

Genus *Hamigera*, six new species and multilocus DNA sequence based phylogeny

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Abstract: Genus *Hamigera* was erected for *Talaromyces* species that make asci singly instead of in chains. Initially it contained two species, *H. avellanea* and *H. striata*. We describe six new species in the genus, *H. fusca*, *H. inflata*, *H. insecticola*, *H. pallida*, *H. paravellanea* and *H. terricola*. *Merimbla ingelheimensis* is a distinct anamorphic species in the *Hamigera* clade. None of our DNA sequence data (*BT2*, calmodulin, ITS, *lsu* rDNA, *RPB2*, *Tsr1* and *Mcm7*) supported the placement of *H. striata* in the same clade as *H. avellanea*, thus we accepted *Talaromyces striatus*. In addition to *Hamigera* species we examined the phylogenetic disposition of *Warcupiellea spinulosa*, *Penicillium megasporum*, *Penicillium arenicola* and *Merimbla humicoloides*. Despite nominal similarity of some of these species to *Merimbla*, none of these species are part of the *Hamigera* clade and *M. humicoloides* is placed in *Penicillium* to have a monophyletic genus *Hamigera*.

Key words: *Hamigera avellanea*, *Hamigera fusca*, *Hamigera inflata*, *Hamigera insecticola*, *Hamigera pallida*, *Hamigera paravellanea*, *Hamigera terricola*, *Merimbla ingelheimensis*, *Penicillium humicoloides*, phylogeny, systematics

mbla ingelheimensis, *Penicillium humicoloides*, phylogeny, systematics

INTRODUCTION

The unresolved taxonomy of *Penicillium avellaneum* Thom and Turesson (1915) has spanned nearly a century. Although this species produces a holomorph, Thom and Turesson (1915) placed it in the anamorphic genus *Penicillium*. Raper and Thom (1949) explicitly recognized the nomenclatural problem of describing a holomorphic fungus in a genus typified by an anamorphic fungus (*Penicillium*) but perpetuated the practice to group in one genus anamorphic and holomorphic fungi that they thought were clearly related. Benjamin (1955) renamed several holomorphic species that Raper and Thom (1949) had placed in *Penicillium*, among these were *Talaromyces striatus* (*Penicillium striatum*) and *Talaromyces avellaneus* (*Penicillium avellaneum*).

Stolk and Samson (1971) created *Hamigera* to contain two holomorphic fungi, *Talaromyces avellaneus* and *Talaromyces striatus*. *Hamigera* species produce asci singly from crosiers, while *Talaromyces* species forms asci in chains. These authors transferred *P. striatum* and *P. avellaneum* into *Hamigera* as *H. striata* and *H. avellanea* although the anamorphs were interpreted respectively as *Paecilomyces* or *Penicillium*. Pitt and Hocking (1979) introduced genus *Merimbla* for the anamorph of *Hamigera avellanea*. Pitt (1980) rejected the placement of *P. striatum* in *Hamigera*, accepting it in *Talaromyces*. Von Arx (1986) transferred *Warcupiellea spinulosa* to *Hamigera* and rejected *Merimbla* as the anamorph of *H. avellanea*, using the anamorphic genus *Raperia*. Subramanian and Rajendran (1979) for the anamorph of *Hamigera*. Von Arx (1986) also transferred *Penicillium striatum* to *Byssochlamys* as *B. striata*. Ogawa and Sugiyama (2000) demonstrated with 18S rDNA sequence analysis that *Hamigera avellanea* was distinct from *Byssochlamys nivea* and that *H. striata* was part of the *Hamigera* clade with 83% bootstrap support. Peterson (2008) showed that *P. avellaneum* is not part of the clade containing *Penicillium* and *Eupenicillium* species and that *Talaromyces leycettanus* and *Warcupiellea spinulosa* are in a clade with *H. avellanea*.

Pitt and Hocking (1985) compared the distinctive features of a number of anamorphic genera in the Trichocomaceae and were able to separate otherwise similar species on the basis of synchronicity of

conidiogenesis, conidial and phialide shape, color, conidiophore roughness and other features. They noted a resemblance of *P. arenicola* to *Merimbla*.

Isolates of *Penicillium striatum* and *P. avellaneum*, *Talaromyces* spp., *P. megasporum*, *P. canadensis*, *P. arenicola*, *Merimbla ingelheimensis*, and *Merimbla humicoloides* were examined. DNA was extracted and the *Mcm7*, *Tsr1* and RNA polymerase beta subunit loci were amplified and sequenced. The sequences were analyzed to determine whether *Hamigera* is a monophyletic genus and to test the hypotheses of prior authors concerning the phylogeny of *Talaromyces* and *Hamigera* and the utility of the phenotypic markers for genus *Merimbla*. In addition the ITS and *lsu-rDNA*, β -tubulin and calmodulin loci were amplified and sequenced for the ingroup species to apply genealogical concordance to the systematics of genus *Hamigera*.

MATERIALS AND METHODS

Fungal isolates examined in this study are available from the Agricultural Research Service Culture Collection (NRRL), Peoria (<http://nrrl.ncaur.usda.gov>). The methods of Pitt (1980) were followed for phenotypic examination of the cultures. Briefly cultures were grown on Czapek's yeast extract agar (CYA), malt extract agar (MEA) and glycerol nitrate agar (G25N) 7 d at 25 C and examined (Pitt 1980). Cultures also were grown on cornmeal agar as formulated and recommended by Raper and Thom (1949) for the production of asci and ascospores. Colony diameters and appearance were recorded and photographs were made from these plates. Colors were based on the Ridgway (1912) guide and are referred to by plate number (e.g. R35). Microscopic examination was performed by pressing and lifting a ca. 10 × 10 mm piece of transparent tape to a colony, rinsing the tape with 70% ethanol and mounting the tape in lactic acid with fuchsin dye. A Zeiss Axioskop microscope with bright field, phase contrast and DIF contrast optics was used to view the slides. A Kodak 420b or DCS pro 14n camera was mounted on the microscope for photomicrography.

Ascospores for scanning electron microscopy (SEM) were mounted on conductive tape on an aluminum stub, air-dried, sputter-coated with gold-palladium and viewed. Photographs were resized, contrast adjusted and fitted into plates with Photoshop 6.0.1.

DNA methods.—Conidia from agar slant cultures were used to inoculate 125 mL Erlenmeyer flasks containing 50 mL malt extract broth. Cultures were grown on a rotary platform (200 rpm) 2–3 d at 25 C. Biomass was collected by vacuum filtration, frozen and freeze-dried in microfuge tubes. Mycelium was ground to a powder, rehydrated with CTAB buffer, extracted with chloroform and DNA was precipitated from the aqueous phase with an equal volume of isopropanol. Total DNA was collected by centrifugation, the pellet was rinsed with 70% ethanol and nucleic acids were dissolved in 100 μ L sterile deionized water.

DNA was diluted ca. 1:100 with sterile deionized water for amplifications. Beta tubulin (*BT2*), calmodulin (*CF*), ITS and partial *lsu-rDNA* (*ID*) and RNA polymerase (*RPB2*) were amplified by standard methods (Peterson 2008). For some isolates the *BT2* amplification did not make product and a new set of primers was devised to amplify a superset of the *BT2* region. The 5' primer BTX1 (5'-cty car acc ggc cag tgt g) is located in exon 2 of *A. nidulans* β -tubulin (GenBank M17519), and the 3' primer BTXR (5'-cat cat acg gtc ggg gaa ct) is located in exon 6. Standard buffer and conditions were used with a thermal profile of 95 C for 2 min followed by 35 cycles of 96 C for 30 s; 51 C for 60 s; 72 C for 60 s; followed by 72 C for 5 min. The resulting fragment was ca. 800 nt long and extended both the 5' and 3' ends of the amplified fragment by ca. 150 nt relative to *BT2*. *Tsr1* and *Mcm7* loci were amplified with the primers and conditions of Schmitt et al. (2009), except annealing temperature for *Tsr1* was 54 C and for *Mcm7* was 58 C. DNA sequencing was performed with dye terminator technology (3.1) and an ABI 3730 sequencer, both from Applied Biosystems (Foster City, California). Raw sequences (bidirectional) were corrected with Sequencher (Gene Codes Corp., Ann Arbor, Michigan). Corrected sequences were aligned for phylogenetic analysis with Clustal W (Thomson et al. 1994). Sequences are deposited in GenBank under accession numbers AF033393, AF033494, AF454073–AF454075, AF454078–AF454080, EF634410, EF634416–EF634420, EF669665–EF669668, EF669679, EF669680, EF669686, EF669687, EF669697, EF669698, EF669700, EF669701, EF669706, EF669712–EF669714, EF669718, EF669859, EF669930, EU014110, EU021627, EU021654, EU021664, EU021682, GU092696–GU092973 and GU111748–GU111760. Tree diagrams and datasets are deposited in TreeBase (<http://www.treebase.org>) under sn4703.

Parsimony analysis was conducted with PAUP* 4.0b10 (Swofford 2003). For single-locus datasets the criterion was parsimony, addition order was random (5000 replications), branch swapping was NNI (nearest neighbor interchange) and MAXTREES was set at 5000. The set of trees generated was used as the starting point for parsimony analysis with addition order "as is" and TBR branch swapping. Bootstrap analysis was conducted with "as is" addition order and TBR branch swapping for 1000 replications.

Bayesian posterior probabilities were calculated with MrBayes 3.12 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). *Mcm7*, *Tsr1* and *RPB2* datasets included only protein coding sequences, and each dataset was partitioned into codon positions 1, 2 and 3. The *BT2* and *CF* loci included protein coding and intron regions, and the data were partitioned accordingly into intron and exon data. *ID* data were partitioned into three regions, ITS1, ITS2, and 5.8s rDNA and 28S rDNA. A GTR (general time-reversible) model was used with a proportion of invariant sites and a gamma-shaped distribution of rates across the sites. Markov chain Monte Carlo (MCMC) analysis was conducted for up to 5×10^6 generations until the chains converged.

Concordance analysis was based on the exclusionary principle of Baum and Shaw (1995) and genealogical concordance phylogenetic species recognition concepts of

Taylor et al. (2000). Noncongruence in the apical branches was interpreted as species boundaries (Baum and Shaw 1995). Congruence was based on strongly supported branches (> 90% bootstrap, > 0.90 posterior probability) at one or more loci that were not contradicted strongly at other loci (Dettman et al. 2006, Sites and Marshall 2004).

RESULTS

The ITS-*lsu* rDNA dataset included two isolates, NRRL 35601 and NRRL 35721, that contained an 18S rDNA insert of ca. 450 bases contained in the 3' region of the 18S rDNA. The inserts of each isolate had identical sequences. BLAST analysis of the GenBank nucleotide database revealed 70–80% sequence similarity of these inserts with type I introns found in several species of *Byssosclamyces*, *Penicillium*, *Geosmithia* and *Cenococcum* (AY265223, AF179227, AB033527, AY818547). Other isolates in this study lacked the 18S rDNA intron.

