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RESEARCH ARTICLE

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Riding with the ants

A.P.M. Duarte[1], D. Attili-Angelis[1,2], N.C. Baron[1], J.Z. Groenewald[3], P.W. Crous[3,4,5], F.C. Pagnocca[1]

Key words

Attini tribe
leaf-cutting ants
multi-gene analyses
systematics
Xenopenidiella

Abstract Isolates of *Teratosphaeriaceae* have frequently been found in the integument of attine ants, proving to be common and diverse in this microenvironment. The LSU phylogeny of the ant-isolated strains studied revealed that they cluster in two main lineages. The first was associated with the genus *Xenopenidiella* whereas the other represented two ant-isolated lineages sister to the taxa *Penidiella aggregata* and *P. drakensbergensis*, which are allocated to the new genus *Penidiellomyces*. The genus *Penidiella* is limited to the lineage containing *P. columbiana*, which is not congeneric with *Penidiellomyces* or *Penidiellopsis*, nor with *Simplicidiella*, a novel genus introduced here to accommodate a strain isolated from ants. For species level analysis, the final 26 aligned sequences of the ITS (498 characters), *cmdA* (389 characters), *tef1* (342 characters) and *tub2* (446 characters) gene regions lead to the introduction of six new species in *Xenopenidiella*, and one in respectively *Penidiellopsis* and *Simplicidiella*. The species described in this study were distinguished by the combination of morphological and phylogenetic data. Novelties on the integument of leaf-cutting ants from Brazil include: *Penidiellopsis ramosus*, *Xenopenidiella clavata*, *X. formica*, *X. inflata*, *X. laevigata*, *X. nigrescens*, *X. tarda* spp. nov., and *Simplicidiella nigra* gen. & sp. nov. Beta-tubulin is recommended as primary barcode for the distinction of species in *Penidiellopsis*, whereas ITS was sufficient to distinguish species of *Xenopenidiella*.

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INTRODUCTION

Seen as important symbionts, fungi interact with a wide range of organisms, including insects (McLaughlin et al. 2009). According to Hawksworth (2012) it is estimated that at least 1.5–3 M species of fungi are living on Earth, of which only around 100 000 are presently known (Crous et al. 2015). Since the symbiotic relationships of attine ants with fungi are not fully understood and comprise a wide fungal diversity (Pagnocca et al. 2008, Rodrigues et al. 2011, Duarte et al. 2014), nests of ants are favourable environments to investigate for undescribed taxa.

The symbiosis between attine ants and their mutualistic fungi (*Leucocoprineae* and *Pterulaceae*), nourished as food source (Mueller & Rabeling 2008) exists for approximately 50 M years (Schultz & Brady 2008). In order to maintain the symbiont, attine ants utilise different agricultural systems (Schultz & Brady 2008). The leaf-cutter agriculture performed by genera *Atta* and *Acromyrmex* is the most common in Brazil and consists of cutting fresh plant material to be incorporated into the fungal garden and used as energy source for the growth of the mutualistic fungus *Leucoagaricus gongylophorus* (Weber 1972).

Recent studies of melanised fungi carried in the integument of leaf-cutting ants have revealed the existence of a wide diversity of undescribed fungal species (Duarte et al. 2014). Little & Currie (2007) were the first to show an association between a black yeast (phialophora-like), classified by the authors as the fifth symbiont, and *Apterostigma* ants (tribe Attini). Thus far, three new black fungal species isolated from leaf-cutting ants have been described: *Phialophora attae*, *P. capiguarae* (Attili-Angelis et al. 2014) and *Ochroconis globalis* (Samerpitak et al. 2015). In addition, Duarte et al. (2014) isolated several unknown species affiliated to *Teratosphaeriaceae*, which are the subject of the present study.

The *Teratosphaeriaceae* was originally separated from *Mycosphaerellaceae* (Crous et al. 2007) and circumscribed to include saprobic, extremophilic, human opportunistic and plant pathogenic fungi (Quaedvlieg et al. 2014). Although often linked to diseases in plants, especially *Myrtaceae* and *Proteaceae* hosts (Pérez et al. 2009, Carnegie et al. 2011, Crous & Groenewald 2011, Hunter et al. 2011), *Teratosphaeriaceae* species do not have reports of specific associations with ants. However, this family is also rich in genera and species associated with rocks and other extreme environments (Egidi et al. 2014), and its ecology remains insufficiently known.

In a recent revision to phylogenetically and morphologically classified lineages in *Teratosphaeriaceae*, Quaedvlieg et al. (2014) generated a multi-locus DNA sequence dataset and introduced 17 new genera within *Teratosphaeriaceae*, including *Xenopenidiella*, a saprobic fungus on leaf litter. *Xenopenidiella* was introduced based on a single species, *X. rigidophora*, which was represented by a single isolate (Quaedvlieg et al. 2014). The discovery of more species of *Xenopenidiella* will thus allow for a more robust generic circumscription of the genus and facilitate a better understanding of its ecology.

The present study aims to provide a taxonomic study of *Teratosphaeriaceae* species obtained from the integument of leaf-cutting ants from Brazil. Here, we describe a novel genus

[1] Institute of Biosciences, UNESP – Univ. Estadual Paulista, Rio Claro, Center for the Study of Social Insects;
corresponding author e-mail: derlene@cpqba.unicamp.br.
[2] Division of Microbial Resources, CPQBA – University of Campinas, SP, Brazil.
[3] CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.
[4] Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.
[5] Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

isolated from ants (*Simplicidiella*), a new species of *Penidiellopsis* (*P. ramosus*), and six new species of *Xenopenidiella* (*X. clavata*, *X. formica*, *X. inflata*, *X. laevigata*, *X. nigrescens*, *X. tarda*). A third genus, *Penidiellomyces*, is also introduced for two species of *Penidiella* s.lat.

MATERIALS AND METHODS

Isolation techniques

In order to isolate the fungi present in the integument of attine ants, three different methods were used as described below.

For the oil flotation technique (Satow et al. 2008) the bodies of sampled ants were immersed in 25 mL of saline solution (NaCl 0.85 %) with antibiotics (200 U penicillin, 200 µg/mL chloramphenicol, 200 µg/mL streptomycin and 500 µg/mL cycloheximide). Tubes were vortexed and after 30 min of incubation at room temperature, approximately 20 % sterile mineral oil was added, followed by vortexing for 5 min. After 20 min of settling, 100 µL aliquots were removed from the oil-solution interface and plated on Mycosel agar using a Drigalsky rod. Plates were incubated in the dark at 25 °C and monitored daily until the appearance of fungal colonies.

For the nitrogen-free cultivation, ants were immersed in distilled water and sonicated for 15 min. Then, 150 µL of the solution containing fungal cells in suspension were plated in a free-nitrogen medium described by Thanh (2006). Plates were incubated in the dark at 25 °C for 3 wk.

In the third method, a dextrose 50 % enrichment technique was performed by immersing the ants in tubes containing 50 % dextrose and 0.5 % yeast extract. After 10 d of incubation in the dark at 28 °C, tubes were shaken and 150 µL of the mixed solution was plated on malt-dextrose agar (1 % malt, 1 % dextrose, 0.01 % chloramphenicol, 2 % agar) and again incubated in the dark at 28 °C for 10 d (Thanh et al. 2013).

Fungal isolates

The 25 isolates analysed are listed in Table 1 and were all obtained from the integument of *Atta capiguara* and *A. laevigata* leaf-cutting ant gynes (primary reproductive female caste) and drones (male caste which role is to mate with a gyne) from Brazil.

All isolates are maintained in the culture collection of the Microbial Resource Centre (CRM-UNESP, Brazil). Ex-type strains were deposited in the CBS-KNAW Fungal Biodiversity Centre (The Netherlands) and/or in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI, Brazil). Sequences derived in this study were lodged at GenBank and taxonomic novelties with MycoBank (Crous et al. 2004).

DNA extraction

Isolates were transferred to fresh 2 % MA (Malt Agar) plates and incubated in the dark at 25 °C for 14–21 d. Approximately 1 cm^2 of mycelium was transferred to a 2 mL Eppendorf tube containing 500 µL of lysis buffer (50 mmol/L Tris-HCl; 250 mmol/L NaCl; 50 mmol/L EDTA; 0.3 % w/v SDS; pH 8) and the equivalent to 100 µL of glass beads (SIGMA-ALDRICH catalogue G8893). After vortexing for 4 min, the microtubes were incubated for 1 h at 65 °C (two replicates). After centrifugation for 15 min at 13 000 rpm the aqueous phase with DNA was transferred into new microtubes. DNA extracts were stored at -20 °C prior to use.

Table 1 Fungal isolates details and GenBank accession numbers of isolates included in this study.

Species	Isolate number[1]	Source	Isolation technique	GenBank Accession numbers[2]				
				tub2	*cmdA*	*tef1*	ITS	LSU
Penidiellopsis radicularis	AP386 = CBS 131976 = CBMAI 1938	*A. capiguara* gyne	Oil flotation	KU216267	KU216292	KU216339	KT833148	KU216314
	AP387 = CBS 131979 = CBMAI 1947	*A. capiguara* gyne	Oil flotation	KU216268	KU216293	KU216340	KT833149	KU216315
	AP389	*A. capiguara* gyne	Oil flotation	KU216269	KU216294	KU216341	KT833150	KU216316
	AP410 = CBS 131963	*A. capiguara* gyne	Oil flotation	KU216272	KU216297	KU216344	KT833153	KU216319
	AP418	*A. capiguara* gyne	Oil flotation	KU216273	KU216298	KU216345	KT833154	KU216320
	AP440 = CBS 132769 = CBMAI 1951	*A. capiguara* gyne	Oil flotation	KU216276	KU216301	KU216348	KT833157	KU216323
	DOC350 = CBMAI 1952	*A. capiguara* drone	Nitrogen-free	KU216277	–	KU216349	KT833158	KU216324
	DOC363 = CBMAI 1953	*A. capiguara* drone	Nitrogen-free	KU216278	KU216302	KU216350	KT833159	KU216325
Penidiellopsis ramosus	AP391 = CBMAI 1937[ET]	*A. capiguara* gyne	Oil flotation	KU216270	KU216295	KU216342	KT833151	KU216317
	AP392 = CBMAI 1948	*A. capiguara* gyne	Oil flotation	KU216271	KU216296	KU216343	KT833152	KU216318
	AP420 = CBMAI 1949	*A. capiguara* gyne	Oil flotation	KU216274	KU216299	KU216346	KT833155	KU216321
	AP421 = CBMAI 1950	*A. capiguara* gyne	Oil flotation	KU216275	KU216300	KU216347	KT833156	KU216322
Simplicidiella nigra	AP 416 = CBMAI 1939[ET]	*A. capiguara* gyne	Oil flotation	KU216266	KU216291	KU216338	KT833147	KU216313
Xenopenidiella clavata	DOC354 = CBMAI 1942[ET]	*A. capiguara* drone	Nitrogen-free	KU216287	–	–	KT833168	KU216334
Xenopenidiella formica	67N = CBMAI 1954	*A. laevigata* drone	Oil flotation	KU216282	KU216306	KU216354	KT833163	KU216329
	AP380 = CBMAI 1946	*A. capiguara* gyne	Oil flotation	KU216283	KU216307	KU216355	KT833164	KU216330
	DOC323 = CBMAI 1941[ET]	*A. capiguara* drone	Nitrogen-free	KU216284	KU216308	KU216356	KT833165	KU216331
	DOC349	*A. capiguara* drone	Nitrogen-free	KU216285	KU216309	KU216357	KT833166	KU216332
	DOC362	*A. capiguara* drone	Nitrogen-free	KU216286	KU216310	KU216358	KT833167	KU216333
Xenopenidiella inflata	87N = CBMAI 1945[ET]	*A. laevigata* drone	Oil flotation	KU216290	KU216312	KU216359	KT833171	KU216337
Xenopenidiella laevigata	89N = CBMAI 1944[ET]	*A. laevigata* gyne	Oil flotation	KU216289	–	–	KT833170	KU216336
Xenopenidiella nigrescens	DOC356 = CBMAI 1943[ET]	*A. capiguara* drone	Nitrogen-free	KU216288	KU216311	–	KT833169	KU216335
Xenopenidiella tarda	DOC248 = CBMAI 1940[ET]	*A. capiguara* drone	Dextrose 50%	KU216279	KU216303	KU216351	KT833160	KU216326
	DOC317	*A. capiguara* drone	Nitrogen-free	KU216280	KU216304	KU216352	KT833161	KU216327
	DOC343	*A. capiguara* drone	Nitrogen-free	KU216281	KU216305	KU216353	KT833162	KU216328

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CBMAI: Brazilian Collection of Environmental and Industrial Microorganisms; all other codes are placed in the culture collection of the Microbial Resource Centre (UNESP, Brazil).

