf (**A**) and on a branch (**B**). **C–F.** Scanning electron micrographs of *S. valliformis*. **C, D.** Palisade defense-like erect mycelial setae, appearing as if protecting underlying groups of glabrous perithecia. **E, F.** Ascospores. **G.** Squashed perithecia releasing ascospores surrounded by setose mycelium. Note apically dichotomously branched, terminally dentate setae. **H.** Setose mycelium. **I.** Close-up of mycelial setae. **J.** Superficial hyphae bearing abundant appressoria. **K.** Superficial hyphae bearing abundant conidiogenous cells. **L–O.** Ascospores immature (hyaline to subhyaline) and mature (brown). Note 2-spored thin-walled evanescent asci (**L** and **M**). Scale bars: C, D, G = 100 μ m; H, I, N = 50 μ m; J–M, O = 20 μ m; E, F = 10 μ m.

cells globose oval or irregular, lobate, 10–26.5 × 9–19 µm, brown to dark brown, smooth. Sexual morph: *Perithecia* numerous, gregarious or dispersed, glabrous, globose to a subglobose, 100–270.5 µm diam., dark brown, verruculose. *Asci* thin-walled, evanescent, 2-spored. *Ascospores* oblong to subellipsoidal, rarely cylindrical, 36.5–45 × 10–16.5 µm, 4-septate, constricted at septa, hyaline to pale brown becoming dark brown at maturity, thick-walled, smooth. Asexual morph: *Phialides* unicellular, opposed or unilateral, intermixed with appressoria, ampulliform, straight or slightly curved, 10.5–25 × 4–8.5 µm, brown, smooth. *Conidia* not observed.

Typus: Brazil, Rio de Janeiro, Nova Friburgo, on leaves and branches of *Cissus sicyoides*, Jan. 2023, R.W. Barreto (**holotype** VIC 49410). GenBank: ITS = PV983261; LSU = PV983264.

Distribution: South America (so far known from Brazil).

Notes: Twenty-six spp. of *Meliolaceae* have been described on member of the *Vitaceae*, including 11 on *Cissus* spp. (Zeng et al. 2017): ten are species of *Meliola* and one belongs to *Asteridiella*. None of these was reported on *C. sicyoides*. Our fungus did not group into any of the valid genera in the *Meliolales* and formed a sister clade to *Asteridiella*, with high support (99/1, ML/BI). The genus *Asteridiella* differs both phylogenetically and morphologically from our fungus. The species *Asteridiella perspicua*, also reported in a species of *Cissus* (*Cissus erosus*), differs from our isolate primarily in having depressed perithecia with an incised lobed margin and does not produce setae (Hansford 1961). *Setoasteridiella valliformis* is characterized by having long, ornate setae, grouped around the perithecia. Besides those differences, *Asteridiella perspicua* (Hansford 1961) also differs from our isolate by presenting hyphae branching at almost right angles, unlike *S. valliformis*, smaller appressoria (18–22 µm vs 18–36.5 µm in *S. valliformis*), smaller conidiogenous cells (15–18 µm vs 10.5–25 µm in *S. valliformis*), smaller perithecia (up to 125 µm in diameter vs 100–270.5 µm in *S. valliformis*), and smaller ascospores (35–38 µm vs 36.5–45 in *S. valliformis*) which are only slightly constricted at the septa in *A. perspicua* as opposed to distinctly constricted at the septa in *S. valliformis*.

Setoasteridiella valliformis was serendipitously found in an abandoned persimmon plantation on a native liana, known popularly as “uva-do-mato” (wild grape) in Brazil, which was hanging on some of the plants in this orchard.

DISCUSSION

There are significant uncertainties about the generic and species delimitation in the *Meliolaceae*. Most taxa have been proposed solely based on morphology and supposed host-specific associations (Jayawardena et al. 2020, Bhunjun et al. 2022). Historically, *Meliolaceae* have been treated as host-specific (e.g. Hansford 1961, Hosagoudar 1996) and identification of the host plant was established (and still remains) central in elucidating the identity of the black mildews (Zeng et al. 2017). Nevertheless, host specificity in the *Meliolaceae* remains an untested hypothesis that has

not been confirmed or rejected experimentally (Hongsan et al. 2015, Marasinghe et al. 2020). Examples where more than one species of *Meliolaceae* are capable of infecting one plant species or of one species of *Meliolaceae* which is capable of infecting more than one species or even different genera are cited by Feng et al. (2025), Khan et al. (2025b) and other authors. Hongsan et al. (2015) although admitting that “*Meliolales* are considered to have a high degree of host specificity because they are biotrophic on living leaves”, concluded their thought stating that “whether *Meliolales* species are host specific needs verifying using molecular techniques”.