The *BT2* aligned sequence set contained 492 sequence positions (219 exon coding positions, 273 intron coding positions). The *CF* sequence set contained 741 aligned sequence positions (398 exon coding positions, 343 intron coding positions). The *ID* sequence dataset contained 1181 aligned positions of the ITS1, ITS2, 5.8S rDNA and 28S rDNA. The *RPB2* aligned sequence set contained 1014 aligned protein-coding positions. The *Tsr1*-aligned sequence set contained 822 protein-coding positions of which 89 were eliminated due to alignment gaps. The *Mcm7* aligned sequence set contained 638 protein-coding positions.

The combined data (*Mcm7*, *RPB2*, *Tsr1*) tree (FIG. 1) depicts *Hamigera* as a clade containing eight species with most species supported by 98–100% of the bootstrap samples and 0.98–1.00 Bayesian probability. *Talaromyces striatus* isolates form another strongly supported clade. There is no evidence in the bootstrap or Bayesian statistics suggesting that the *Hamigera* clade and *T. striatus* clade are siblings. *Merimbla humicoloides* is in a strongly supported clade with *P. canadensis* and *P. arenicola* and *M. ingelheimensis* in the *Hamigera* clade. *Warcupiella spinulosa* is distinct from the *Hamigera* clade.

Trees from all loci support the sibling relationship of *H. terricola* and *H. insecticola*, the unresolved trichotomy of *H. avellanea*, *H. paravellanea* and *M. ingelheimensis*, and the sibling relationship of these two branches (FIG. 1 and SUPPLEMENTAL FIGS. 1–6).

The multilocus analysis also supports the sibling relationship of *P. megasporum* and *P. giganteum*. A polytomy composed of the *H. avellanea* clade, the *T. striatus* clade, the *P. megasporum* clade, the *P. humicoloides*, *P. arenicola* and *P. canadensis* clade

and each of the other species was evident in the combined data tree (FIG. 1). None of the loci analyzed individually resolve the sibling relationships of the strongly supported clades (FIG. 1).

TAXONOMY

Hamigera avellanea Stolk & Samson, *Persoonia* 6:345, 1971

≡ *Penicillium avellaneum* Thom & Turesson, *Mycologia* 7:284, 1915.

≡ *Talaromyces avellaneus* (Thom & Turesson) C.R. Benjamin, *Mycologia* 47:682, 1955.

Type: CBS 295.48 designated lectotype for *Hamigera avellanea* by Stolk and Samson (1971). Cultures ex-type: NRRL 1938, ATCC 10414, CBS 295.48, DSM 2208, FRR 1938, IMI 40230, QM 1849.

Anamorph. *Merimbla* state of *H. avellanea*.

Colonies attaining 50–70 mm diam on CYA (FIGS. 2, 54) after 7 d at 25 C, lanose to velvety, low, sulcate in some isolates, conidial areas pinkish near avellaneous or vinaceous buff (R40), exudate present in some isolates, vinaceous, soluble pigments not seen, reverse in deep red shades near Indian red (R37). Attaining 45–70 mm diam on MEA (FIGS. 3, 54) after 7 d at 25 C, thin, low, pale yellow to pinkish, forming denser radial aggregates, sporulating well, ascomata present, no soluble pigments or exudate, reverse vinaceous purple (R38). Attaining 23–24 mm diam on G25N after 7 d at 25 C, plane, low, thin, producing scattered conidiophores, sporulation light, no sclerotia, no ascomata, no soluble pigments, reverse light buff (R15). Attaining 60–70 mm diam on CYA after 7 d at 37 C, low, velutinous, near light russet vinaceous (R39), sporulation heavy with no exudate, soluble pigments, sclerotia or ascomata, reverse Hellebore red to vinaceous purple (R38). Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 5, 6) rarely branched arising from colony surface, (40–)200–500(–700) × 3–5 μm, walls smooth to finely roughened or finely encrusted at base, older conidiophores appearing vesiculate up to 14 μm diam, hyphae occasionally swollen, septate, often with an apical whorl of *metulae* 5–12(–16) × 3–5(–8) μm, bearing 3–6(–8) ampulliform *phialides*, 5–9(–12) × (2–)2.5–3.5 (–4.5) μm, producing prolate to spheroidal *conidia* 3–5(–7) × 2.0–3.0(–4.5) μm with smooth walls, *metulae* and sessile *phialides* often seen subapically.

Ascomata maturing after 2 wk, pale buff, often surrounded by dark red encrusted hyphae, (60–)100–200 μm diam, ascospores (FIG. 4) prolate spheroids 6.0–7.5 × 4.0–5.0 μm, thick-walled, ornamented with short rod-like projections (FIG. 7).

Cultures examined. USA, Texas, San Antonio, isolated from soil by Mary Clare Sep 1943, ex type, NRRL 1938. SPAIN, Tarragona, Amposta, isolated from soil by A.M.

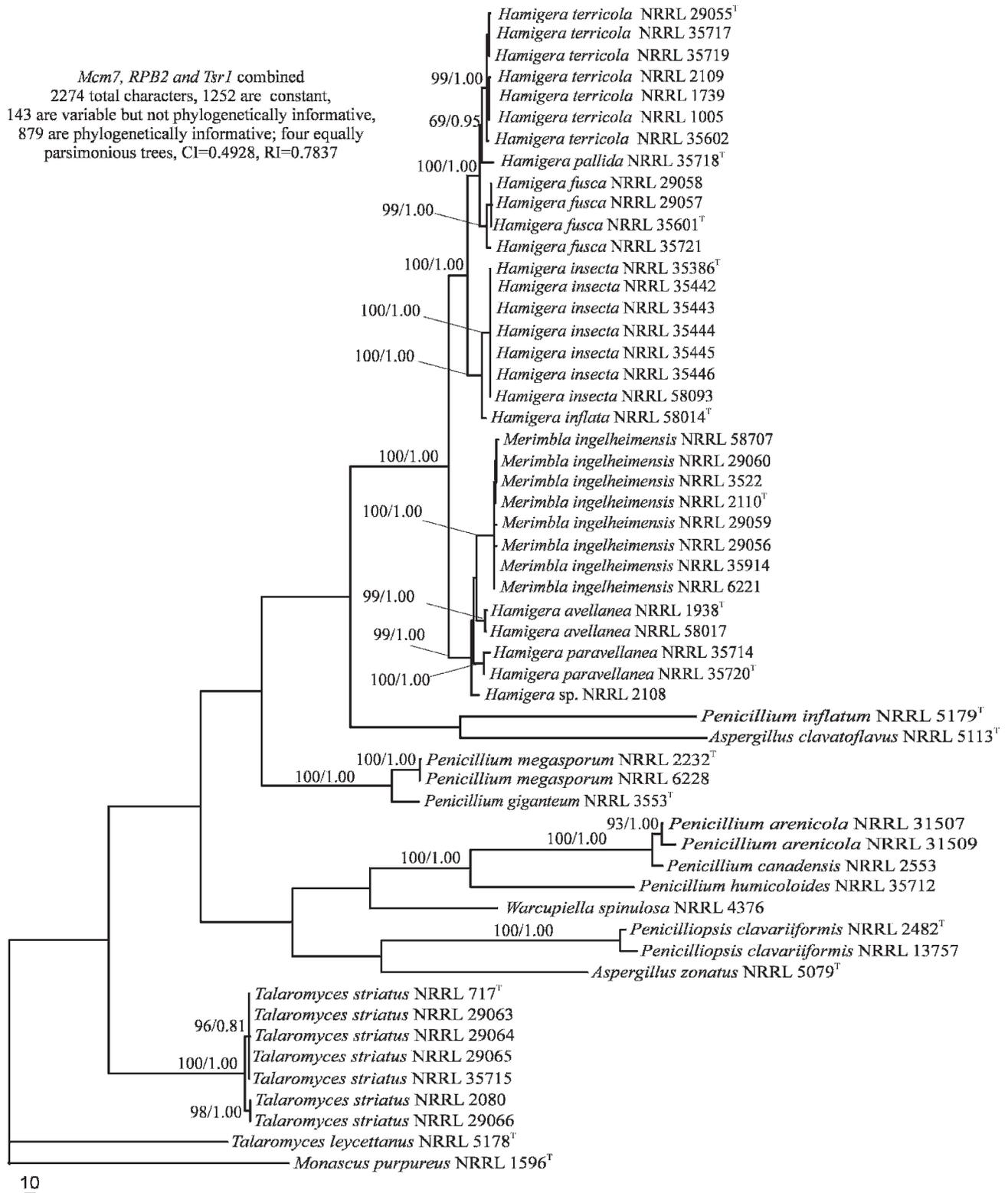
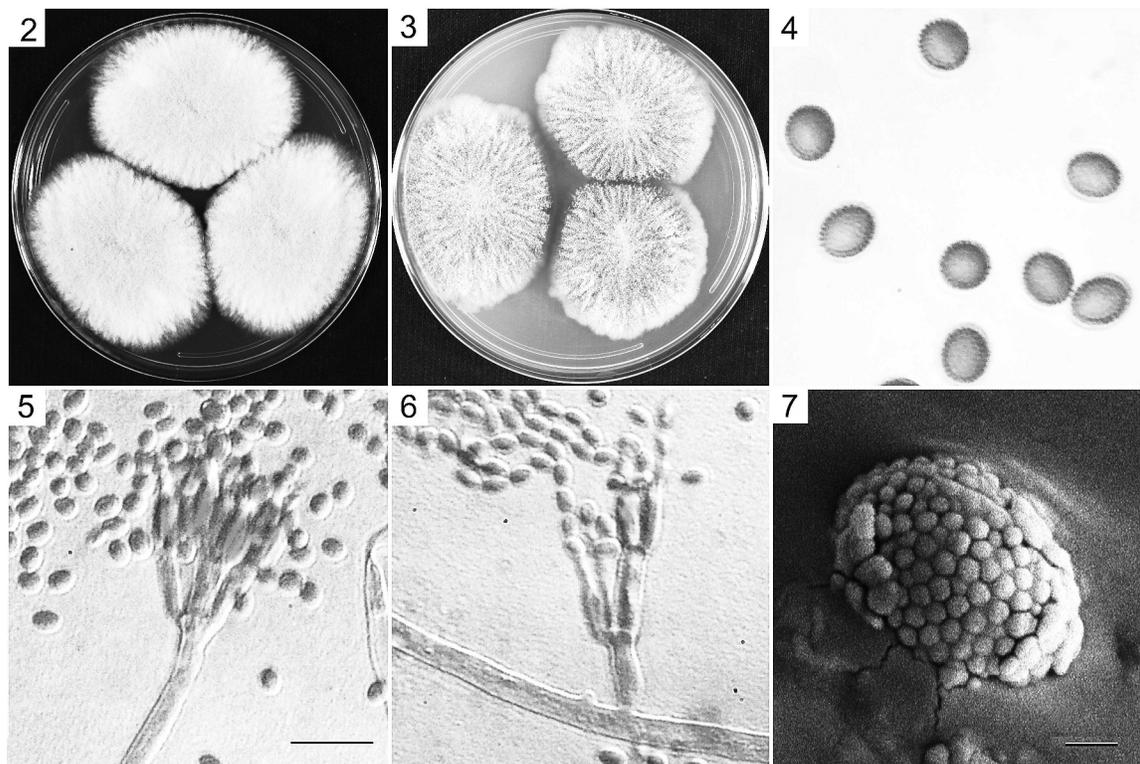


FIG. 1. Phylogenetic tree calculated from combined *Mcm7*, *RPB2* and *Tsr1* data. Bootstrap values/Bayesian posterior probabilities are placed above internodes. Most close relationships are resolved with statistical support; the more basal relationships are not resolved. *Monascus purpureus* was chosen as outgroup species on the basis of the wider tree published by Peterson (2008). Scale is number of nucleotide changes.