[2] *cmdA*: partial calmodulin gene; ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: partial 28S nrRNA gene; *tef1*: partial translation elongation factor 1-alpha; *tub2*: partial beta-tubulin gene.

[ET] ex-type.

Table 2 Primer combinations used for amplification and sequencing.

Locus	Primer	Primer sequence 5' to 3'	Annealing temperature (°C)	Orientation	Reference
β-tubulin (*tub2*)	T1	AACATGCGTGAGATTGTAAGT	52	Forward	O'Donnell & Cigelnik (1997)
	β-Sandy-R	GCRCGNGGVACRTACTTGTT		Reverse	Stukenbrock et al. (2012)
Calmodulin (*cmdA*)	CAL-235F	TTCAAGGAGGCCTTCTCCCTCTT	50	Forward	Quaedvlieg et al. (2012)
	CAL2Rd	TGRTCNGCCTCDCGGATCATCTC		Reverse	Groenewald et al. (2013)
ITS	ITS1	TCCGTAGGTGAACCTGCGG	52	Forward	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC		Reverse	White et al. (1990)
LSU (28S nrDNA)	LSU1Fd	GRATCAGGTAGGRATACCCG	52	Forward	Crous et al. (2009a)
	LR5	TCCTGAGGGAAACTTCG		Reverse	Vilgalys & Hester (1990)
Translation elongation factor-1α (*tef1*)	EF1-728F	CATCGAGAAGTTCGAGAAGG	52	Forward	Carbone & Kohn (1999)
	EF-2	GGARGTACCAGTSATCATGTT		Reverse	O'Donnell et al. (1998)

Multi-locus PCR amplification and sequencing

Isolates were screened for five loci (ITS, LSU, *cmdA*, *tef1* and *tub2*) using the primer sets listed in Table 2. Amplification reactions were performed in a total volume of 25 µL containing 1 µL of 50 mM $MgCl_2$, 4 µL of 1.25 mM of dNTP Mix, 2.5 µL of 10× PCR buffer, 2 µL of 10 µM of each primer (100 µM stock concentration), 0.2 µL of 5 U/µL of Taq polymerase (Invitrogen), 5 µL of DNA template (diluted 1 : 100) and 8.3 µL PCR water. PCR conditions were set as follows: an initial denaturation temperature of 96 °C for 2 min, followed by 35 cycles of denaturation at 96 °C for 45 s, primer annealing at the temperature stated in Table 2, primer extension at 72 °C for 90 s and a final extension step at 72 °C for 2 min. PCR products were analysed on a 1 % agarose gel and a negative control (DNA free) was also included to check for possible contaminations. Amplicons were purified using a NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel), according to the manufacturer's instructions. The PCR products were sequenced in both directions using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA, USA) and an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems). Consensus sequences were generated using the BioEdit Sequence Alignment Editor v. 7.0.5.3 (Hall 1999).

Alignment and phylogenetic reconstruction

A preliminary identification of the isolates to genus level was made by comparing the consensus LSU sequences against NCBIs GenBank nucleotide database using the megaBLAST algorithm. The most similar sequences were downloaded in FASTA format and subsequently combined with the *Teratosphaeriaceae* alignment of Quaedvlieg et al. (2014) (TreeBASE study S16145). A draft phylogeny was created from this alignment (data not shown), after which a reduced dataset was used to generate the final overview phylogeny presented here. The species level phylogeny was created from a concatenated alignment consisting of ITS, *cmdA*, *tub2* and *tef1* sequences. All loci were aligned individually using the MAFFT v. 7 online portal (http://mafft.cbrc.jp/alignment/server/index.html; Katoh & Standley 2013), after which they were manually checked and improved in MEGA v. 6.06 (Tamura et al. 2013). The phylogenetic reconstructions were conducted using the command line versions of MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) and PAUP v. 4.0b10 (Swofford 2003), while MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings for MrBayes. A parsimony analysis was performed on the overview LSU alignment and the combined 4-locus alignment; prior to concatenation of the four loci, individual gene trees derived from each locus were examined for topological conflict compared to the trees from the other loci. Gaps were treated as a new state, missing data were indicated as missing data in the analyses and statistics such as tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC, respectively) were calculated. For the Bayesian analysis of the overview LSU phylogeny, the heating parameter was set to 0.2 and the search was stopped when convergence was reached (stopval = 0.01). Trees were saved every 100 generations and the Markov Chain Monte Carlo (MCMC) analysis of 4 chains started in parallel from a random tree topology. The optimal substitution model under the Akaike's Information Criterion was the GTR model with dirichlet (1,1,1,1) state frequency distribution and inverse gamma-shaped rate variation across sites. Sequences derived from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1), and the alignments and trees in TreeBASE (https://treebase.org/treebase-web/home.html). The resulting phylogenetic trees were imported into and printed with Geneious v. 7.1.8 (http://www.geneious.com, Kearse et al. 2012), and the layout of the tree for publication was done using Adobe Illustrator v. CS6.

Morphology

Colony characters and pigment production were observed on 2 % MA (Malt Agar), PDA (Potato Dextrose Agar, Acumedia®) and CMA (Corn Meal Agar, Himedia®) at 25 °C in the dark for 21 d. Microscopic observations were based on slide culture techniques using CMA due to the rapid induction of sporulation. Agar blocks of ~1 cm^2 were placed on a sterile Petri dish and inoculated at the four edges. The block was subsequently covered with a sterile cover slip (~2 cm^2). Plates were incubated at 25 °C in the dark for 14–21 d. Slides were made in distilled water. Micrographs were taken using a phase-contrast microscope (DM 750, Leica; software Leica Application Suite v. 3.5.0, Leica).

RESULTS

Phylogeny

The LSU alignment consisted of 75 sequences (including the outgroup sequence, *Toxicocladosporium rubrigenum* GenBank FJ790305) and 776 characters were included in the analysis. Of the 776 analysed characters, 174 were parsimony-informative, 85 variable characters were parsimony-uninformative and 517 characters were constant. A maximum of 1 000 equally most parsimonious trees were saved, one of which is shown in Fig. 1 (TL = 799 steps; CI = 0.458; RI = 0.777; RC = 0.356). The Bayesian analysis sampled 255 unique site patterns and lasted 775 000 generations, after which convergence was achieved. The posterior probability values mapped unto Fig. 1 were calculated from the 11 628 trees sampled from the 15 502 trees generated; the remainder of the trees were discarded as burn-in. A similar topology was observed between the Bayesian and parsimony analyses. The LSU phylogeny revealed that the strains from ants are present in two main clades, one of which is associated with the genus *Xenopenidiella*, whereas the other

has as closest neighbour '*Penidiella' aggregata* (GenBank JF499862), '*Penidiella' drakensbergensis* (GenBank KC005792) and the recently described genus *Penidiellopsis* (GenBank LN834445). As the genus *Penidiella* is restricted to the lineage containing *P. columbiana* (represented here by GenBank EU019274, obtained from the ex-type culture CBS 486.80), which is not congeneric with the lineage containing *P. aggregata*, *P. drakensbergensis* and the ant strains, one novel genus, *Simplicidiella*, is described below to accommodate one of the strains isolated from ants and another new genus. *Penidiellomyces*, is proposed for the two '*Penidiella*' species.

For the species level analysis, a concatenated sequence alignment from 26 strains (including the outgroup species *Parapenidiella pseudotasmaniensis* strain CBS 124991) was subjected to maximum parsimony analyses. The final aligned sequences of the ITS (498 characters), *cmdA* (389 characters), *tef1* (342 characters) and *tub2* (446 characters) gene regions had a total length of 1 675 characters (including alignment gaps) which were included in the analyses. The gaps in the alignment were treated as fifth base for the parsimony analyses. From the analysed characters, 902 were constant (ITS: 293; *cmdA*: 257; *tef1*: 152; *tub2*: 200), 255 were variable and parsimony-