Unfortunately, molecular information is available in GenBank for only for 67 spp. of the *Meliolaceae* and few publications dealt with their phylogenetic position (Hongsan et al. 2015, Rodríguez Justavino et al. 2015, Hyde et al. 2020, Marasinghe et al. 2020, Zeng et al. 2020, Bhunjun et al. 2022, Zeng et al. 2022, Feng et al. 2025, Khan et al. 2025b). This situation is likely to remain unchanged for very long, considering the enormous challenge of recollecting and extracting DNA and, particularly, sequencing protein-coding regions from such a plethora of non-culturable highly melanized fungi.

The morphological concept still prevails while molecular information remains very scarce and fragmentary. For generic delimitation the key characters used for have been: the presence of setae only on mycelium (supposedly restricted to members of *Meliola*); the presence of setae both on mycelium and perithecia (*Setameliola*); the absence of appendices (*Asteridiella*); the presence of larviform appendices on perithecia and absence of setae (*Appendiculella*); the presence of setae only on perithecia (*Irenopsis*); the coverage of an irradiating mycelial layer on flattened perithecia and absence of setae (*Amazonia*), the presence of hyphal setae with bulbous tips covering the perithecia (*Cryptomeliola*), and the presence of intercellular hyphae (*Endomeliola*) (Hansford 1961, Hongsan et al. 2015, Rodríguez Justavino et al. 2015, Zeng et al. 2017). Hongsan et al. (2015) provided a key to most genera based on those simple morphological features. Nevertheless, we are already entering a “transition period” and taxonomists are tentatively applying the polyphasic approach to the systematics of the black mildews. Our work is a case in point.

It can be observed a trend among the taxonomists studying the *Meliolaceae* of attributing a higher weight to molecular data than to morphology and other characteristics. Nevertheless, this may be premature. The molecular information available in public databases for the *Meliolales* is still very scarce and most phylogenetic studies of the *Meliolaceae*, including ours, rely on ITS, LSU and/or SSU regions. Although ITS is recognized and treated as the primary fungal barcode (Schoch et al. 2012) and this locus (along with LSU and SSU) is considered useful for inferring higher-level relationships, it provides limited resolution at the species level and for resolving closely related genera for many fungal groups (Tekpinar & Kalmer 2019). The inclusion of protein-coding genes (e.g. *tub2*, *tef1*) would likely improve phylogenetic resolution; however, such data are currently unavailable for most taxa in this family.

One additional complication is the complete lack of sequences for the type species of each genus. Most of the

accepted genera of *Meliolaceae* were erected more than a century ago. Recollecting samples of the type species of each genus, indicating neotypes and epitypes, whenever necessary, and obtaining sequences for relevant genome regions from those samples, although challenging, is critical for the consistent advance of the taxonomy of the *Meliolaceae* and the reevaluation of the main genera. Until then, major taxonomic changes such as the proposal of lumping of *Appendiculella* and *Asteridiella* (Zeng *et al.* 2022) are, in our opinion, premature.

According to Zeng *et al.* (2022), “both genera (*Appendiculella* and *Asteridiella*) lack setae and have raised ascomata surface, while the two typical features, larviform appendages and warty spines, are both cylindrical shapes”. Raised ascoma are found in most genera of the *Meliolaceae* and is not a useful morphological feature for lumping the two genera. The absence of setae might be a valid point until we found and described here *A. setosa*. It is not clear what the authors meant by considering the “warty spines” and larviform appendages as having cylindrical shapes and hence being similar. Zeng *et al.* (2022) also stated that the projecting conical structures predominantly found on the surface of the pseudothecia of *Asteridiella* may be homologous to larviform appendages in *Appendiculella*, according to Rodríguez Justavino *et al.* (2015). The latter authors did, in fact, include this hypothesis in their discussion. Nevertheless Rodríguez Justavino *et al.* (2015) went on to conclude that molecular data did not support a close relationship between the two genera and also added that “monophyly of *Asteridiella* is not supported”. They also added that “compared to setae or appendages in other genera the conical ascomata projections of *Asteridiella* appears less evolved and might be a plesiomorphic characteristic”. Zeng *et al.* (2022) also stated that Hongsanan *et al.* (2015) reexamined the type specimens of *Appendiculella* and *Asteridiella* and observed conoid cells in *Appendiculella*, which might extend to form larviform appendages. This might then indicate that the larviform appendages could be specialized conoid cells. Nevertheless, this is only partly correct. In their review of the genera of *Meliolales*, Hongsanan *et al.* (2015), examined the type of *Asteridiella* – *Asteridiella solani*, but were not able to examine the type material of *Appendiculella calostroma* (the type of *Appendiculella*) in their work. Their observations were made on a specimen of *A. calostroma* on a different host (*Geum chilense*) from what that species was originally described (*Rubus fruticosus*) and from a different geographical origin (Chile) instead of the original locality (France). Ideally, the study should include Demazière’s original material of “*Sphaeria calostroma*”, such as those housed in the fungarium of the U.S. National Fungus Collections (BPI) or fresh collections on *Rubus* sp. in northern France. Additionally, Hongsanan *et al.* (2015) also quoted the work of Rodríguez Justavino *et al.* (2015) and stated that “in this study, the phylogenies indicate a [*sic*] close relationship between *Appendiculella lozanellae* and *Asteridiella nitidae* (60 % ML support). However, the morphology of these two genera differs, and therefore we maintain these genera as distinct”. Most published phylogenies of the *Meliolales* (even if limited for the various reasons discussed earlier) suggest *Asteridiella* to be polyphyletic and in need of revision (e.g. Feng *et al.* 2025). Lumping a possibly well delimited genus (*Appendiculella*) with a likely polyphyletic