FIGS. 2-7. *Hamigera avellanea*. 2. Colonies grown 7 d at 25 C on CYA. 3. Colonies grown 7 d at 25 C on MEA. 4. Punctate ascospores. 5. Conidiophore with metulae and phialides. 6. Subapical metula and phialides on a conidiophore. 7. SEM view of ascospore. Bars: 7 = 1 μ m; 4-6 = 10 μ m.

Stchigel, NRRL 58017. Also examined, *Hamigera* sp., USA, California, Salinas, isolated from retting guayule by Ralph Emerson Aug 1945, NRRL 2108.

Comments: Phylogenetically NRRL 2108 is an undescribed species. Phenotypically this isolate resembles *M. ingelheimensis* in colony color, but the MEA colonies of NRRL 2108 are velutinous, deeply sulcate and produce ascomata whereas *M. ingelheimensis* colonies are deep, loose, plane and make no ascomata. Description of the species represented by NRRL 2108 is deferred until additional isolates are obtained and can be used to better circumscribe the species.

Merimbla ingelheimensis (F.H. Beyma) J.I. Pitt, Can J Bot 57:2394, 1979.

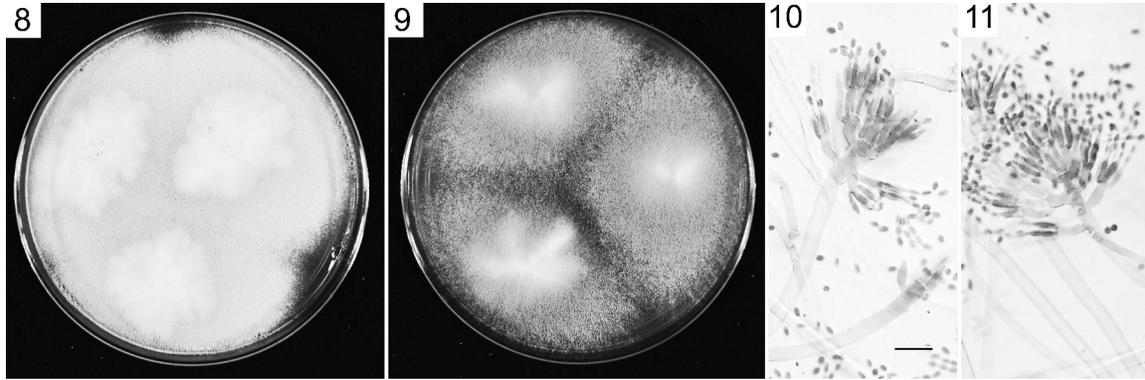
= *Penicillium ingelheimensis* F.H. Beyma Antonie van Leeuwenhoek 8:109, 1942.

Type IMI 234977 designated for *Merimbla ingelheimensis* by Pitt and Hocking, Can J Bot 57:2394, 1979. Cultures ex-type NRRL 2110, CBS 163.42, FRR 2110, IMI 234977, QM 7490.

Colonies attaining 55-65 mm diam on CYA (FIGS. 8, 55) after 7 d at 25 C, plane, some isolates sulcate, velutinous, light salmon orange (R2) to capucine

orange (R3), sporulation heavy, no exudate, sclerotia, ascomata or soluble pigments, thin sterile overgrowth centrally; reverse color influenced by surface color, pale pink near Safrano pink (R2). Attaining 70 mm diam on MEA (FIGS. 9, 55) after 7 d at 25 C, thin, plane, velutinous, low, sporulation heavy, light pinkish cinnamon to cinnamon-buff (R29), no exudate, sclerotia, ascomata or soluble pigments; reverse color mostly from surface growth, pale pinkish. Seven days incubation on G25N at 25 C produced no growth. Attaining 55-65 mm diam on CYA after 7 d at 37 C, low, velutinous, radially sulcate, some isolates overgrown centrally by a thin, white, sterile hyphae, cinnamon to orange-cinnamon (R29), sporulation heavy, exudate commonly present, clear to reddish orange, no sclerotia, no ascomata; reverse pale pinkish buff (R29), some isolates with a light brown central area. Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 10, 11) rarely branched, arising from colony surface, 100-800(-1000) \times (3-)4-7(-10) μ m, walls smooth, finely roughened or finely encrusted near base, older conidiophores appearing vesiculate up to 20 μ m diam, commonly with an apical whorl of metulae 3-14(-20) \times 3-6(-11) μ m, bearing



FIGS. 8–11. *Merimbla ingelheimensis*. 8. Colonies grown 7 d at 25 C on CYA. 9. Colonies grown 7 d at 25 C on MEA. 10, 11. Conidiophores showing subapical proliferation of metulae and phialides as conidiophores mature. Bar = 10 µm.

five or more *phialides*, $7\text{--}9(-14) \times 2\text{--}3.0(-5)$ µm, acerose to ampulliform, producing prolate occasionally spheroidal to subspheroidal *conidia* $(3\text{--})3.5\text{--}5.0(-9.5) \times 2.0\text{--}3.0(-5)$ µm with smooth walls. Metulae and sessile phialides often seen subapically.

Cultures examined. GERMANY, Ingelheim am Rhein, isolated from air by C.M. Böhringer Sohn, 1941, NRRL 2110 ex-type. INDIA, isolated from soil by D.I. Fennell, ca. 1969, NRRL 3522. ROMANIA, isolated from tobacco-field soil 1967, NRRL 6221. USA, New York, New York Botanical Garden, received from B.O. Dodge Jun 1952, NRRL 29056. SWITZERLAND, Basel, received as CBS 107.66 = NRRL 29059. USA, Illinois, Peoria County, isolated from swine-dung fermentation by L.K. Nakamura Nov 1977, NRRL 29060.

The epithet *ingelheimensis* is an orthographic correction, ICBN recommendation 60D

Hamigera paravellanea S.W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F.E. Vega, sp. nov. FIGS. 12–18.

Species nova coloniis in CYA post 7 dies temperatura 25 C crescentibus luteolis, in parte aversa coccineis vel brunneorubris distinguenda.

Holotype BPI 879311. Dried colonies of NRRL 35720 grown 7 d at 25 C on CYA and MEA. Culture isolated from unidentified dung in Spain.

Etymology. Alongside *avellanea* refers to the position of *H. paravellanea* in the phylogeny.

Anamorph. *Merimbla* state of *H. paravellanea*. MycoBank MB516015

Colonies attaining 70 mm diam on CYA (FIGS. 12, 56) after 7 d at 25 C, plane, velutinous, orange buff to capucine yellow (R3), sporulation heavy, no exudate, sclerotia, ascomata or soluble pigments; reverse raw sienna to antique brown (R3). Attaining 70 mm diam on MEA (FIGS. 13, 56) after 7 d at 25 C, thin, plane, velutinous, low, sporulation heavy, no exudate, sclerotia, ascomata or soluble pigments; reverse near raw sienna to Sudan brown or raw umber. Attaining 25–26 mm diam on G25N after 7 d at 25 C, thin, plane, little sporulation, no sclerotia, ascomata or

soluble exudate. Attaining 40–45 mm diam on CYA after 7 d at 37 C, lobate, irregularly raised, buff brown centrally (R40) to light orange-yellow at margin, sporulation heavy, no exudate, no sclerotia, no ascomata; reverse darker than mummy brown (R15). Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 14–16) occasionally branched arising from surface hyphae $200\text{--}500(-1200) \times 3\text{--}5(-7)$ µm, less commonly $30\text{--}150$ µm if borne on aerial hyphae, wall smooth to finely roughened or finely crustose near base, occasionally vesiculate apically up to 12 µm bearing a whorl of metulae $5\text{--}10(-21) \times 3\text{--}6(-7)$ µm diam, each with $3\text{--}6(-8)$ ampulliform to cylindrical phialides $5\text{--}10(-21) \times (2\text{--})2.5\text{--}3.5(-5)$ µm, producing prolate spheroidal to subspheroidal or pyriform conidia $3\text{--}5(-10) \times 2\text{--}3(-5)$ µm, smooth-walled (FIG. 17).

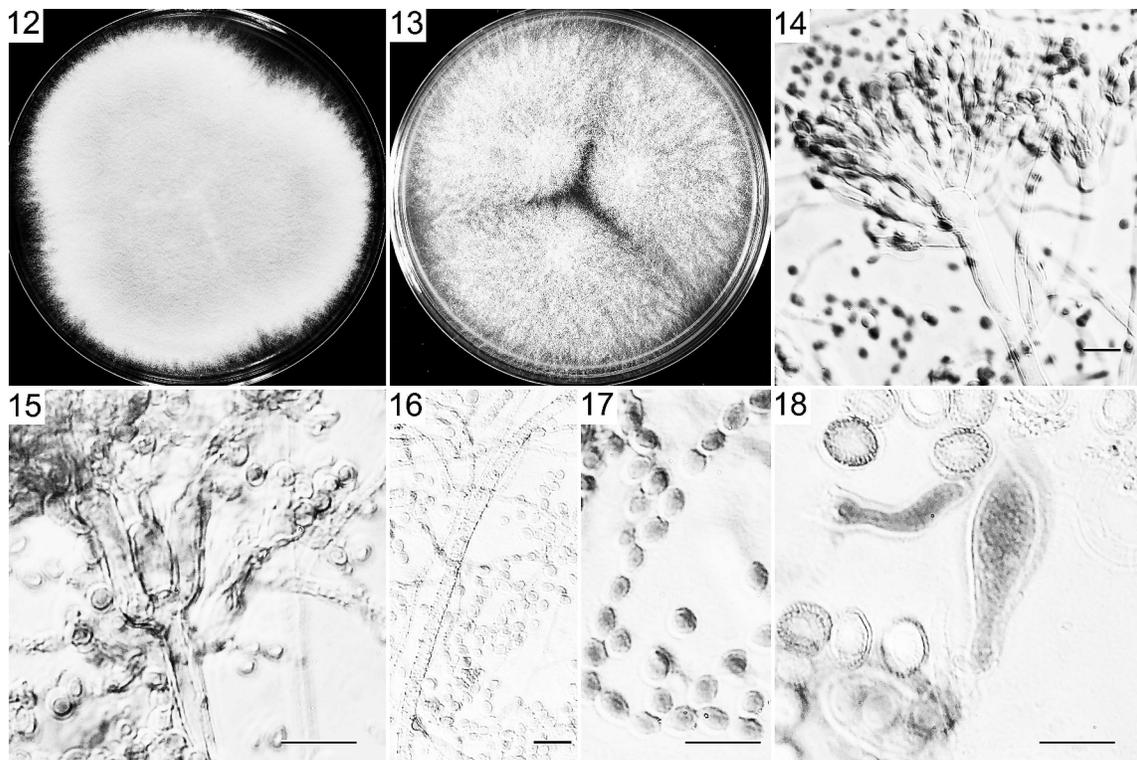
Ascomata white, spheroidal, $(80\text{--})100\text{--}200(-240)$ µm, with some dark red hyphae around the ascomata, containing numerous asci, initially clavate $6\text{--}7 \times 10\text{--}15$ µm but at maturity irregularly ovoid $10\text{--}12 \times 15\text{--}20$ µm, containing eight prolate spheroidal ascospores $7\text{--}7.5 \times 5\text{--}6$ µm, wall surface appearing punctate (FIG. 18).