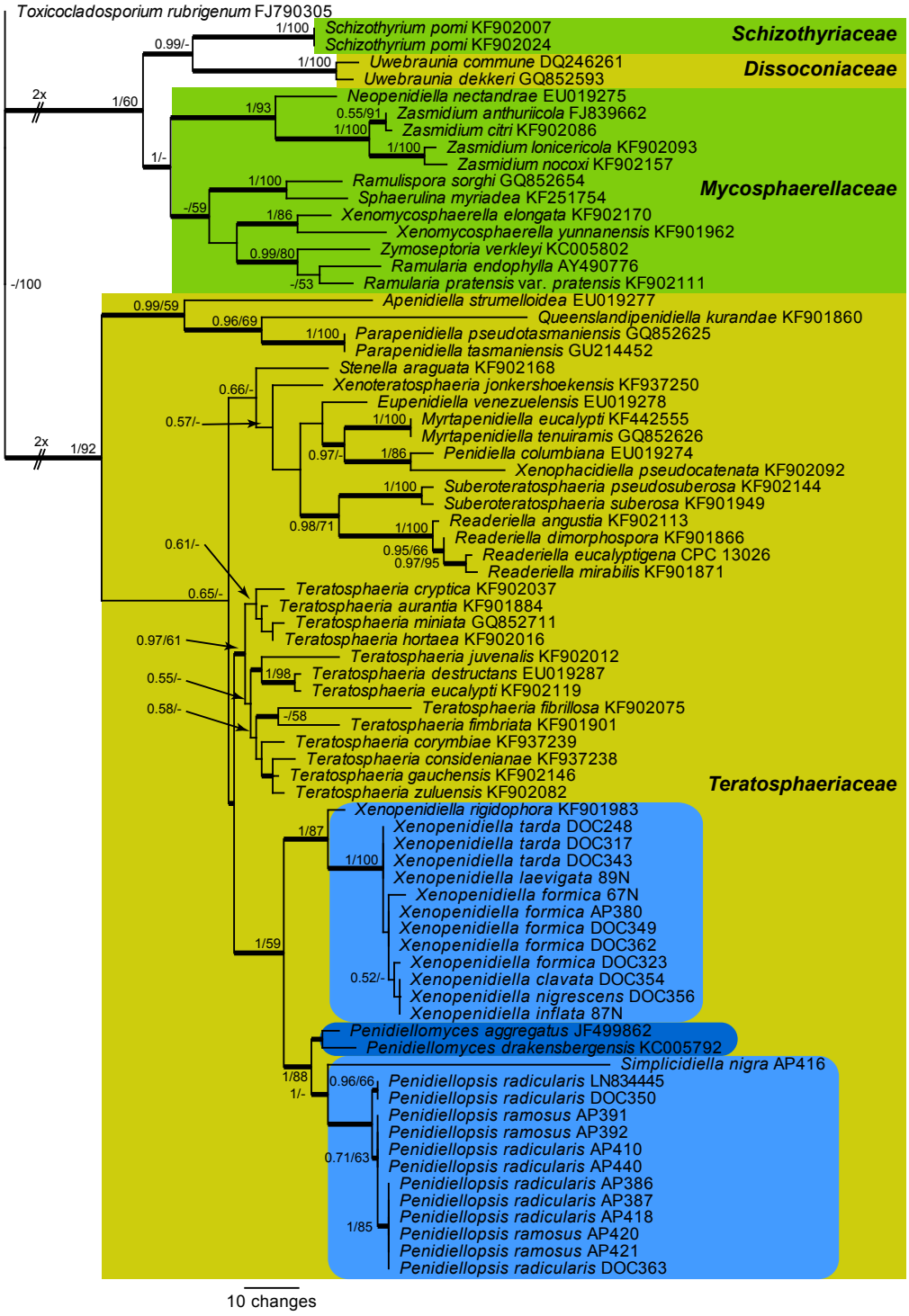

Fig. 1 One of 1 000 equally most parsimonious trees obtained from a maximum parsimony analysis of the LSU sequence alignment. The scale bar shows 10 changes, and posterior probability values from a Bayesian analysis (PP) and parsimony bootstrap support (PBS) values from 1 000 replicates are shown at the nodes (PP/PBS). Thickened lines represent those branches also present in the strict consensus tree and families are indicated with coloured square blocks and the clades with ant isolates with light blue-coloured blocks with rounded corners. The darker blue block with rounded corners represents the novel genus introduced here to accommodate '*P.*' *aggregata* and '*P.*' *drakensbergensis*. The lengths of some branches were halved to facilitate easier layout. The tree was rooted to *Toxicocladosporium rubrigenum* (GenBank accession number FJ790305).

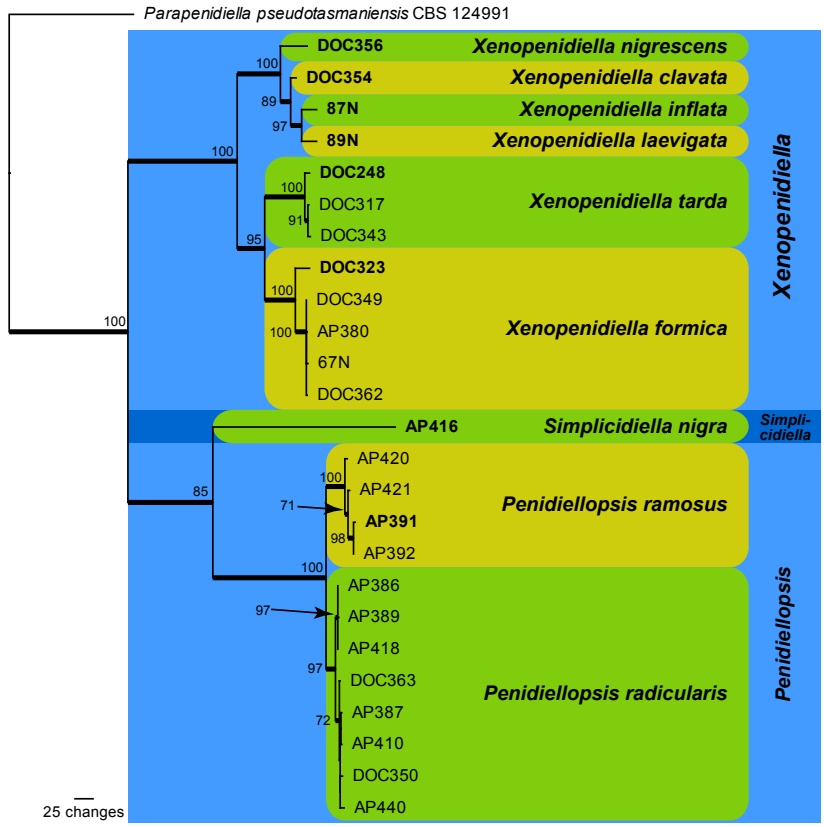

Fig. 2 One of 59 equally most parsimonious trees obtained from a maximum parsimony analysis of the combined ITS, *cmdA*, *tub2* and *tef1* sequence alignment. The scale bar shows 100 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines represent those branches also present in the strict consensus tree and species are indicated with coloured square blocks with rounded corners and the genera with coloured blocks. The tree was rooted to *Parapenidiella pseudotasmaniensis* strain CBS 124991 (GenBank accession numbers KF901522, KF902589, KF902855, KF903152, respectively).

uninformative (ITS: 93; *cmdA*: 44; *tef1*: 65; *tub2*: 53) and 518 were parsimony-informative (ITS: 112; *cmdA*: 88; *tef1*: 125; *tub2*: 193). A total of 59 equally most parsimonious trees (TL = 1 343 steps; CI = 0.832; RI = 0.944; RC = 0.786) were saved from the parsimony analysis, one of which is shown in Fig. 2. Rerunning the analysis without the *tef1* sequences (the least complete dataset) did not result in an overall topology that was much different from the topology depicted in Fig. 2; only some internal rearrangements were observed between the isolates representing each of the two multi-strain species in *Penidiellopsis* (data not shown). In addition, a comparison of the individual gene trees revealed the same generic and terminal species clades, with some rearrangements in the order of the species clades within the different genera (data not shown). The only exception was the genus *Penidiellopsis*, where the most support for the two species recognised in Fig. 2 was obtained from the *tub2* sequences. The ITS sequence of strain DOC 350 was 100 % identical to *Penidiellopsis radicularis* (GenBank LN834441); unfortunately no other loci were available to include the ex-type culture of this species in the multi-gene phylogeny. Several unique lineages are present in the obtained phylogeny (Fig. 2), and these are described as novel species in the Taxonomy section below.

Taxonomy

Penidiellomyces Crous, Attili-Angelis, A.P.M. Duarte, Pagnocca & J.Z. Groenew., *gen. nov.* — MycoBank MB817411

Etymology. Named after its morphological similarity to the genus *Penidiella*.

Type species. *Penidiellomyces aggregatus* (Crous) Crous & A.P.M. Duarte.

Hyphomycetous. *Mycelium* consisting of branched, septate, smooth, pale brown hyphae. *Conidiophores* solitary, arising from superficial mycelium, erect, brown, smooth, septate, straight to irregularly geniculate-sinuous. *Conidiogenous cells* terminal, subcylindrical, unbranched, medium brown, smooth, tapering to a flattened or rounded apical region, scars unthickened, aggregated, somewhat darkened, not refractive. *Ramoconidia* 0–1-septate, medium brown, smooth, ellipsoidal to obclavate, obovoid or subcylindrical, with 1–3-apical hila. *Intermediate and terminal conidia* subcylindrical to ellipsoid, 0–1-septate, brown, in chains of up to 6; hila truncate, unthickened, somewhat darkened.

Notes — *Penidiellomyces* resembles *Penidiella* in having solitary conidiophores with an apical apparatus that can appear penicillate, giving rise to branched chains of conidia. It differs, however, by having a terminal conidiogenous cell with a more well-developed apical rachis that gives rise to ramoconidia. The recent introduction of several penidiella-like genera in this complex (Quaedvlieg et al. 2014), however, will make it difficult to identify species of *Penidiellomyces* without the aid of molecular data. Ecologically, both known species share a foliicolous habitat on plants known from South Africa (Crous et al. 2007, 2012, Crous & Groenewald 2011), and are presumed to be saprobic.

Penidiellomyces aggregatus (Crous) Crous & A.P.M. Duarte, *comb. nov.* — MycoBank MB817412

Basionym. *Penidiella aggregata* Crous, Persoonia 26: 78. 2011.

Description and illustration — See Crous & Groenewald (2011).

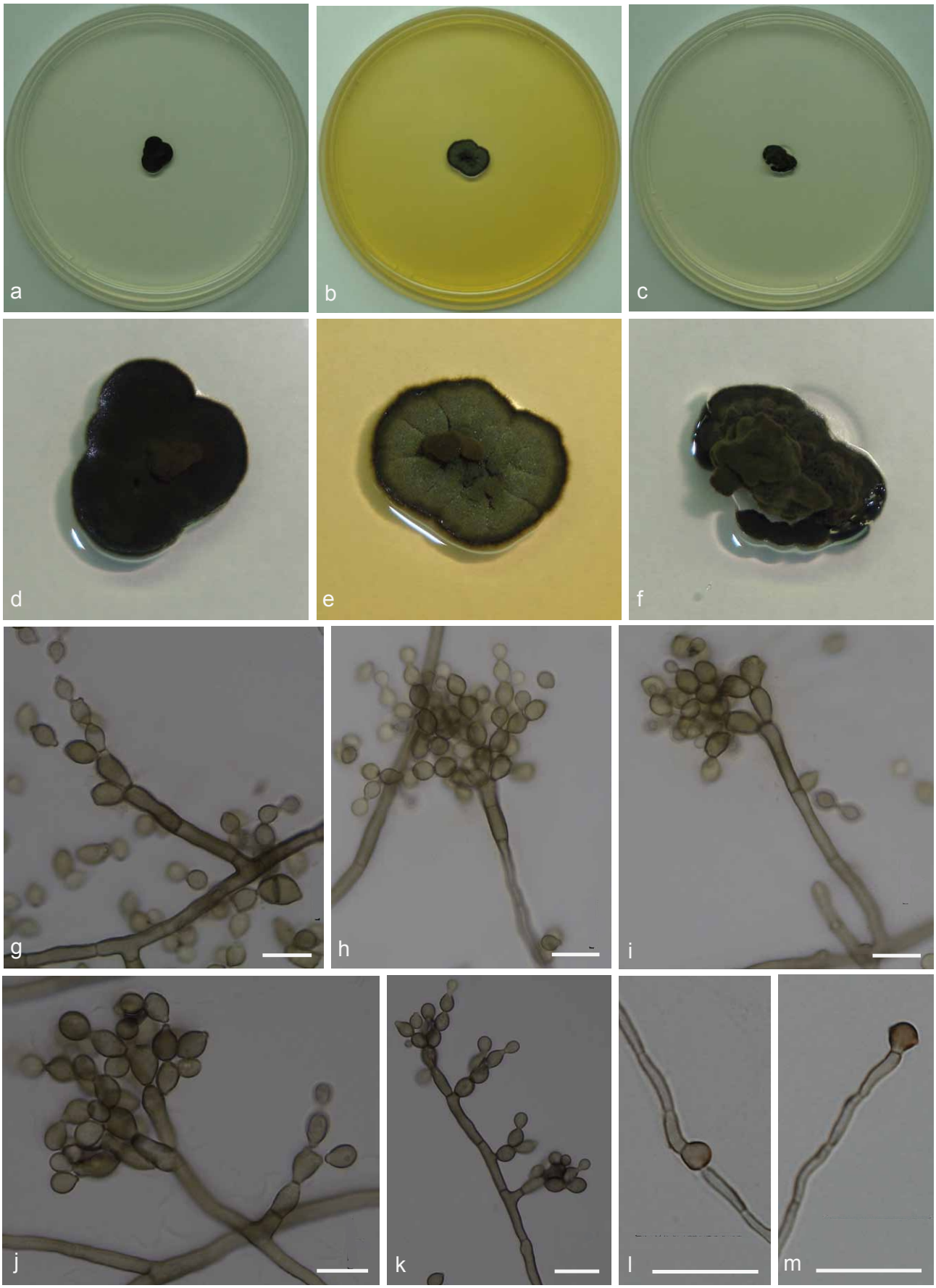

Fig. 3 *Penidiellopsis radicularis*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–k. conidiophores with conidia in chains; l–m. chlamydospores. — Scale bars = 10 µm.