genus (*Asteridiella*) is, in our opinion, inadequate, premature and an unsupported taxonomic decision. Although the proposal of Zeng *et al.* appeared in the literature in 2022, *Appendiculella* and *Asteridiella* remain being treated as separate taxa both in Index Fungorum and in MycoBank and no formal proposals of new combinations of species of *Appendiculella* into *Asteridiella* have appeared in the literature. Khan *et al.* (2025a) kept species of *Appendiculella* and *Asteridiella* under distinct generic names in their recent work. This is interpreted here as an indication that the mycologists dedicated to the taxonomy of the *Meliolaceae* have not accepted the merging of the two genera.

We hope that, eventually, *Asteridiella solani* will be recollected in Australia and *Appendiculella calostroma* will be recollected in France, and successful sequencing of such epitypes will follow, allowing for a better understanding of the affinity between the two genera. Meanwhile, we regard it as better option to fit our fungi into new genera as this will not unnecessarily deteriorate the (still much needed) morphological taxonomic boundaries of genera in the *Meliolaceae*.

Herein, we have described four additions to the *Meliolaceae* resulting from *ad hoc* collections of black mildew samples. We have attempted to follow closely the polyphasic approach by considering morphological, ecological (host associations), geographical and molecular evidence for the proposal of those new taxa.

The two newly described genera, *Parappendiculella* and *Setoasteridiella*, would fit into *Meliola* if only morphological features were considered. However, the former is phylogenetically closer to *Appendiculella* (but has no larviform appendages), and the latter is phylogenetically closer to *Asteridiella* (but produces hyphal setae and has no conical cells on the surface of its perithecia). The weakness of the support appearing in our phylogenetic study is related to the availability of ITS and LSU sequence only and their well-known lack of resolution for closely related taxa, as previously discussed. Nevertheless, the affinities of VIC 49607 with *Appendiculella*, and VIC 49410 with *Asteridiella*, are clear as seen in Fig. 1. Additionally, our newly described species of *Appendiculella* (*A. setosa*) produces mycelial setae, which were unknown for the genus. None of our species present the conoid cells on their pseudothecia (see SEM in Figs 2–5) which are regarded as key for members of *Asteridiella*. Additionally, several studies have indicated that *Asteridiella* is polyphyletic (e.g. Feng *et al.* 2025). Placing VIC 49607 into *Meliola* based on its morphology alone, and ignoring the molecular data, or placing VIC 49410 into *Asteridiella* while ignoring the morphological evidence against it, would be incorrect. Therefore, our choice was to propose the new genera *Parappendiculella* and *Setoasteridiella* as placements for our fungi.

Despite the large size of the *Meliolaceae*, it is reasonable to conjecture that a plethora of black mildew taxa are still awaiting discovery in Brazil and other tropical countries. This has already been highlighted by Macedo *et al.* (2010) and Pinho *et al.* (2012a, b). Unfortunately, little has been published on Brazilian *Meliolaceae* since those publications. The supposedly large existing black mildew diversity belongs to the broad category named by Grossart *et al.* (2016) as the “dark matter fungi”. Most Brazilian “dark matter *Meliolaceae*” may still be awaiting to be discovered by mycologists.

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Conflict of interest: The authors declare that there is no conflict of interest.

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