Cultures examined. POLAND, Drawa Wilderness Forest complex, isolated from soil by A.M. Stchigel, Apr 1999, NRRL 35714. SPAIN, Tarragona Province, Prades Mountains, isolated from unidentified dung by A.M. Stchigel, Oct 1997, NRRL 35720, ex-type.

Hamigera insecticola S.W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F.E. Vega, sp. nov. FIGS. 19–26.

Species nova coloniis in CYA post 7 dies temperatura 25 C crescentibus pallide luteolis, sulcatis, in parte aversa brunneis distinguenda.

Holotype BPI 879309. Dried colonies of NRRL 35386 grown 7 d at 25 C on CYA and MEA. Isolated from cuticle of a coffee berry borer, *Hypothenemus*



FIGS. 12–18. *Hamigera paravellanea*. 12. Colonies grown 7 d at 25 C on CYA. 13. Colonies grown 7 d at 25 C on MEA. 14. Conidiophores with vesicular apex, metulae and phialides. 15, 16. More mature phialides showing wall roughening and yellow brown pigmentation. 17. Ellipsoidal conidia. 18. Asci and punctate ascospores. Bars = 10 μ m.

hampei (Ferrari) (Coleoptera: Curculionidae), by F. Posada, Beltsville, Maryland, USA, Dec 2003.

Etymology. From insects.

Anamorph. *Merimbla* state of *H. insecticola*.

Mycobank MB516016

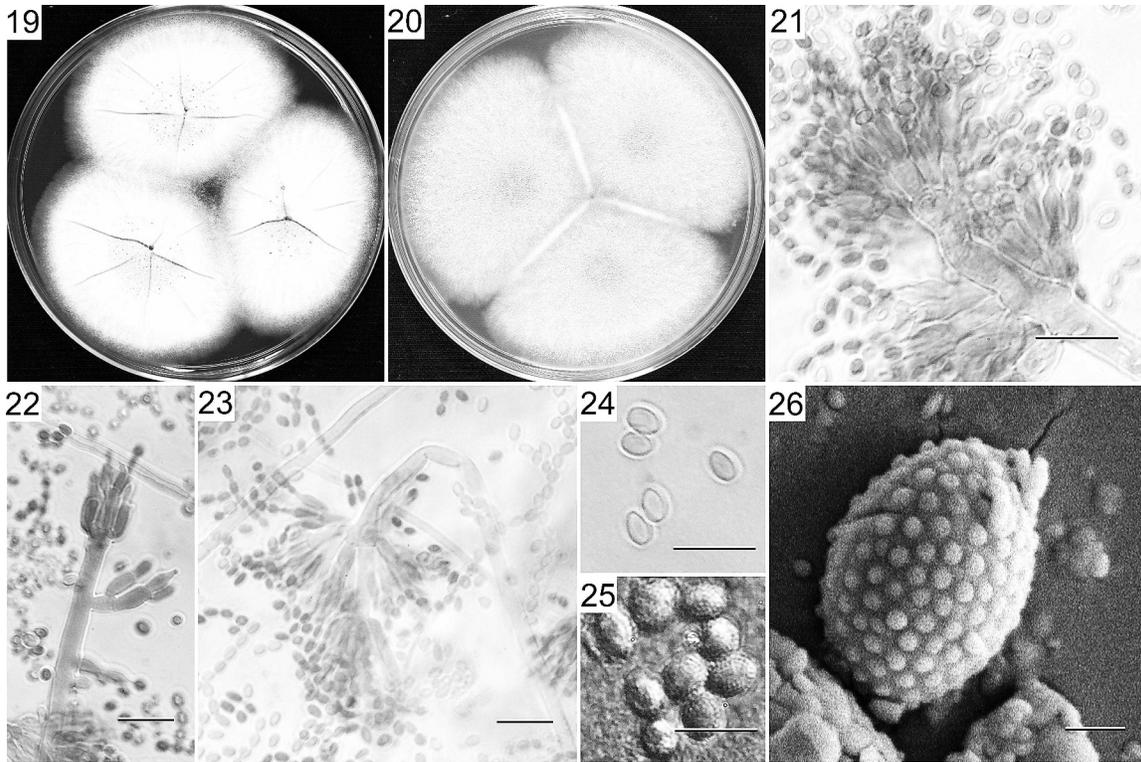
Colonies attaining 55–62 mm on CYA (FIGS. 19, 57) after 7 d at 25 C, irregularly radially sulcate, velutinous, consisting of a low felt, sporulating well, near deep olive buff (R40), small amounts of clear to reddish brown exudate present in most isolates, no soluble pigments, no sclerotia, no ascomata; reverse buff near margins, fawn to Army brown (R40) centrally, occasional isolate bone brown or buff brown. Attaining 58–65 mm diam on MEA (FIGS. 20, 57) after 7 d at 25 C, thin, plane, loose, consisting of a thin felt of basal hyphae and aerial conidiophores, sporulating well, sayal brown to Isabella (R30) to light brownish olive, sporulation from short basal conidiophores, margin ragged, submerged; no exudate, no soluble pigments, no sclerotia, no ascomata; reverse near cartridge-buff (R30) or buckthorn brown (R15). Attaining 14–20 mm diam on G25N after 7 d at 25 C, velutinous, consisting of a low dense felt, sporulating well, near antimony yellow (R15) with a white margin 1–2 mm wide, submerged colony growth extending 3–5 mm beyond aerial growth, no soluble pigments, no

exudate, no ascomata or sclerotia produced; reverse light buff to warm buff (R15). Attaining 60–70 mm diam on CYA after 7 d at 37 C, similar to colonies grown at 25 C, but reverse developing fewer brown hues, sporulation moderate, occasionally producing clear exudate. Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 21–23) rarely branched arising from colony surface, (150–)300–600(–700) \times (3–)4–6(–9) μ m, walls smooth, older conidiophores appearing vesiculate up to 12 μ m diam, often with an apical whorl of metulae 5–10(–15) \times 3–5(–9) μ m, bearing 3–6 ampulliform *phialides* 5–7(–12) \times (2–)2.5–3.0(–5) μ m, producing subspheroidal to prolate spheroidal conidia (3–)3.5–5(–9) \times 2.0–3.0(–5.5) μ m with smooth to finely roughen walls (FIG. 24). Metulae and sessile phialides often seen subapically (FIG. 22).

Ascomata white, (40–)80–160(–240) μ m diam, appearing after ca. 6–7 d, mature ascospores at ca. 14 d, asci subglobose 15–20 μ m diam, containing eight prolate spheroidal ascospores 5–7 \times 3–5 μ m, surface lightly ornamented, appearing punctate in light microscopy (FIG. 25) appearing warty in SEM (FIG. 26).

Cultures examined. USA, Maryland, on cuticle of coffee berry borer beetle *Hypothenemus hampei*, Dec 2003, NRRL



FIGS. 19–26. *Hamigera insecticola*. 19. Colonies grown 7 d at 25 C on CYA. 20. Colonies grown 7 d at 25 C on MEA. 21. Mature conidiophore with metulae, phialides and conidia (note the subapical proliferation of metulae and phialides). 22. Conidiophore displaying subapical metula and phialides, and a sessile phialide. 23. Conidiophore displaying the ampulliform phialides with long necks. 24. Prolate spheroidal conidia. 25. Ascospore showing the characteristic punctate roughening. 26. SEM view of an ascospore. Bars: 19–25 = 10 μ m; 26 = 1 μ m.

35386, ex-type. Cultures NRRL 35442, NRRL 35443, NRRL 35444, NRRL 35445 and NRRL 35446, each isolated by F. Posada from a different *Hypothenemus hampei* at Beltsville, Maryland, USA, Indiana, isolated from air by Z. Jurjevic, Jun 2008, NRRL 58093.

Hamigera inflata S.W. Peterson, Jurjevic, Bills, Stichel, Guarro & F.E. Vega, sp. nov. FIGS. 27–32.

Species nova coloniis in CYA post 7 dies temperatura 25 C crescentibus pallide carneolis, in parte aversa in centro brunneis, zona pallide bubalina circumdatis distinguenda.

Holotype BPI 879308. Dried colonies of NRRL 58014 grown 7 d at 25 C on CYA or MEA. Isolated from soil, Gran Canaria Island.

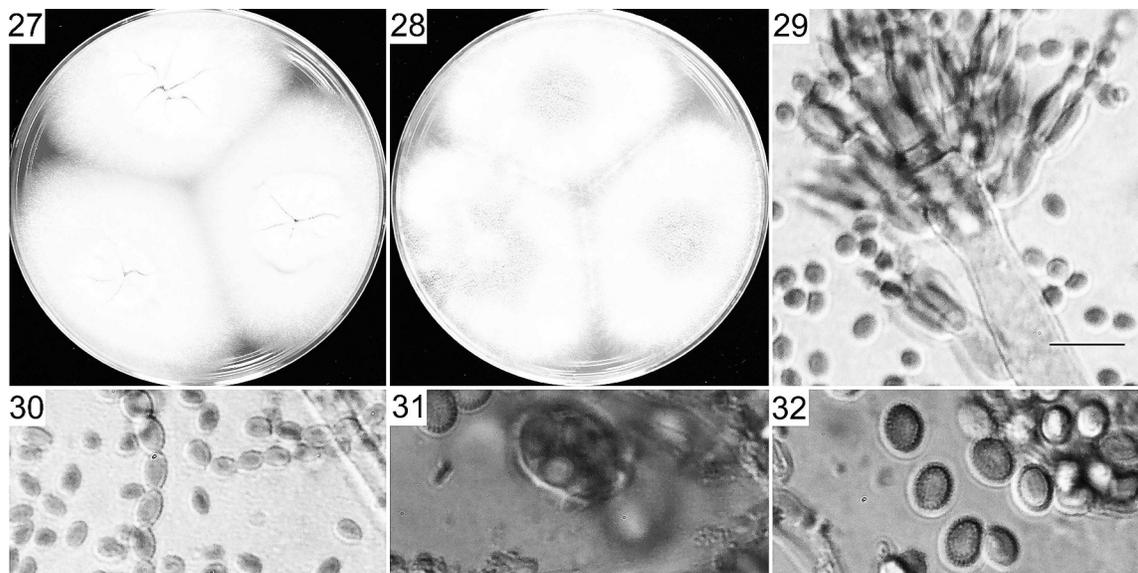
Etymology. Referring to the inflated appearance of the conidiophore apex.