Penidiellomyces drakensbergensis (Crous) Crous & Attili-Angelis, *comb. nov.* — MycoBank MB817413

Basionym. *Penidiella drakensbergensis* Crous, Persoonia 29: 161. 2012.

Description and illustration — See Crous et al. (2012).

Penidiellopsis Sandoval-Denis et al., Persoonia 36: 439. 2016

Type species. *Penidiellopsis radicularis* Sandoval-Denis et al.

Conidiophores differentiated, solitary, erect, straight to geniculate-sinuous, rarely branched, pale to medium brown, smooth- and thick-walled. *Conidiogenous cells* integrated, terminal or intercalary, pale to medium brown, smooth, mono- and polyblastic, giving rise to one or more sets of ramoconidia, scars truncate, slightly darkened, unthickened and not refractive. *Ramoconidia* 0–1-septate, obovoid, ellipsoid or slightly clavate, pale to medium brown, smooth- and thick-walled, apical part with denticle-like loci, basal scar flattened, slightly darkened, unthickened and not refractive. *Conidia* in branched acropetal chains, 0-septate, obovoid, ellipsoid or limoniform, pale to medium brown, smooth, thick-walled, with conidial scars truncate or protuberant, somewhat darkened, unthickened and not refractive. *Sexual morph* unknown.

Notes — *Penidiellopsis* was introduced as a new genus to accommodate an isolate from a human nail, collected in South Carolina, USA (Crous et al. 2016). Phylogenetically it is closely related to *Penidiellomyces aggregatus* and *P. drakensbergensis.* Conidiophores in *Penidiella* are penicillate with well-developed apical branches, characteristics that were absent in *Penidiellopsis*. Morphological data are only known from slow-growing colonies in artificial media.

Penidiellopsis radicularis Sandoval-Denis et al., Persoonia 36: 439. 2016 — Fig. 3

Isolated from gynes and drones of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale brown, 1.5–3 µm wide hyphae. *Conidiophores* erect, solitary, from superficial mycelium, branched or not, 25–53 × 3.5–4 µm. *Conidiogenous cells* mostly elongate, aseptate, smooth-walled, 10.5–13 × 3.5–4 µm. *Conidia* catenate in branched chains, 0–1-septate, smooth, lemon-shaped with prominent hila, not thickened, slightly darkened, 4.5–6.5 × 3–4 µm. *Chlamydospores* terminal and intercalary. Hila present but neither thickened nor darkened.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies flat with velutinous central portion, dark olivaceous to black, lobed margin, reaching 10 mm diam. Colonies on MA greyish green with darker mycelium in the centre and margin, crenate surface, reaching 11.5 mm diam. On PDA, dark olivaceous folded colonies, with a raised, velutinous central portion, reaching 11 mm diam. Colonies sporulating on all media, with dark mycelium and olivaceous black in reverse on all three media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* gyne, Nov. 2009, *F.C. Pagnocca*, CBMAI 1938.

Notes — Similar to *P. ramosus* although colonies are smaller. *Penidiellopsis radicularis* (originally isolated and described from an infection of a human nail) was found on gynes and drones, while *P. ramosus* only occurs on gynes of *Atta capiguara*.

Penidiellopsis ramosus Attili-Angelis & A.P.M. Duarte, *sp. nov.* — MycoBank MB817886; Fig. 4

Etymology. Name refers to its apically branched conidiophores.

Isolate from gynes of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale olivaceous-brown, 2–3.5 µm wide hyphae. *Conidiophores* ascending to erect, apically branched, bearing conidia in short chains, irregularly geniculate-sinuous, 27–63.5 × 3–4 µm. *Conidiogenous cells* aseptate, smooth-walled, 7–17 × 3–4 µm; scars not thickened, slightly darkened. *Conidia* catenate in branched chains, aseptate, smooth, limoniform to fusiform, 4–7.5 × 3–4 µm. *Chlamydospores* not observed. Hila present but neither thickened nor darkened.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies flat with velutinous central portion, dark olivaceous to black, reaching 13 mm diam. Colonies on MA greyish green with darker mycelium in the centre and margin, sulcate surface, reaching 14 mm diam. On PDA, dark olivaceous folded colonies, raised with an entire edge, velutinous central portion, reaching 12 mm diam. Colonies sporulating on all media, with dark mycelium and olivaceous black in reverse on all three media.

Specimens examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* gyne, Nov. 2009, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1937, preserved as metabolically inactive).

Notes — Similar to *P. radicularis*, but chlamydospores were not observed. Although conidia are similar in shape and size, those of *P. ramosus* are aseptate.

Simplicidiella Crous, Attili-Angelis, A.P.M. Duarte, Pagnocca & J.Z. Groenew., *gen. nov.* — MycoBank MB817414

Etymology. Named after the presence of simple and poorly differentiated conidiophores (Simplici-, *Latin* = simple).

Type species. *Simplicidiella nigra* A.P.M. Duarte & Attili-Angelis.

Hyphomycetous. *Mycelium* consisting of septate, smooth-walled, pale brown hyphae. *Conidiophores* erect, solitary, poorly branched or unbranched. *Conidiogenous cells* elongate, aseptate, smooth-walled; scars not thickened, slightly darkened. *Conidia* catenate, in branched chains, 0–1-septate, smooth, ellipsoidal to broadly ellipsoidal with prominent hila. *Sexual morph* unknown.

Notes — The genus *Simplicidiella* is described to accommodate ant-isolated melanised fungi in Brazil, with simple and poorly differentiated reproductive structures. Its few phenotypic characteristics underly the need of molecular tools for identification. The genus is presumed here to be saprobic. *Simplicidiella* is distinct from *Penidiella* and other penidiella-like genera for not sharing the following characteristics: penicillate conidiophores with branches (as in *Penidiella* s.str. and *Queenslandipenidiella*); being described as foliicolous (as in *Neopenidiella* and *Myrtapenidiella*); associated with opportunistic human infections with dimorphic conidiophores (as reported for *Eupenidiella*); production of ramoconidia (as in *Apenidiella*), and mycelium strongly branched (as observed in *Xenopenidiella*).

Simplicidiella nigra A.P.M. Duarte & Attili-Angelis, *sp. nov.* — MycoBank MB817415; Fig. 5

Etymology. Name refers to very dark colonies on CMA.

Isolated from gyne of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale brown, 1.5–3 µm wide hyphae. *Conidiophores* erect, solitary, poorly branched or unbranched, 27–47 × 3–3.5 µm. *Conidiogenous cells* elongate, aseptate, smooth-walled, 8–18.5 × 3–4 µm; scars not thickened, slightly darkened. *Conidia* catenate in

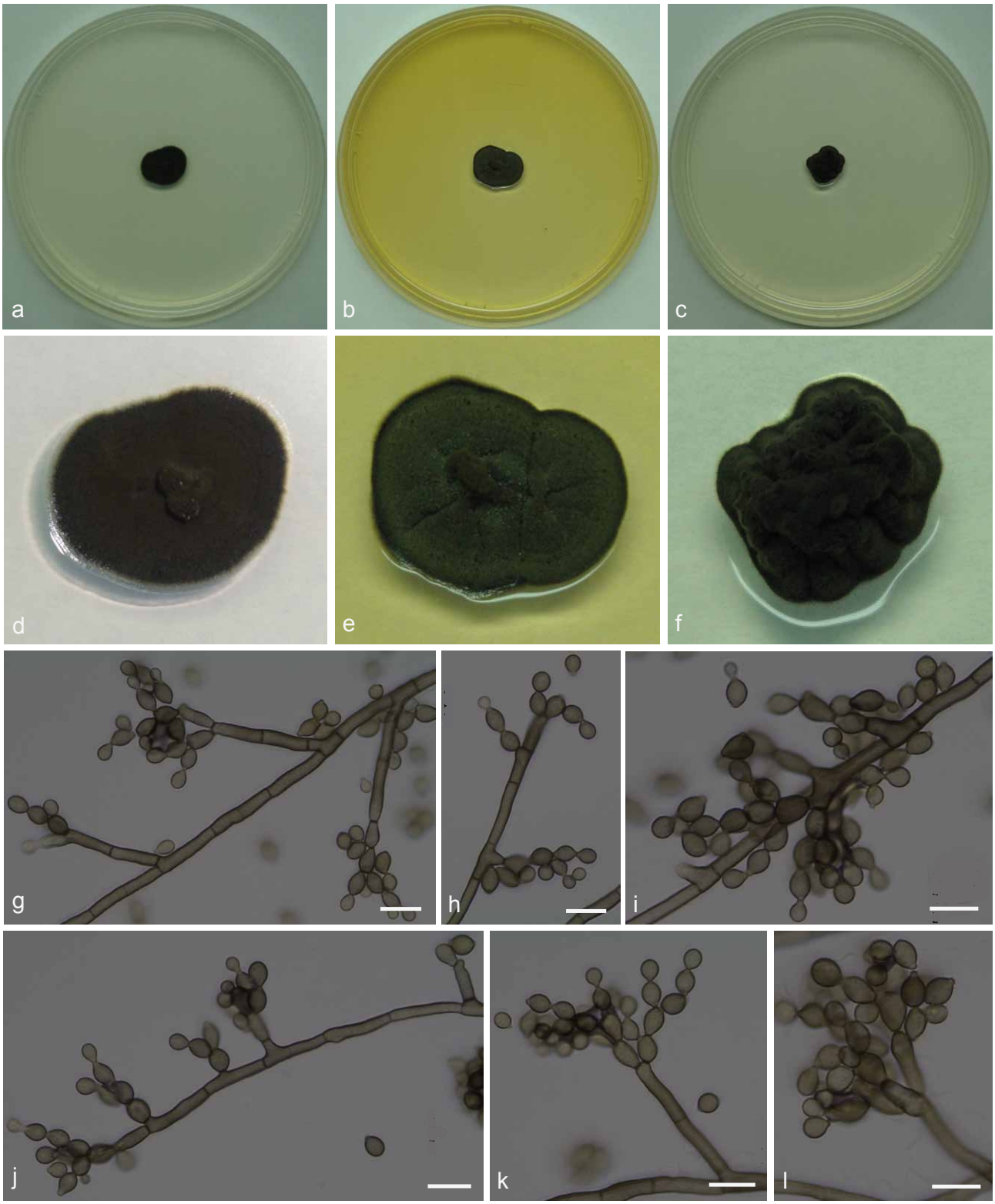

Fig. 4 *Penidiellopsis ramosus*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–l. conidiophores with conidia in chains. — Scale bars = 10 µm.

branched chains, 0–1-septate, smooth, ellipsoidal to broadly ellipsoidal with prominent hila, 5.5–9 × 3–4 µm. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies flat with velutinous central portion, colony margin dark olivaceous to black, reaching 14 mm diam. Colonies on MA flat with raised velutinous central portion, intermediate section with olivaceous green mycelium, and darker margins, reaching 11 mm diam. Exudate produced. On PDA, colonies with raised greyish central portion producing exudate, black mycelium and darker margins, reaching 11 mm diam. Sporulation and olivaceous black reverse were present in all media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* gyne, Nov. 2009, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1939, preserved as metabolically inactive).