Anamorph. *Merimbla* state of *H. inflata*. MycoBank MB516017

Colonies attaining 70 mm diam (FIGS. 27, 58) on CYA after 7 d at 25 C, composed of a dense sulcate central area ca. 25 mm diam, sporulating densely, pale orange-yellow (R3) with peripheral area less dense, light sporulation and aerial hyphae ropy, exudate moderate, clear, no soluble pigments, no

sclerotia, no ascomata, reverse warm buff (R15) but mummy brown (R15) where sulcations are deepest. Attaining 70 mm diam on MEA (FIGS. 28, 58) after 7 d at 25 C, deep olive buff to citrine drab (R40), thin, low velutinous, sporulation dense, no exudate, no soluble pigments, no sclerotia, no ascomata, reverse dark olive to clove brown (R40) centrally, deep olive buff peripherally. Attaining 17–18 mm diam on G25N after 7 d at 25 C, thin, plane, low, white, sparse conidiophores, no exudate, no soluble pigments, no sclerotia, no ascomata, reverse white. Attaining 70 mm diam on CYA after 7 d at 37 C, low, velutinous, sulcate, sporulating well, light brown (cacao brown R28 to sayal brown R29) to orange-rufous (R2), no exudate, no soluble pigments, no sclerotia, no ascomata, reverse in light brown shades (warm buff R15). Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIG. 29) arising from basal hyphae, (250–)500–1000 \times 3–7(–8.5) μ m, rarely from aerial hyphae (20–)70–120 μ m, wall smooth, often vesiculate up to 14 μ m diam, terminated by a whorl of metulae 5–16(–20) \times 3–7 μ m, each supporting 3–6 ampulliform phialides 5–7 (–10) \times 2.0–3.0 μ m, conidia



FIGS. 27–32. *Hamigera inflata*. 27. Colonies grown 7 d at 25 C on CYA. 28. Colonies grown 7 d at 25 C on MEA. 29. Mature conidiophore with metulae, phialides and conidia, note the subapical proliferation of metulae and phialides. 30. Conidia. 31. Mature ascus. 32. Punctate ascospores. Bars = 10 μ m.

(FIG. 30) prolate spheroidal to ovoid, smooth 3–4.5 (–8) \times 2–3 (–5) μ m, phialides and metulae also arise subapically along the conidiophore.

Ascomata spheroidal, loosely covered with hyphae, brittle in age, ca. 80–160(–240) μ m diam, asci (FIG. 31) subspheroidal 15–20 μ m diam or ovoid 15–18 \times 8–12 μ m, containing eight prolate spheroidal ascospores 5–6 \times 4–5 μ m appearing punctate in light microscopy (FIG. 32).

Culture examined: SPAIN, Grand Canary Island, Las Palmas from soil by A.M. Stchigel, NRRL 58014, ex-type.

Hamigera terricola S.W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F.E. Vega, sp. nov. FIGS. 33–40.

Species nova coloniis in CYA post 7 dies temperatura 25 C crescentibus vivide flavis, diametrum restrictum attingentibus, in parte aversa variabiliter coloratis sed plus minusve rufulo-brunneis distinguenda.

Holotype BPI 879312. Dried colonies of NRRL 29055 grown 7 d at 25 C on CYA or MEA. Isolated from a textile sample in contact with soil, Barro Colorado Island, Panama, December 1944 by E. S. Barghoorn.

Etymology. From or of the soil.

Anamorph. *Merimbla* state of *H. terricola*.

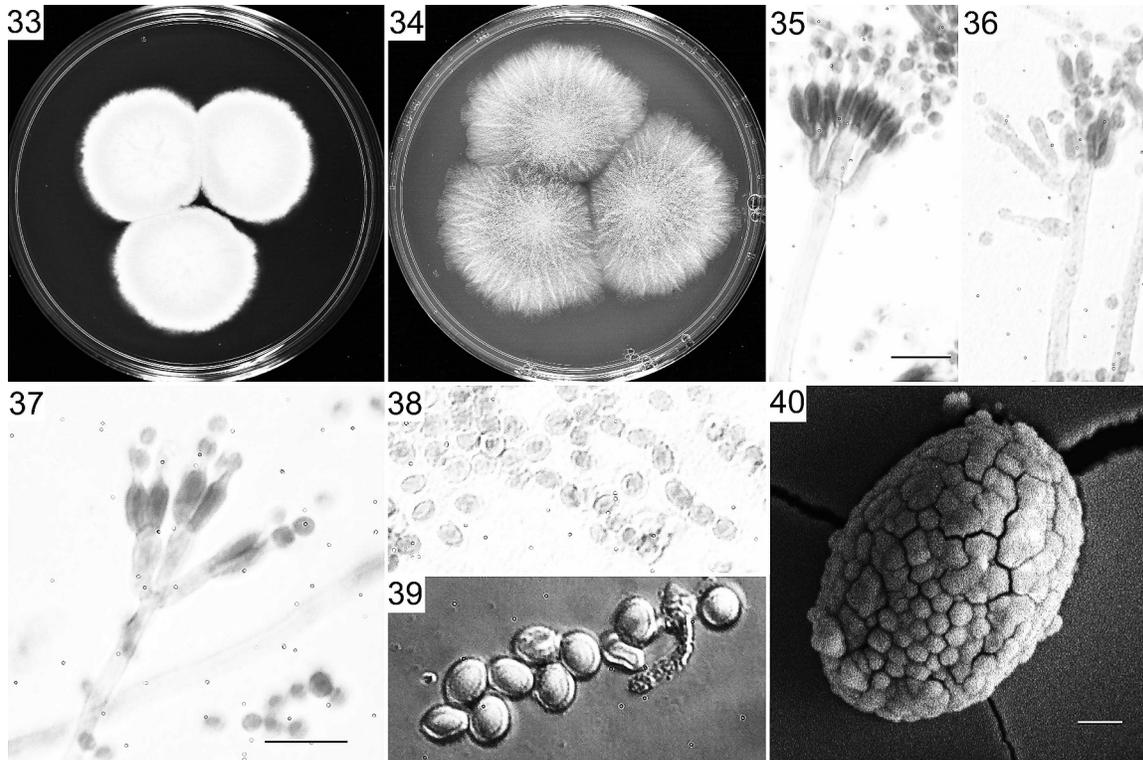
Mycobank MB516018

Colonies attaining 45–65 mm diam on CYA (FIGS. 33, 59) after 7 d at 25 C, irregularly radially sulcate, velutinous, consisting of a low felt, sporulating well, ranging from light orange yellow (R3) to ochraceous buff (R15), no soluble pigments, no exudate, reverse pale orange yellow (R3) near

margins, raw sienna to raw umber (R3) or dusky brown (R45) centrally. Attaining 44–68 mm diam on MEA (FIGS. 34, 59) after 7 d at 25 C, velutinous with thin overgrowth of sterile hyphae, pale orange buff, but much darker where submerged hyphae show through, approximately raw umber (R3) to deep olive buff (R40), no soluble pigments, no exudate, no sclerotia; no ascomata, reverse pale yellow-orange (R3) marginally becoming dark brown near raw umber (R3) or dusky brown (R45) centrally. Attaining 6–15 mm diam on G25N after 7 d at 25 C, with most growth hyaline and submerged in the agar, surface growth velutinous, thin, near light orange-yellow, no exudate, no sclerotia, no ascomata, reverse near light yellow-buff. Attaining 60–70 mm diam on CYA after 7 d at 37 C, low, sulcate, sporulating well near margins, light buff centrally, orange-rufous (R2) in sporulating areas, no soluble pigments, no exudate, no sclerotia, no ascomata, reverse in reddish-brown shades near burnt-sienna (R2). Incubation for 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 35–37) occasionally branched arising from surface, 200–500(–750) \times (3–)4–6(–7) μ m, wall smooth, sometimes vesiculate up to 11 μ m diam, bearing a whorl of metulae 5–16 \times (2.5–)4–7 μ m (FIG. 35), each supporting 3–6(–8) ampulliform phialides 5–8(–14) \times 2.5–4(–5) μ m (FIG. 37), conidia prolate spheroidal to rarely ovoid, smooth 3–4.5(–8) \times 2–3.5(–5) μ m (FIG. 38), phialides and metulae also arise subapically along the conidiophore (FIG. 36).

Ascomata spheroidal, loosely covered with hyphae, ca. 80–160(–240) μ m diam, asci subspheroidal 15–



FIGS. 33–40. *Hamigera terricola*. 33. Colonies grown 7 d at 25 C on CYA. 34. Colonies grown 7 d at 25 C on MEA. 35. Young conidiophore with typical furcate penicillus structure. 36. Conidiophore showing subapical metula and sessile phialide. 37. Ampulliform phialides with long necks. 38. Ellipsoidal conidia. 39. Punctate ascospores. 40. SEM view of ascospore. Bars: 19–25 = 10 μ m; 40 = 1 μ m.

20 μ m diam, containing eight prolate spheroidal ascospores 6–7 \times 4–5 μ m appearing punctate in light microscopy (FIG. 39) but wall with low spinose projections under SEM (FIG. 40).

Cultures examined. GUATEMALA, Guatemala City, isolated by Sernita ca. 1920, NRRL 1005 and NRRL 1739. PANAMA, Barro Colorado Island, isolated from cloth in contact with soil by E.S. Barghoorn, Dec 1944, NRRL 29055, ex-type. COSTA RICA, sent by R.D. Goos, Norwood, Massachusetts 1959, NRRL 35602. FRENCH GUIANA, near Rémire-Montjoly, isolated from phenol-treated soil 2003, F-147860 = NRRL 35717. EQUATORIAL GUINEA, south of Bata, isolated from phenol-treated soil 2005, F-191093 = NRRL 35719.

Observations: *Hamigera terricola* is apparently widespread in tropical soils. Strain NRRL 35717 was isolated on DG 18 medium (Pitt and Hocking 1997) at 37 C. Culture data indicates that the fungus is mildly thermotolerant and osmotolerant.

Hamigera pallida S.W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F. E. Vega, sp. nov. FIGS. 41–47.

Species nova coloniis in CYA post 7 dies temperatura 25 C crescentibus pallide subbubalinis, in parte aversa fulvis distinguenda.

Holotype BPI 879310. Dried colonies of NRRL 35718 grown 7 d at 25 C on CYA and MEA. Isolated from phenol-treated soil, Zio River, Togo, 2005.