Notes — Due to few phenotypic characteristics available, identification of *S. nigra* requires molecular data. This species is presumed to be saprobic and a potential hydrocarbon-degrader, based on the selective method used for isolation and due to the known presence of a complex mixture of hydrocarbons on the

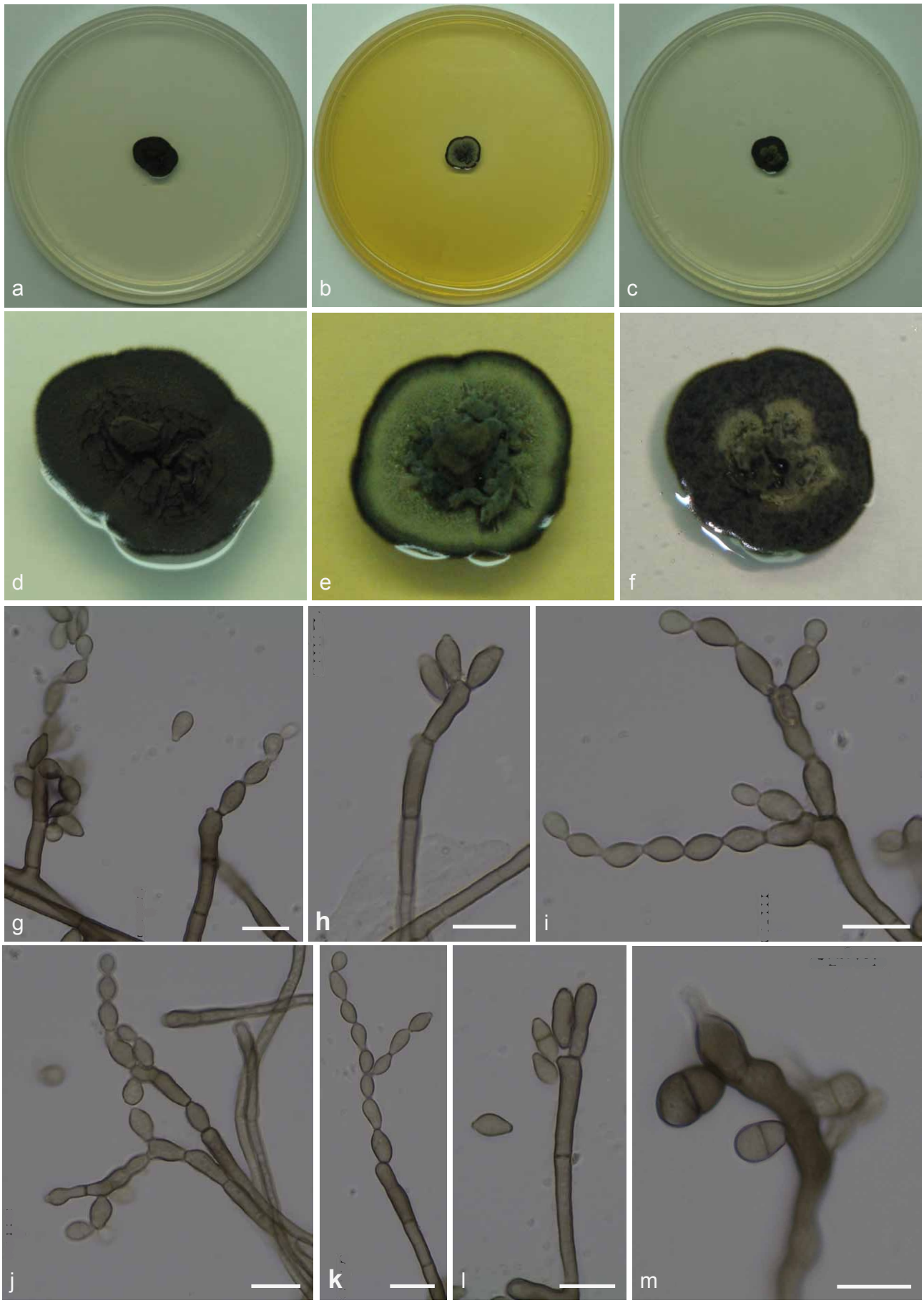

Fig. 5 *Simplicidiella nigra*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–k. conidiophores with conidia in chains; l–m. conidiogenous cells. — Scale bars = 10 µm.

cuticle of insects (Howard & Blomquist 2005). The species is described based on a single isolate, although its phylogenetic placement was supported by all loci and analyses.

Xenopenidiella Quaedvlieg & Crous, Persoonia 33: 33. 2014

Type species. *Xenopenidiella rigidophora* (Crous et al.) Quaedvlieg & Crous.

Xenopenidiella clavata Attili-Angelis, A.P.M. Duarte & Pagnocca, *sp. nov.* — MycoBank MB817416; Fig. 6

Etymology. Named after its clavate conidia.

Isolated from drone of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale brown, 3–4 µm wide hyphae. *Conidiophores* mostly reduced to conidiogenous cells arising directly from hyphae, rarely 1–5-septate,

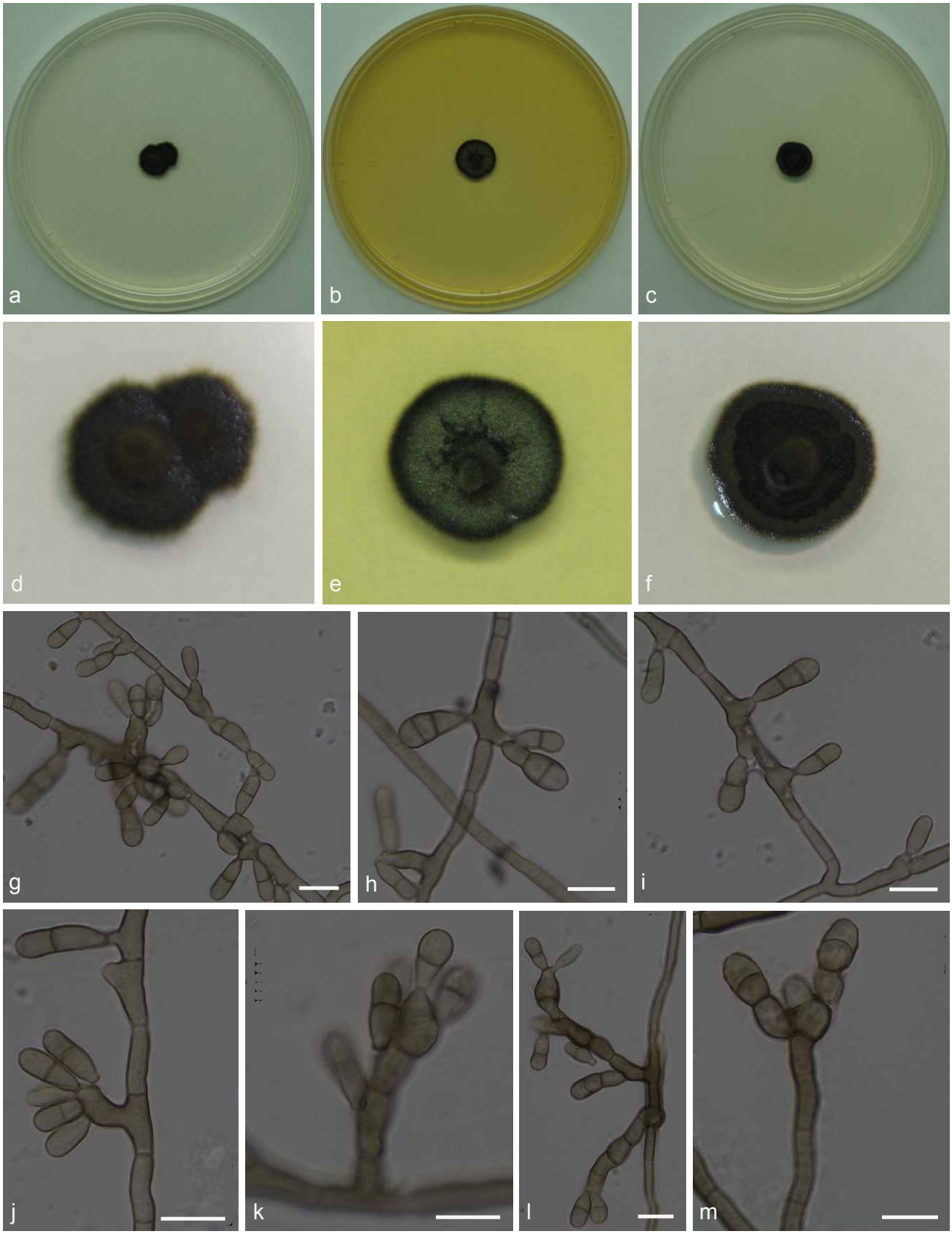

Fig. 6 *Xenopenidiella clavata*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–m. conidiophores with conidia. — Scale bars = 10 µm.

brown, smooth-walled, straight to geniculate-sinuous, 11–34 × 3.5–4.5 µm. *Conidiogenous cells* intercalary or terminal, brown, smooth-walled, 11–12 × 3.5–4 µm, with several aggregated terminal scars, not thickened nor darkened. *Conidia* 2–3-celled, smooth-walled to finely verrucose, clavate, apex obtuse, base truncate, constricted at both septa, 8–15.5 × 3–4 µm; hila not thickened, slightly darkened, no conidial scars observed. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. Colonies on CMA flat and dark olivaceous to brown, with a velutinous central portion, reaching 10 mm diam. Colonies on MA with a slightly elevated velutinous central portion, greenish mycelium and black margins, reaching 11 mm diam. On PDA, flat velutinous dark olivaceous to brown colonies, reaching 10 mm diam. Colonies with low to moderate sporulation and olivaceous black in reverse on all media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* drone, Nov. 2013, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1942, preserved as metabolically inactive).

Notes — *Xenopenidiella clavata* is ecologically distinct from *X. rigidophora* (type species) in its association to the leaf-cutting ants, which represents a new niche for the genus. Morphologically, conidiophores of *X. clavata* are not strongly branched, hyphae are not guttulate, and its mycelium is not strongly branched. Dimorphic conidiophores are present in both species, but in *X. clavata* conidiogenous cells are predominantly intercalary and conidia are only finely verrucose.

Xenopenidiella formica A.P.M. Duarte, Attili-Angelis & N.C. Baron, *sp. nov*. — MycoBank MB817417; Fig. 7

Etymology. Named after the isolation source (ants).

Isolated from gynes and drones of *Atta capiguara* and *A. laevigata* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale olivaceous, 2–3 µm wide hyphae, producing poorly differentiated conidiophores. *Conidiophores* erect, solitary, poorly branched or unbranched, smooth, brown, straight to geniculate-sinuous, 28.5–75 × 2–3.5 µm. *Conidiogenous cells* smooth-walled, bearing conidia from flat to semi-denticulate scars, 13–17 × 3.5–4 µm. *Conidia* 1-septate, smooth-walled, clavate, apex obtuse, base truncate, constricted at septum, 7–12 × 2.5–4 µm. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. Colonies on CMA were dark olivaceous and flat, velutinous, reaching 12 mm diam. On MA, flat colonies with velutinous central portion, greenish mycelium and darker margins, reaching 12.5 mm diam. On PDA, velutinous colonies with raised olivaceous green central portion, reaching 11 mm diam. Poor sporulation and olivaceous black in reverse on all media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* drone, Nov. 2013, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1941, preserved as metabolically inactive).