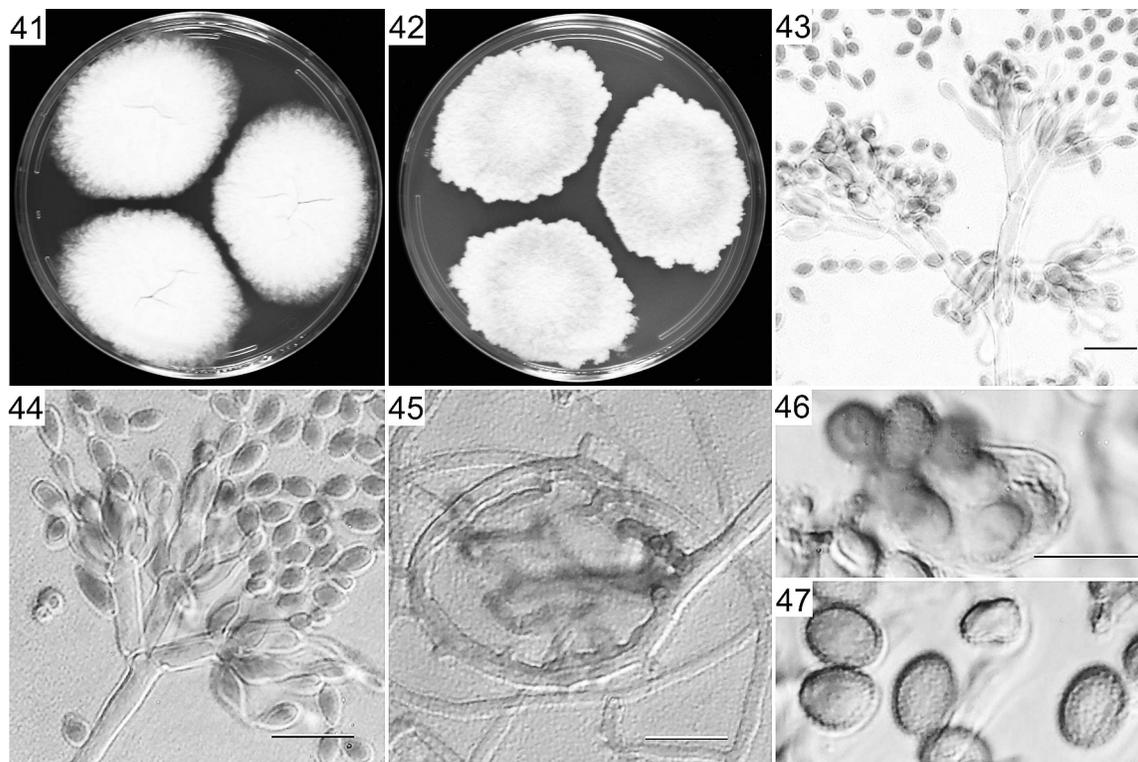
Etymology. Pale.

Anamorph. *Merimbla* state of *H. pallida*.

Mycobank MB516019

Colonies attaining 51–53 mm diam on CYA (FIGS. 41, 60) after 7 d at 25 C, sulcate and mounded, white to pallid, sporulation sparse, producing small amounts of clear exudate, no ascomata or sclerotia; reverse dark reddish brown, near liver brown (R14). Attaining 50 mm diam on MEA (FIGS. 42, 60) after 7 d at 25 C, lanose, ca. 1 mm deep, pallid, sporulation sparse, no exudate, no sclerotia, no ascomata; reverse dark brown, mummy brown (R15) or even darker. Attaining 12–13 mm diam on G25N after 7 d at 25 C, radially sulcate, thin, white to slightly yellow in conidial areas, sporulation moderate, reverse light brown. Attaining 65–70 mm diam on CYA after 7 d at 37 C, in appearance very similar to cultures grown at 25 C except reverse is light tawny brown. Incubation 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIGS. 43, 44) arising from basal



FIGS. 41–47. *Hamigera pallida*. 41. Colonies grown 7 d at 25 C on CYA. 42. Colonies grown 7 d at 25 C on MEA. 43, 44. Conidiophores with metulae, ampulliform phialides and ellipsoidal conidia. 45. Coiled ascogenous hyphae. 46. Ascus releasing ascospores. 47. Punctate ascospores. Bars: 41 = 1 μ m; 42–47 = 10 μ m.

hyphae (30–)70–300(–400) \times 2.5–5(–7) μ m, wall smooth to finely crustose at base, bearing an apical whorl of metulae 5–16(–26) \times 2.5–5(–7) μ m, each bearing 3–6(–8) ampulliform *phialides*, 5–9(–12) \times 2–3.5(–5) μ m, *conidia* smooth, prolate spheroidal 3–4.5(–9) \times 2–3.5(–6) μ m. Sessile phialides present on subapical conidiophore.

Ascomata subspheroidal, white 120–200 μ m diam, coiled hyphae (FIG. 45) give rise to numerous non-dehiscent, subspheroidal asci (FIG. 46) 10–12 \times 14–16 μ m, containing eight prolate spheroidal ascospores 5–7 \times 4–6 μ m, finely spinose surface (FIG. 47).

Culture examined. TOGO, Zio River, isolated from phenol-treated soil, 2005, F-189696 = NRRL 35718, ex-type.

Hamigera fusca S.W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F.E. Vega, sp. nov. FIGS. 48–53.

Species nova diametro coloniae in CYA post 7 dies temperatura 25 C crescentibus restricto atque coloniis in parte aversa fusco-brunneis distinguenda.

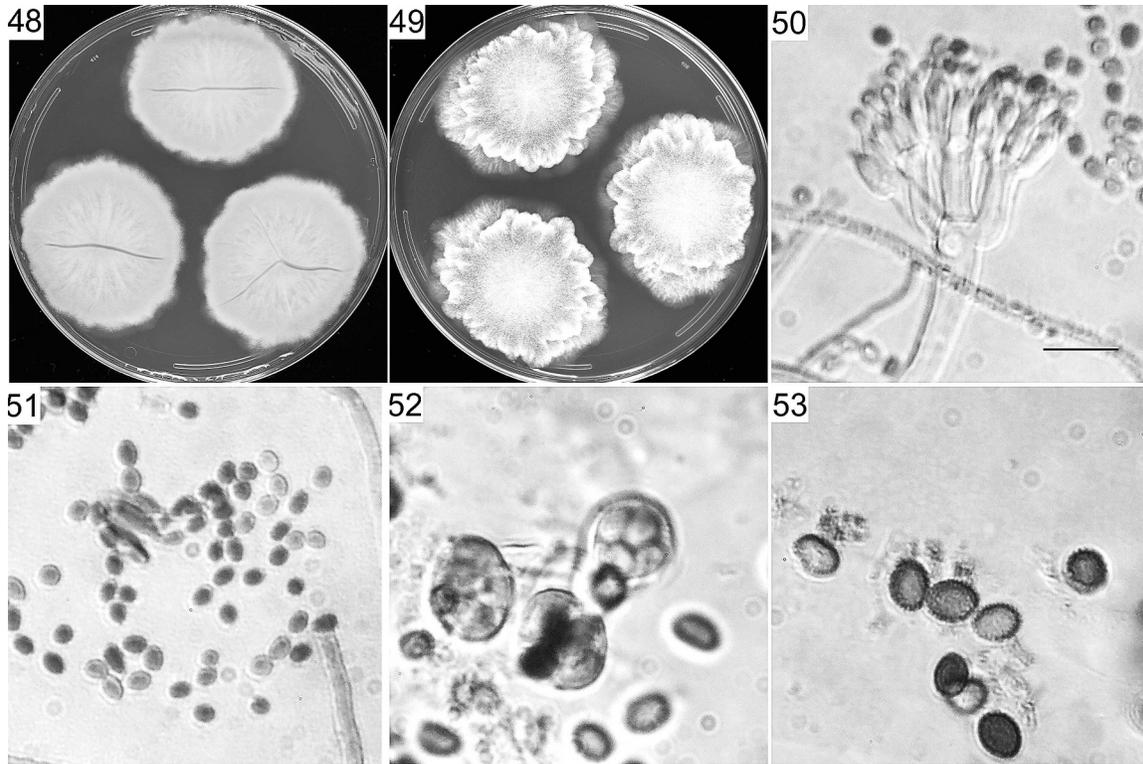
Holotype BPI 879307. Dried colonies of NRRL 35601 grown 7 d at 25 C on CYA and MEA. Type isolated from soil, Adelaide, Australia.

Etymology. Refers to the dark, dusky colony.

Anamorph. *Merimbla* state of *H. fusca*.

MycoBank MB516020

Colonies attaining 23–45 mm diam on CYA (FIGS. 48, 61) after 7 d at 25 C, velutinous, low, deeply sulcate, margin dissected, ochraceous buff (R15), sporulation heavy, no exudate, no soluble pigments, no sclerotia, no ascomata, reverse dark brown centrally (Dresden brown to mummy brown R15) diminishing to warm buff (R15) peripherally. Attaining 25–45 mm diam on MEA (FIGS. 49, 61) after 7 d at 25 C, low, velutinous, medium brown (buckthorn brown to cinnamon brown R15), sporulation heavy, thin, sterile overgrowth centrally, margins deeply dissected, no exudate, no soluble pigments, no sclerotia, no ascomata, reverse dark brown (mummy brown R15). Attaining 14–16 mm diam on G25N after 7 d at 25 C, thin, plane, low, sporulation heavy, ochraceous buff (R15) no exudate, no soluble pigments, no sclerotia, no ascomata, reverse warm buff (R15). Attaining 55–65 mm diam on CYA after 7 d at 37 C, low, velutinous, sulcate, sporulating well, light brown centrally (buckthorn brown R15), orange-rufous (R2) in sporulating areas, exudate moderate, golden, no soluble pigments, no sclerotia, no ascomata, reverse in light brown shades (cinnamon-brown R15) centrally to warm buff (R15) peripherally.



FIGS. 48–53. *Hamigera fusca*. 48. Colonies grown 7 d at 25 C on CYA. 49. Colonies grown 7 d at 25 C on MEA. 50. Young conidiophore with typical furcate penicillus structure. 51. Subspheroidal to ovoidal conidia. 52. Subspheroidal asci. 53. Punctate ascospores. Bars = 10 µm.

Incubation for 7 d on CYA at 5 C produced no growth or germination of conidia.

Conidiophores (FIG. 50) arising from finely roughened to crustose basal hyphae, 200–500(–800) × (2.5–)3–5(–6) µm, rarely from aerial hyphae (20–)70–120 µm, wall smooth, often terminated by a whorl of metulae 5–10(–22) × (2–)3–4(–6) µm, each supporting (2–)3–6(–8) ampulliform phialides 5–7(–12) × (2–)2.5–3.5(–4.5) µm, occasionally cylindrical with short and long collula, conidia (FIG. 51) prolate spheroidal to ovoid, smooth (2.5–)3–4(–6) × 2–3(–5) µm, phialides and metulae also arise subapically along the conidiophore.

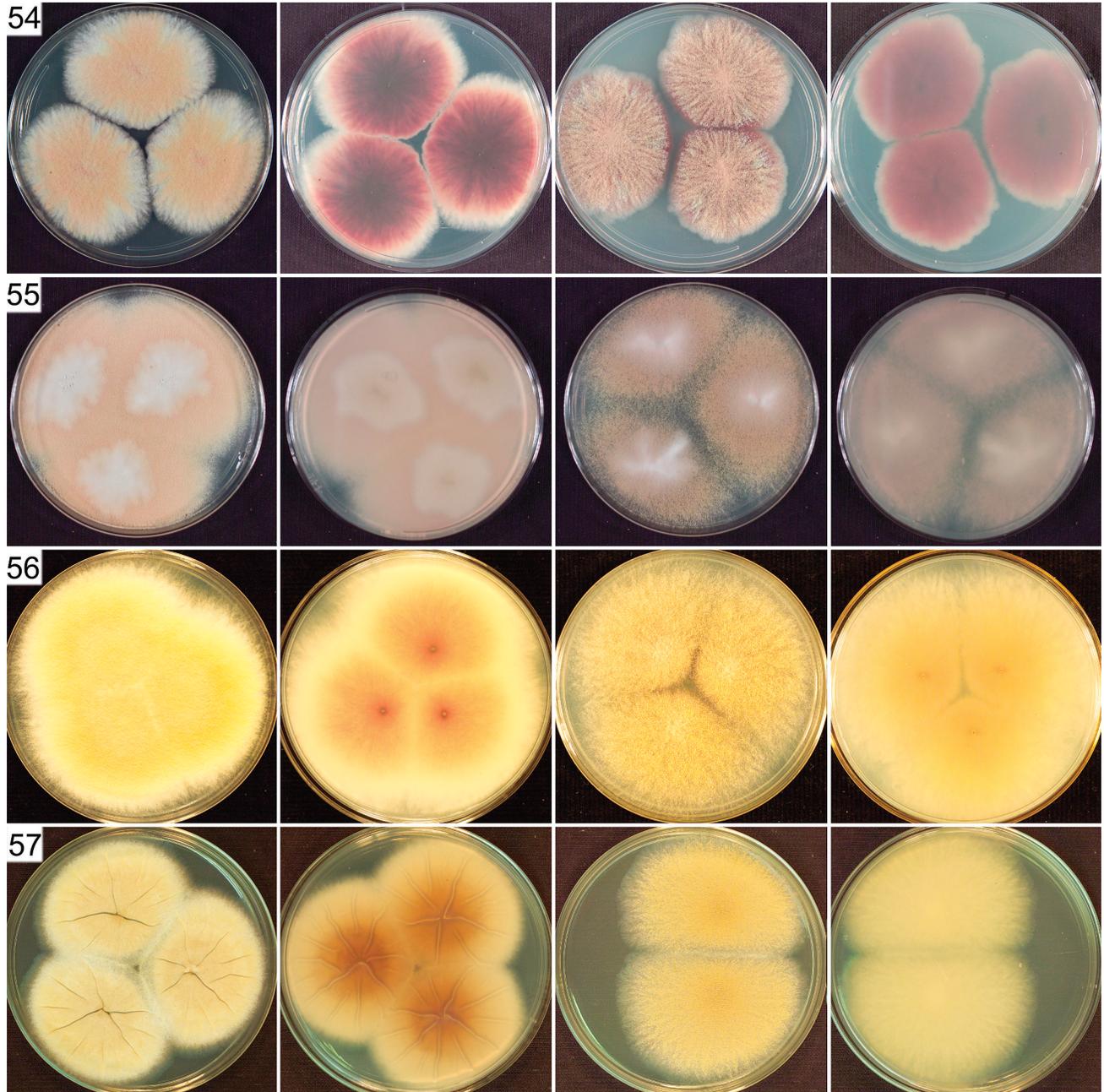
Ascomata subspheroidal, arising from coiled hyphae, white 120–200 µm diam, containing numerous nondehiscent, subspheroidal asci (FIG. 52), 9–15(–17) × 9–12 µm, containing eight prolate spheroidal ascospores, 5–7 × 4–5 µm, finely ornamented surface (FIG. 53).

Cultures examined: AUSTRALIA, Adelaide, isolated from soil, J.H. Warcup ca. 1950, NRRL 29057, NRRL 29058 and NRRL 35601 ex-type. THE COMOROS, Grande Comore, Maouéni, isolated from phenol-treated soil 2006, F-225356 = NRRL 35721. NRRL 35721 is strongly floccose, growing at the lowest end of the size range and failed to grow on G25N. CYA cultures with nil sporulation produce light gray colonies.

KEY TO *HAMIGERA* SPECIES BY COLONY DIAMETER AND COLORATION

- 1a. Colony diameter greater than 40 mm on CYA at 7 d growth 2
- 1b. Colony diameter less than 40 mm on CYA at 7 d growth 7
- 2a. CYA colonies salmon to pinkish
. *Merimbla ingelheimensis*
- 2b. CYA colonies not salmon or pink 3
- 3a. CYA colonies vinaceous to avellaneous, reverse purple red *Hamigera avellanea*
- 3b. CYA colonies in drab or yellow shades 4
- 4a. CYA colonies light yellow, reverse scarlet to brownish red *Hamigera paravellanea*
- 4b. CYA reverse another color 5
- 5a. CYA colonies light buff, CYA and MEA reverse tawny *Hamigera pallida*
- 5b. Colony reverse another color 6
- 6a. CYA colony reverse dark brown *Hamigera fusca*
- 6b. CYA colony light brown to buff *Hamigera inflata*
- 7a. CYA colonies light buff, CYA and MEA reverse light buff to pale orange-brown *Hamigera insecticola*
- 7b. CYA colonies bright yellow, warm buff or orange buff, reverse chamois, orange, dark red or brown *Hamigera terricola*

Penicillium humicoloides (Bills & Heredia) S. W. Peterson, Jurjevic, Bills, Stchigel, Guarro & F.E. Vega, comb. nov.



FIGS. 54–57. Colony growth on CYA, CYA reverse, MEA and MEA reverse after 7 d at 25 C. 54. *Hamigera avellanea*. 55. *Merimbla ingelheimensis*. 56. *Hamigera paravellanea*. 57. *Hamigera insecticola*.

Basionym: *Merimbla humicoloides* Bills & Heredia, Mycol Res 105:1276, 2001.

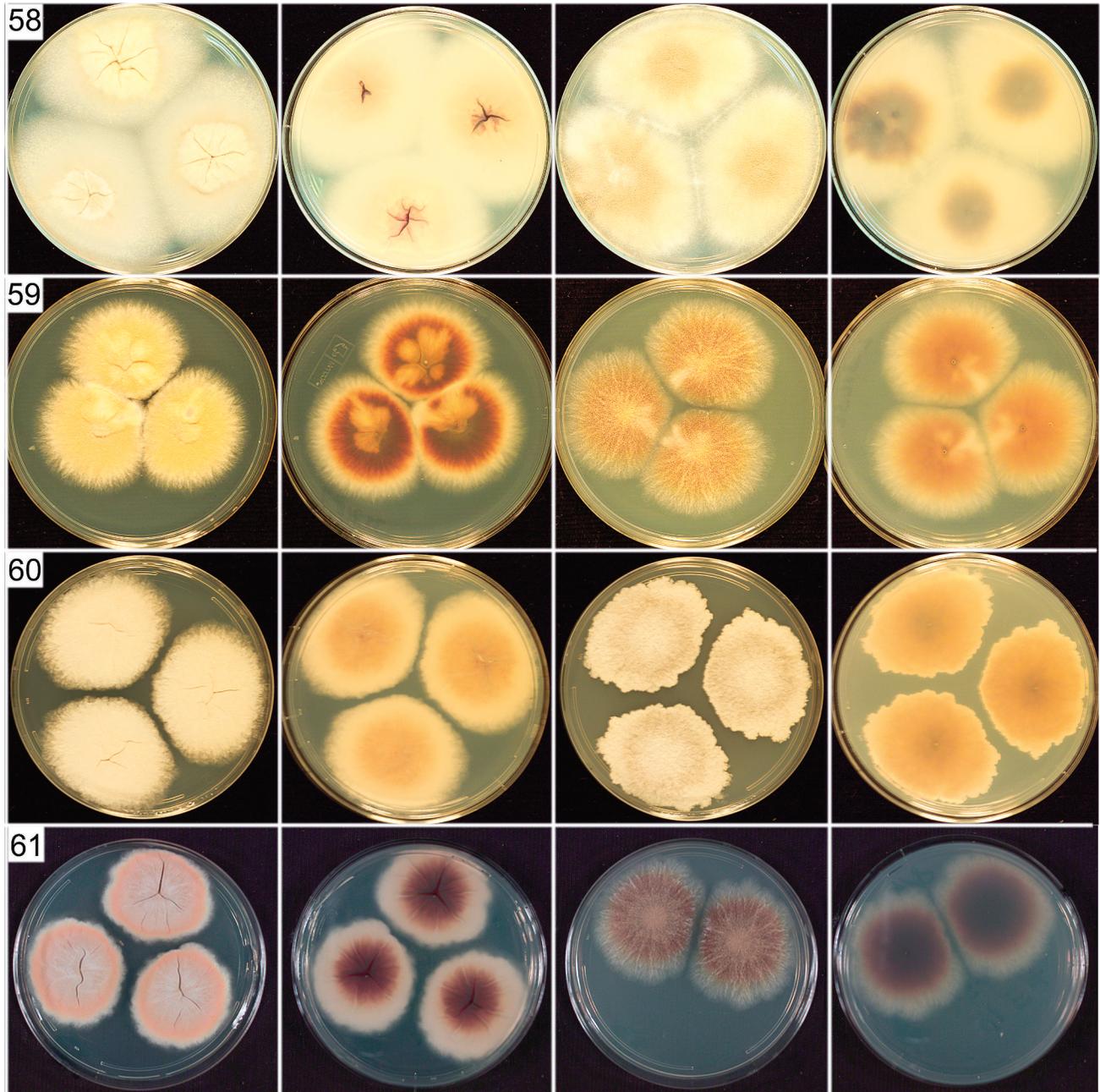
Holotype BPI 748244. Cultures ex-type CBS 102854, NRRL 35712.

Cultures examined: MEXICO, Veracruz, Las Vigas, San Juan de Monte Ecological Reserve, isolated from heat-treated soil 1999, NRRL 35712.

Mycobank MB516021

Phylogenetic analysis of the DNA sequences dem-

onstrates that *P. humicoloides* is not part of the *Hamigera/Merimbla* clade (FIG. 1) and to maintain a monophyletic genus *Merimbla* it must be moved to another genus. Bills et al. (2001) discussed the difficulty of placing their new species into existing taxonomy because of the unique form of the penicillus in *P. humicoloides*. The complex penicillus of *P. humicoloides* resembles that of *Merimbla* but differs in containing rami, metulae and phialides instead of just metulae and phialides found in



FIGS. 58–61. Colony growth on CYA, CYA reverse, MEA and MEA reverse after 7 d at 25 C. 58. *Hamigera inflata*. 59. *Hamigera terricola*. 60. *Hamigera pallida*. 61. *Hamigera fusca*.

Merimbla. *P. humicoloides* is a sibling of *P. arenicola* and *P. canadensis* as indicated by the strong statistical support in tree diagrams.

Penicillium arenicola Chalab., Bot Mater. Otd Sporov Rast 6:162, 1950

Neotype. IMI 117658, designated by Pitt 1980. Cultures ex-neotype: NRRL 3392, ATCC 18321, ATCC 18330, CBS 220.66, DSM 2435, FRR 3392, IMI 117658, NRRL 6169, VKM F-1035.

Cultures examined: UKRAINE, Kiev, pine forest soil, NRRL 3392, ex-type isolate. CANADA, Ontario, Petawawa, pine forest soil, UAMH 2878=NRRL 31507. CANADA, Northwest Territories, Norman Wells, oil-soaked soil, UAMH 4054=NRRL 31509.