Notes — Phylogenetically, *X. formica* is closely related to *X. tarda*, but with a faster growth rate in culture, and smaller conidia. It is represented by five isolates from gynes and drones of *A. capiguara* and *A. laevigata*, while *X. tarda* was found only on *A. capiguara*. In contrast to *X. rigidophora*, *X. formica* does not exhibit strongly branched hyphae, and its conidia are not verrucose.

Xenopenidiella inflata A.P.M. Duarte, N.C. Baron, Pagnocca & Attili-Angelis, *sp. nov.* — MycoBank MB817418; Fig. 8

Etymology. Named after its swollen conidiogenous cells (Inflatus, *Latin* = swollen).

Isolated from drone of *A. laevigata* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth, pale brown, 1.5–3 µm wide hyphae. *Conidiophores* reduced to conidiogenous cells, or subcylindrical, smooth, brown, up to 3-septate, straight to geniculous-flexuous, 16–52 × 4–5 µm. *Conidiogenous cells* swollen or elongated, arising from mycelium or terminal on conidiophores, sometimes rejuvenating and elongating to have lateral and terminal conidiogenous cells, smooth-walled, 9–14 × 3.5–4.5 µm; scars not thickened nor darkened. *Conidia* 1-septate, smooth-walled, clavate, constricted at septum, truncate hila, 7.5–10 × 3–4 µm. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies with black velutinous mycelium and pronounced central portion, reaching 12.5 mm diam. Colonies on MA greyish green, velutinous and sulcate, black margins, reaching 12 mm diam. On PDA colonies were greenish and raised at the centre, olivaceous green towards the sulcate and darker margin, reaching 12.5 mm diam. Poor to moderate sporulation and olivaceous black reverse on all media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta laevigata* drone, Nov. 2011, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1945, preserved as metabolically inactive).

Notes — *Xenopenidiella inflata* is described based on a single isolate from an *A. laevigata* drone. Although phylogenetically closely related to *X. laevigata*, it differs based on its swollen conidiogenous cells and slower growth rate on cultural media. *Xenopenidiella inflata* differs from the *X. rigidophora* in the following morphological aspects: pale brown mycelium present (not pale olivaceous to medium brown); thinner hyphae (1.5–3 µm wide, not up to 6 µm diam); conidiogenous cells terminal and intercalary; conidia not verrucose (not appearing like small spines under light microscope); thicker hila.

Xenopenidiella laevigata *N.C.* Baron, A.P.M. Duarte, Pagnocca & Attili-Angelis, *sp. nov.* — MycoBank MB817419; Fig. 9

Etymology. Name refers to the first isolation source: the ant species *Atta laevigata*.

Isolated from gyne of *A. laevigata* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, olivaceous-brown, smooth and thick-walled, 3–3.5 µm wide hyphae. *Conidiophores* brown, smooth to finely verrucose, thick-walled erect, straight to geniculous-sinuous, 1–10-septate, 10–64 × 2–4.5 µm. *Conidiogenous cells* in loose branches, smooth-walled to finely verrucose, terminal or lateral, 10.5–14 × 2.5–3.5 µm; scars unthickened and not darkened. *Conidia* 1-septate, smooth-walled to finely verrucose, clavate, constricted at somewhat darker septum, 9.5–17.5 × 3–5 µm; hila unthickened and not darkened. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies with black velutinous mycelium and aerial central portion, reaching 18 mm diam. Colonies on MA greyish green, velutinous and sulcate, black margins, reaching 19 mm diam. On PDA colonies were dark olivaceous, sulcate and folded, with raised central portion and velutinous mycelium, reaching 18 mm diam. Poor sporulation and olivaceous black reverse on all three media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta laevigata* gyne, Nov. 2011, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1944, preserved as metabolically inactive).

Notes — In this study both species *X. laevigata* and *X. inflata* are known from single strains isolated from *A. laevigata* via the oil flotation method. The parsimony tree shows that they are closely related, but the former differs in the melanisation

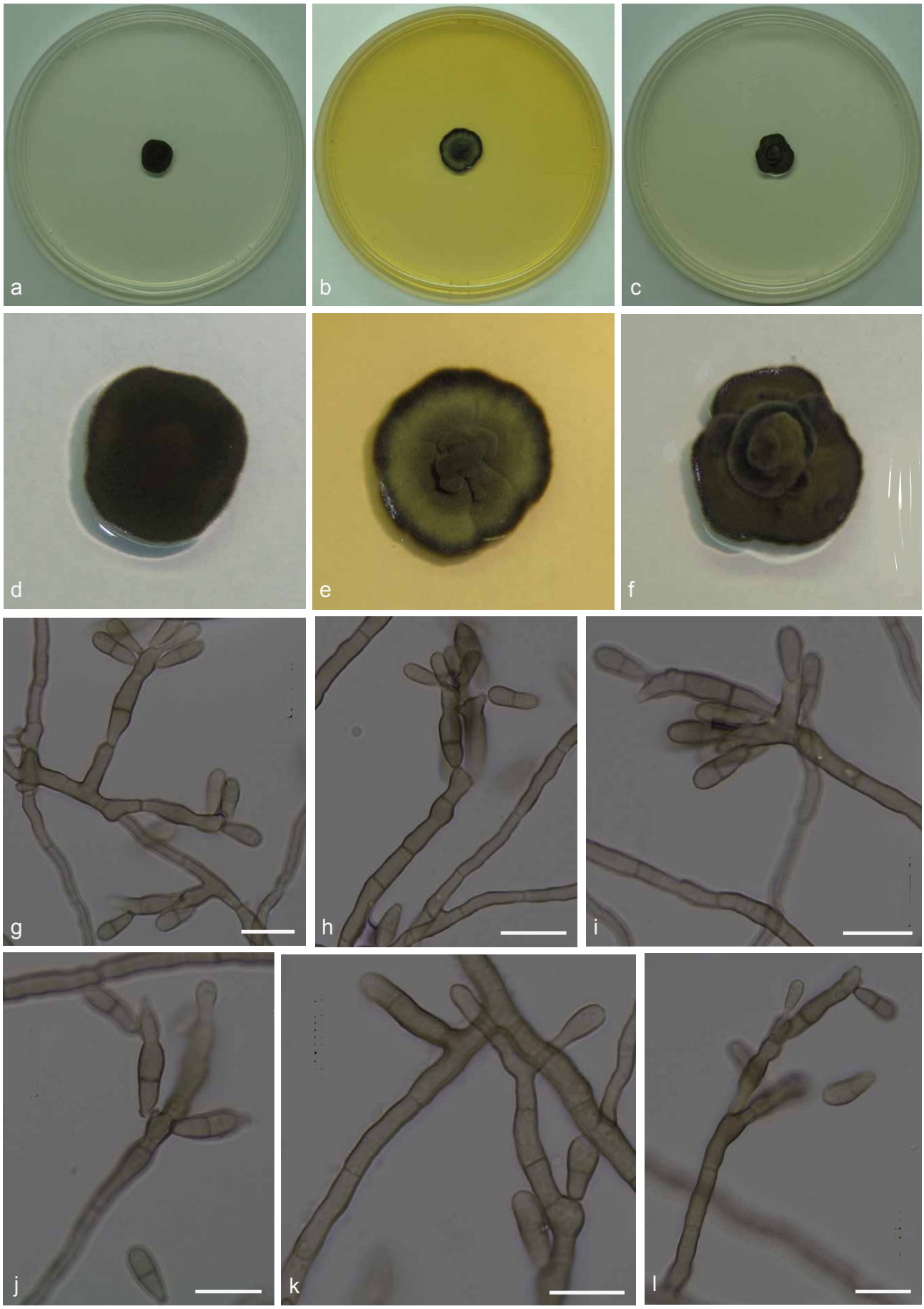

Fig. 7 *Xenopenidiella formica*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–l. conidiophores with conidia. — Scale bars = 10 µm.

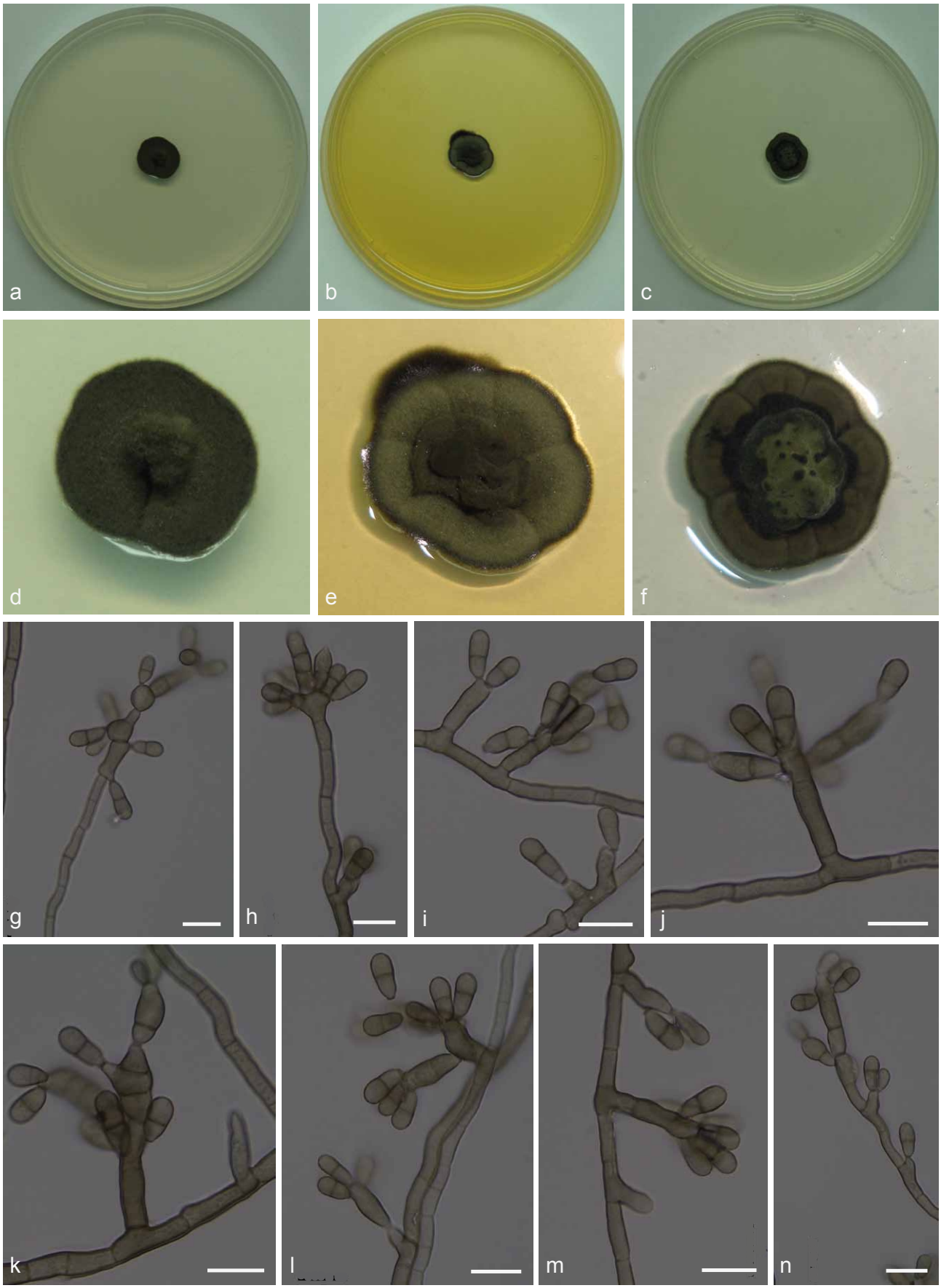

Fig. 8 *Xenopenidiella inflata*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–n. conidiophores with conidia. — Scale bars = 10 µm.

of the mycelium (colonies are darker and hyphae are thicker), growth rate and conidial size. A comparison with *X. rigidophora* shows differences such as less-branched and thinner hyphae, rare macronematous conidiophores that are loosely penicillate, and conidiogenous cells frequently intercalary.