Ramírez (1982) placed *Penicillium arenicola* in the *Penicillium brevicompactum* series because of the complex penicillus structure. Similarly Raper and Thom (1949) stated that they would have placed the anamorph of *P. avellaneum* in the *P. brevi-compactum*

series except for the production of the perfect state. Pitt (1980) regarded this species as being a *Penicillium* related to *Thysanophora* or *Merimbla*, while Pitt and Hocking (1985) regarded the similarity of *Merimbla* and *P. arenicola* as convergence.

The conidiophore of *P. arenicola* is septate apically, broad, and fertile branches composed of rami, metulae and phialides are thickly produced from different cells near the apex of the conidiophore. Lateral branches also may arise subapically that produce rami, metulae and phialides. Conidia ellipsoidal, $2.5\text{--}3.0 \times 3.5\text{--}4.0 \mu\text{m}$, smooth to finely roughened. Colonies on Czapek's agar attained 30–40 mm diam and are pale fawn, with no reverse pigmentation. *Merimbla ingelheimensis* and the anamorphs of the *Hamigera* species are similar to *P. arenicola* but produce metulae and phialides on the conidiophore while *P. arenicola* and *P. canadensis* form rami, metulae and phialides. Phylogenetic analysis does not statistically support the placement of the species in *Merimbla* (FIG. 1).

Penicillium canadensis G. Sm., Trans Br Mycol Soc 39:113, 1956.

Holotype specimen designated by Smith (1956) and deposited at IMI. Cultures ex type: NRRL 2553, ATCC 18424, CBS 245.56, FRR 2553, IMI 61834, LSHB BB300, LSHTM BB.300, NRRL 6167, QM 6970, WB 4155.

Phylogenetic analysis of six loci (supplemental trees) places this species as a sibling of *P. arenicola*. Attesting to the close relationship, Pitt (1980) reduced these two species to synonymy. Phylogenetically they are distinct and we accept *P. canadensis*. The epithet *canadensis* is an orthographic correction, ICBN recommendation 60D.

Penicillium megasporum Orpurt & Fennell, Mycologia 47:233, 1955.

Living syntype NRRL 2232 designated by Orpurt and Fennell (1955). Dried holotype IMI 216904, based on NRRL 2232, designated by Pitt 1980 as lectotype of *P. megasporum*. Cultures ex-type: NRRL 2232, ATCC 12322, CBS 256.55, FRR 2232, IMI 216904, MUCL 38804, QM 6879, WB 2232.

Cultures examined: UK, Suffolk, Lakenheath Warren, isolated from heath soil by J.H. Warcup 1947, NRRL 2232 ex-type. UK, isolated from an unrecorded substrate by G. Smith, London School of Hygiene and Tropical Medicine 1953, NRRL 6228.

Conidiophores of *P. megasporum* are thin (2–4 μm diam), apically vesiculate, producing metulae with 3–5 phialides, spiny conidia 6–10 μm diam; metulae and phialides produced subapically, either singly or in whorls. Pitt (1980) created *Penicillium* series *Megaspora* to contain this species and *P. asperosporum* with

the comment that these species have little affinity with other groups in *Penicillium*. The penicillus structure described here is consistent with placement of the species in genus *Merimbla*, but the phylogenetic analysis does not support inclusion of *P. megasporum* in the *Hamigera*/*Merimbla* clade. *P. megasporum* typically appears in trees as the sibling of the *Hamigera* clade but with no statistical certainty.

Penicillium giganteum R.Y. Roy & G.N. Singh, Trans Br Mycol Soc 51:805, 1968.

Holotype IMI 132774. Cultures ex-type: NRRL 3553, ATCC 48996, CBS 144.69, FRR 535, IMI 132774.

Cultures examined. INDIA, West Bengal, isolated from soil by G. N. Singh, 1967, NRRL 3553.

Conidiophores vary from sessile phialides to biverticillate, according to Roy and Singh (1968). When present, metulae are oblong to obpyriform 7–15 \times 2.5–4.0 μm bearing 2–3 phialides 6–9(–20) \times 1.5–3.0 μm , producing spherical, spiny conidia 6–9(–12) μm diam. Our culture sporulated poorly but was consistent with the description of Roy and Singh (1968). Pitt (1980) considered *P. giganteum* to be a synonym of *P. megasporum*, but analysis of each of the six loci examined here (supplemental figures) depict *P. giganteum* and *P. megasporum* as sibling species. We accept *P. giganteum* as a distinct species.

The synonymy of *Raperia* and *Merimbla* hypothesized by von Arx (1986) was not supported in analysis of any of the individual loci or in the combined data analysis.

Talaromyces striatus (Raper & Fennell) C.R. Benj., Mycologia 47:682, 1955.

\equiv *Penicillium striatum* Raper & Fennell, Mycologia 40:521, 1948 (nom. holom.)

\equiv *Hamigera striata* (Raper & Fennell) Stolk & Samson, Persoonia 6:347, 1971.

Lectotype IMI 39741, designated by Pitt 1980:487 for *Talaromyces striatus*. *Cultures ex-type*: NRRL 717, ATCC 10501, CBS 377.48, FRR 717, IFO 6106, IMI 39741, QM 1857.

Anamorph. *Penicillium lineatum* J.I. Pitt (1980).

Cultures examined: USA, New Jersey, isolated by E.J. Cameron from canned blue berries 1938, ex-type, NRRL 717. SWEDEN, soil isolate sent by E. Velander 1946, NRRL 2080. USA, Illinois, Maywood, isolated from canned blueberries by R.W. Pilcher 1949, NRRL 29063. UK, Suffolk, Brandon Park, isolated from soil under *Pinus sylvestris* by J.H. Warcup 1950, NRRL 29064. USA, North Carolina, Durham, isolate sent by F.A. Wolf 1951, NRRL 29065. SWEDEN, soil isolate sent by E. Velander 1946, NRRL 29066. JAPAN, Tokyo, isolated from soil by S. Udagawa, received from Banyu Research Laboratories, F-129157=NRRL 35715.

Phylogenetic analysis shows that this species is

distinct from the clade that includes the type species of *Hamigera*, *H. avellanea*. Statistical analysis of the DNA sequence data shows no support for a sibling relationship between *H. avellanea* and *T. striatus* and consequently we accept Benjamin's (1955) placement of the latter species in *Talaromyces*.

Talaromyces leycettanus H.C. Evans & Stolk, Trans Br Mycol Soc 56:45, 1971.

Holotype CBS 398.68. Cultures ex-type: NRRL 5178, CBS 398.68, ATCC 22469, IMI 178525.

Cultures examined: ENGLAND, Staffordshire, isolated by H. C. Evans from Leycett coal spoil tips, May 1968, NRRL 5178, ex-type.

Talaromyces leycettanus grows poorly at 25 C but much better at 37 C or up to about 52 C (Evans and Stolk 1971). Colony and micromorphology also easily distinguish *T. leycettanus* from other species (Evans and Stolk 1971). Evans and Stolk (1971) designated *Paecilomyces leycettanus* H.C. Evans & Stolk as the name of the anamorph of this fungus. Although the complex conidiophore resembles *Merimbla*, the long, narrow, acerose phialides and the subcylindrical conidial argue strongly that the anamorph is properly placed in *Paecilomyces*. Phylogenetically it is distinct from the *Hamigera* clade.

DISCUSSION

Stolk and Samson (1971) regarded *Penicillium ingelheimensis* as a species distinct from *H. avellanea* based on conidial shape and colony color production. Pitt and Hocking (1979) concluded from the similarity of the anamorph of *H. avellanea* and *M. ingelheimensis* that the two were conspecific. The multilocus DNA sequence dataset (FIG. 1) shows that the two are sibling species. Morphologically these species are most easily distinguished by colony colors; *H. avellanea* displays a dark reddish-purple brown reverse, while *M. ingelheimensis* cultures display a slightly pinkish reverse sometimes with a faint tint of red. Also *M. ingelheimensis* isolates do not make ascomata, while all examined isolates of *H. avellanea* form the teleomorph. It remains to be seen whether *M. ingelheimensis* is truly an anamorphic species or whether like *Aspergillus flavus* (Horn et al. 2009) or *Aspergillus fumigatus* (O'Gorman et al. 2008) it is a heterothallic species and ascomata form when the compatible mating types are crossed under adequate conditions.

The size and shape of conidia among the newly described species of *Hamigera* overlap considerably. The size, shape and wall ornamentation of the ascospores of these species also exhibit great similarity. While the yellow ascomata typical of *H. avellanea*

are distinctive, most of the species make white ascomata loosely covered with hyphae of approximately the same size, 100–200 µm diam. The species do have distinctive colony colors and colony reverse colors. They can be divided into two groups, the species producing pinkish, orange or flesh on CYA and the species that produce bright-yellow to drab yellow colonies on CYA. Colony reverse ranges from bright purple-red, fleshy pink, orange, light brown and deep brown. The combinations of colony size, colors and colors in reverse have been adequate in the current sample of isolates to satisfactorily distinguish the species on these bases. DNA sequence analysis can unambiguously identify the isolates within the framework of phylogenetic species recognition (Taylor et al. 2000; Dettman et al. 2006).

Penicillium arenicola, *P. humicoloides*, *P. canadensis*, *P. megasporum* and *P. giganteum* are unusual among *Penicillium* species because of the form of the conidiophore. It has not been clear in the past exactly where these species fit in with other species in the genus (Pitt 1980, Pitt and Hocking 1985). We demonstrate that they do not fit phylogenetically with other *Penicillium* species, and they do not fit with *Merimbla*. Ogawa et al. (1997), Ogawa and Sugiyama (2000), Geiser et al. (2006) and Peterson et al. (2008) have contributed to resolving relationships in Trichocomaceae, but additional loci or taxa must be included to resolve the relationships of the species and genera.

The relationship of *Talaromyces striatus* and *Byssochlamys nivea* hypothesized by von Arx (1986) when he placed *T. striatus* in *Byssochlamys* was not supported in this analysis, nor was the simultaneously hypothesized synonymy of *Merimbla* and *Raperia* (supplemental figures).

Two isolates of *Hamigera fusca* have a type I intron in the 18S rDNA, while two other isolates, NRRL 29057 and NRRL 29058, lack this intron. A similar situation was found in *Monilinia fructicola* isolates (Cote et al. 2004) where only a proportion of them contained an 18S intron. Possession of the intron is not a singular character defining fungal species.

It has been a common practice in dealing with holomorphic species in the Trichocomaceae to provide separate formal names for the anamorphs and teleomorphs of each species. In recent years some have criticized giving two separate and valid names to different stages of a single biological species. In the case of *Hamigera* species and in light of recommendation 59A.3 of the current Botanical Code (Vienna Code, McNeill et al. 2006) it is unnecessary to provide separate anamorph names. There is no advantage to having anamorph names for *Hamigera* species where ascomata are produced in all isolates seen

to date. Only *Merimbla ingelheimensis* isolates fail to make ascomata. So to provide the simplest most useful taxonomy we do not provide *Merimbla* names for the anamorphs of *Hamigera* species.

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