Xenopenidiella nigrescens Attili-Angelis, A.P.M. Duarte & Pagnocca, *sp. nov.* — MycoBank MB817420; Fig. 10

Etymology. Named after its dark brown mycelium.

Isolated from drone of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, olivaceous-brown, smooth and thick-walled 3–4 µm wide hyphae. *Conidiophores* brown, smooth to finely verrucose, thick-walled, erect, straight to flexuous, 0–6-septate, 7–79 × 3.5–4.5 µm. *Conidiogenous cells* arising from mycelium, loosely branched, smooth-walled to verrucose, terminal and lateral, 13–18 × 3–3.5 µm. *Conidia* 1-septate, smooth-walled to finely verrucose, dark brown, clavate and constricted, 9–13 × 3.5–4 µm; hila unthickened and not darkened. *Chlamydospores* not observed.

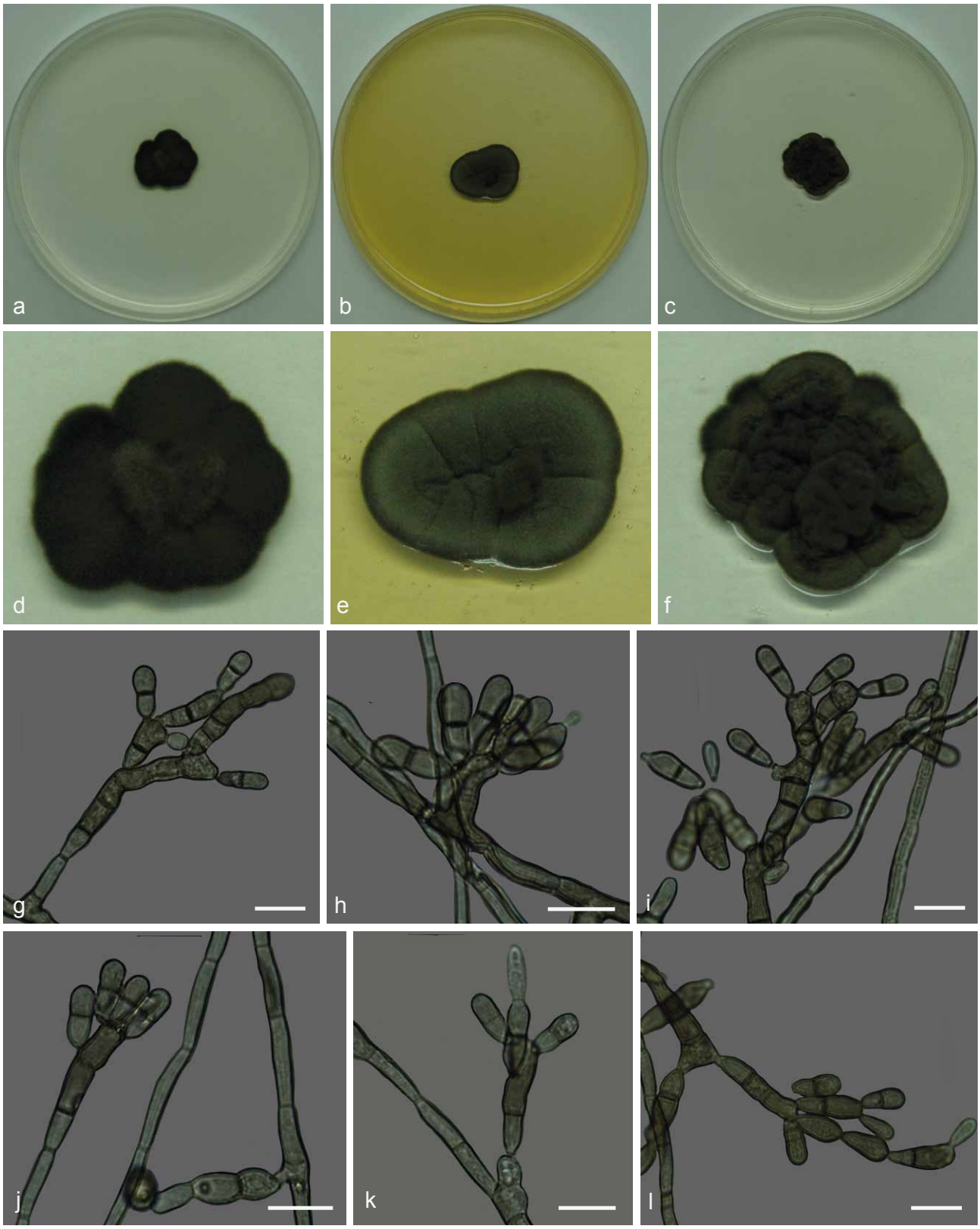

Fig. 9 *Xenopenidiella laevigata*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–l. conidiophores with conidia. — Scale bars = 10 µm.

Culture characteristics — Colonies were grown at 25 °C for 21 d. Colonies on CMA were flat and dark olivaceous to brown, velutinous with raised central portion, irregular margins, reaching 14 mm diam. Colonies on MA with velutinous and slightly folded surface, raised central portion, olivaceous mycelium, reaching 13 mm diam. On PDA, dark olivaceous highly folded colonies, raised velutinous central portion, reaching 13 mm diam. Colonies presented low to moderate sporulation and olivaceous black reverse on all media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* drone, Nov. 2013, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1943, preserved as metabolically inactive).

Notes — *Xenopenidiella nigrescens* was found to consistently have the darkest colonies on all three media tested when compared to other *Xenopenidiella* species. A combination of previously mentioned characteristics is also found in this species: slow growth rate (as in *X. tarda*), clavate conidia (as in *X. clavata*), 1-septate, smooth-walled to finely verrucose conidia

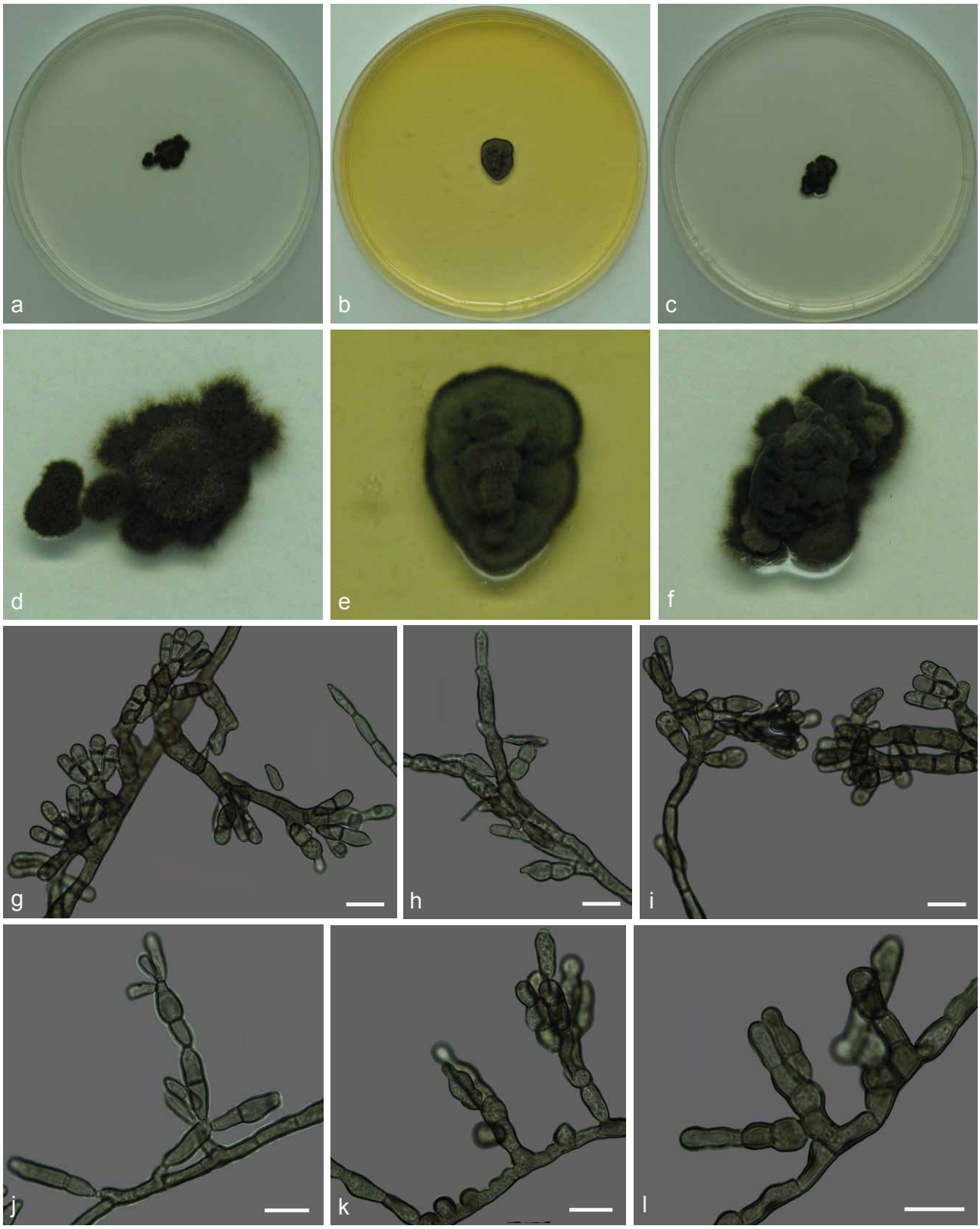

Fig. 10 *Xenopenidiella nigrescens*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–l. conidiophores with conidia. — Scale bars = 10 µm.

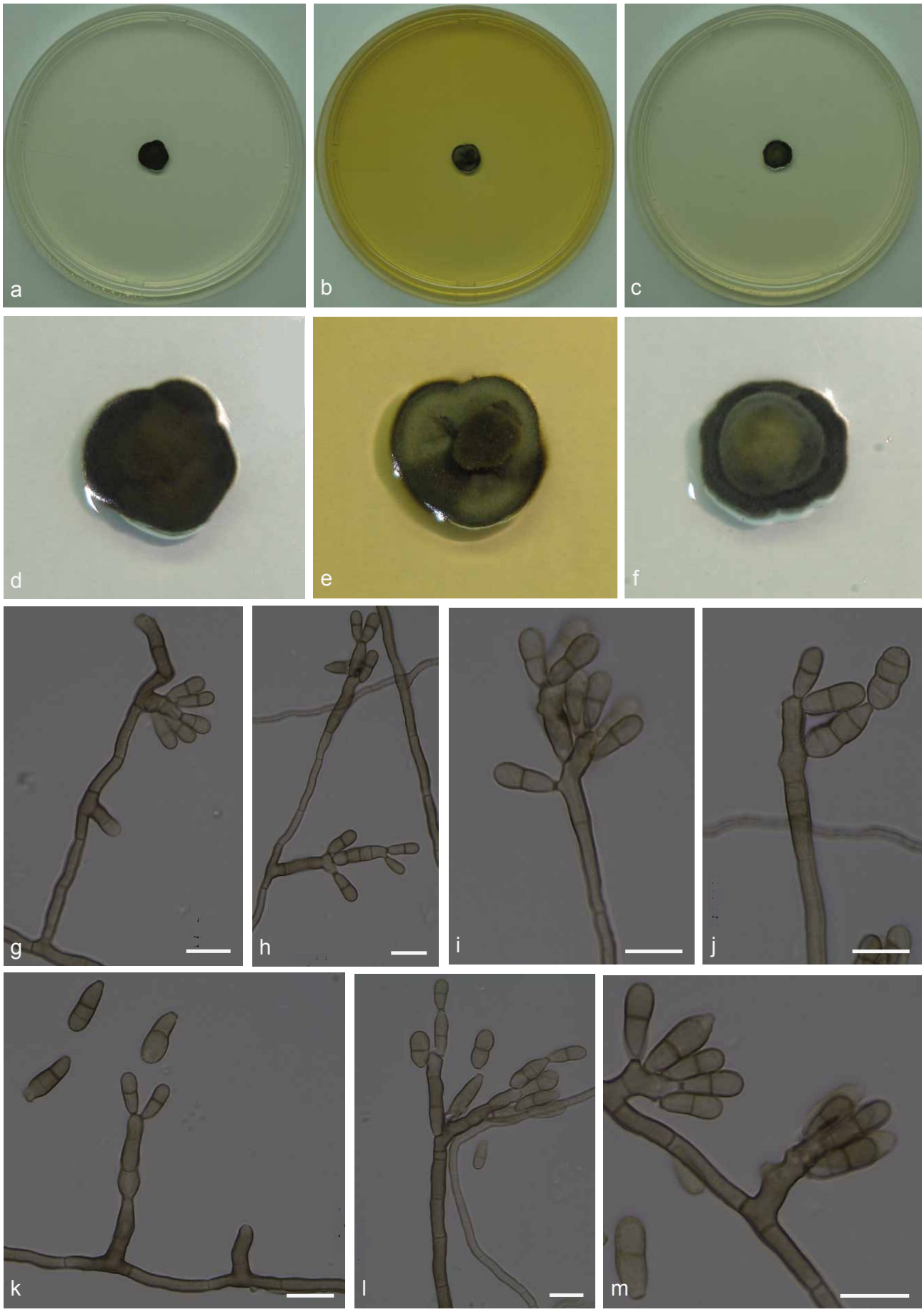

Fig. 11 *Xenopenidiella tarda*. a, d. Colony on CMA; b, e. colony on MA; c, f. colony on PDA; g–m. conidiophores with conidia. — Scale bars = 10 µm.

(resembling *X. laevigata*), conidiophores poorly differentiated (as in *X. formica*). In comparison to *X. rigidophora*, *X. nigrescens* differs in having darker mycelium, conidiogenous cells smooth to verrucose, and conidia rarely occurring in branched chains. Molecular data are required to confirm its identification.

Xenopenidiella tarda Pagnocca, A.P.M. Duarte & Attili-Angelis, *sp. nov.* — MycoBank MB817421; Fig. 11

Etymology. Named due to very slow growth rate (Tardus, *Latin* = slow).

Isolated from drones of *Atta capiguara* (*Myrmicinae*, Attini tribe). *Mycelium* consisting of septate, smooth-walled, pale brown, 2–3 µm wide hyphae. *Conidiophores* ascending to erect, solitary, poorly branched or unbranched, 0–4-septate, 5.5–51 × 3–4 µm. *Conidiogenous cells* elongate, aseptate, smooth-walled, 5.5–21.5 × 3–3.5 µm; scars unthickened and not darkened. *Conidia* 1-septate, smooth-walled to finely verrucose, clavate, constricted at septum, 8–11.5 × 3–5 µm; hila truncate, unthickened and not darkened. *Chlamydospores* not observed.

Culture characteristics — Colonies were grown at 25 °C for 21 d. On CMA, colonies dark olivaceous, velutinous, flat and small, reaching 9 mm diam. Colonies on MA flat with aerial velutinous central portion, olivaceous green mycelium and black margin, reaching 9 mm diam. On PDA colonies with raised greyish green central portion, and black margins, reaching 9 mm diam. Moderate sporulation and olivaceous black reverse present on all three media.

Specimen examined. Brazil, São Paulo (S22°50.6" W48°26.1", elev. 798 m), isolated from the integument of *Atta capiguara* drone, Nov. 2013, *F.C. Pagnocca* (holotype and culture ex-type CBMAI 1940, preserved as metabolically inactive).

Notes — *Xenopenidiella tarda* is represented by two isolates from drones of *A. capiguara*. It is morphologically similar to *X. formica*, although colonies have a slower growth rate in culture. This species can be distinguished from *X. rigidophora* because mycelium of *X. tarda* does not show to be strongly branched, not necessarily constricted at septa and hyphae are not so wide. Furthermore, conidiogenous cells are less wide and pale brown; conidia mostly smooth-walled or finely verrucose.

DISCUSSION

Leaf-cutting ants from the Attini tribe are social insects limited to the New World, widely distributed from Argentina to Southern USA, with a range of latitude from N10° to S25° (Mayhé-Nunes & Jaffé 1998). These ants have been investigated for the huge economic losses they can cause by damaging plant leaves, and for their unique ability for fungiculture, which has become a model system for coevolutionary studies (Chapela et al. 1994, Currie et al. 2003).

These insects were found to harbour a large and unknown diversity of microorganisms (Pagnocca et al. 2008, Rodrigues et al. 2008, 2011, Guedes et al. 2012, Duarte et al. 2014), and some novel species of filamentous fungi and yeasts were already described from the ants' microenvironment (Middelhoven et al. 2003, Carreiro et al. 2004, Pagnocca et al. 2010, Augustin et al. 2013, Attili-Angelis et al. 2014, Melo et al. 2014, Masiulionis et al. 2015, Meirelles et al. 2015, Samerpitak et al. 2015, Montoya et al. 2016). However, the occurrence of species of *Teratosphaeriaceae* in the ants' environment has never been documented.

The family *Teratosphaeriaceae* was established based on molecular phylogenetic analyses, which strongly supported its separation from *Mycosphaerellaceae*. *Teratosphaeria* and related genera were initially associated with leaf diseases of *Eucalyptus* (*Myrtaceae*) and *Proteaceae*, but their ecological habitat vary from saprobic, human opportunistic and plant pathogen to extremophilic species (Crous et al. 2007, Ruibal et al. 2009, Teodoro et al. 2012, Egidi et al. 2014, Quaedvlieg et al. 2014). Investigators in Brazil have isolated plant pathogenic fungi (Guedes et al. 2012) and opportunistic melanised representatives (Duarte et al. 2014) from workers of *Atta laevigata* and gynes of *Atta* spp., respectively, which were shown here to belong to the *Teratosphaeriaceae*.

One genus and eight new species of *Teratosphaeriaceae* were isolated when investigating the diversity of fungi occurring on the integument of pre-nuptial flight gynes and drones. The aim of these investigations was to further explore and better understand the primary microbial community that is dispersed into a young ant colony by these ants.

This is therefore the first study to provide a taxonomic treatment of *Teratosphaeriaceae* from ants, which has significantly expanded the number of *Xenopenidiella* species in addition to just the type species, *X. rigidophora*, thereby allowing for a more robust amended description of the genus. Previous reports on filamentous fungi from ant niches characterised similar isolates as found in the soil community (Pagnocca et al. 2008, Rodrigues et al. 2008), which is different from the results found in this study, where the fungi were isolated directly from the insects themselves. It can be hypothesized that the isolation methods used in the other studies may have contributed to this difference.

The lack of knowledge about ant nest microenvironments encouraged studies with a focus on this microenvironment, but even less is known about the ant integument from which the new species were obtained. One interesting ecological aspect of the *Teratosphaeriaceae* is that the family harbours isolates from extreme environments. However, the present scientific information is still insufficient to answer several questions about the studied substrate, for instance, if it should be treated as extreme or not. It is known that the pH of fungal gardens ranges from 4.35 to 5 (Powell & Stradling 1986), but nothing has been published about the pH of the integument itself. On the other hand, the presence of cuticular hydrocarbons and alkaloids on the integument was already reported (Roux et al. 2009).

The main lineages in the *Teratosphaeriaceae* remained obscured until the extensive revision published by Quaedvlieg et al. (2014). In this publication, a previous discussion point regarding the phylogenetic position of *Piedraiaceae* was once again raised as this fungal family appears to cluster within the *Teratosphaeriaceae* (Crous et al. 2009a). Other reports studying rock-inhabiting fungi (RIF) showed that these are phylogenetically highly diverse, and highlighted the importance of larger taxon samplings to define questions about generic boundaries (Ruibal et al. 2009, 2011). All these results demonstrate the importance of dedicated projects on fungal isolation from diverse niches.

Although this study still under-represents the total biodiversity on leaf-cutting ants, it does reveal yet another source of *Teratosphaeriaceae* species. It is known that the morphology of many genera of saprobic and plant pathogenic fungi have evolved several times independently (Crous et al. 2009b), which explains how these penidiella-like isolates could adapt to such an unlikely environment such as gynes and drones of attine ants.

The species phylogeny presented in Fig. 2 is based on four genomic loci (ITS, *cmdA*, *tef1* and *tub2* gene regions). For the species of *Xenopenidiella*, all loci for which data was available could distinguish the included species (data not shown, trees for individual loci were deposited in TreeBASE). However, the amplification success rate of *tef1* and *cmdA* for this genus was not as high as for ITS and *tub2*. The ex-type culture of *X. formica*, CBMAI 1941, clusters slightly distant to the rest of

the isolates of this species; this is due to the *tub2* sequence which is different (93 % identical; 371/401 nucleotides) from the other isolates of the species. The phylogenetic placement of the single isolate of *Simplicidiella* was supported by all loci and analyses (Fig. 1, 2). Both species of *Penidiellopsis* are only reliably distinguishable by their *tub2* sequences; all other loci either lack resolution to resolve the species or show an intermixing of isolates from the two species. It is quite possible that with a broader sampling these two species might turn out to be synonymous; however, on *tub2* they are only 92 % identical (394/428 nucleotides) based on fixed nucleotide differences and therefore we maintain them as separate species for now. A broader sampling from wider geographic regions is being planned to determine whether this difference is significant or not. Since all four loci together did not provide 100 % amplification success rate, it is recommended that an ideal identification protocol includes both ITS and *tub2* sequences.

Concluding remarks

Representatives of the *Teratosphaeriaceae* include species of agricultural importance and wide ecological plasticity with ability to colonise diverse substrates, including ants and extreme environments. Fungal richness among social insects is still underestimated despite the increasing number of new species found in this microenvironment. Over the last 10–15 yr it was concluded that knowledge on fungal diversity contributes greatly to safeguard global genetic resources, which will have a great impact on human lives in future (Hawksworth 2004, Lange et al. 2012). The present study contributes to this knowledge, which is a fundamental tool to plan strategies aimed at the protection of species and environments, especially in tropical and subtropical regions. A significant number of fungi are part of the complex interactions in leaf-cutting ant colonies and further studies will be required to determine their roles in this specific niche.

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