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Taxonomy and phylogeny of *Brachyconidiellopsis fimicola*, *gen. et sp. nov.*, a sporodochial to synnematous coprophilous fungi related to the Microascales (Ascomycetes) from Nepal

Cony DECOCK[1a], *Rafael CASTAÑEDA RUIZ*[b] *& Mahesh K. ADHIKARI*[b]

[a] *Mycothèque de l'Université catholique de Louvain (MUCL*[2]*, MBLA),*
Place Croix du Sud 3, B-1348 Louvain-la-Neuve, Belgium.
decock@mbla.ucl.ac.be.

[b] *INIFAT, Calle 1 esq. 2, Santiago de las Vegas,*
C.P. 17200, Ciudad de La Habana, Cuba.

[c] *National Herbarium and Plant Laboratories,*
Godawari, Lalitpur, GPO Box 21758, Katmandu, Nepal.

Abstract – The anamorphic fungus *Brachyconidiellopsis fimicola gen. et sp. nov.*, collected in Chitwan, Terai Belt, southern Nepal on dung of an unidentified Cervidae, is described and illustrated. The fungus is mainly characterized by having a sporodochial to synnematal conidiomata, with a narrow, dark brown to black stipe, ending in a slightly fasciculate fertile apex. The conidiogenesis is holoblastic, with (mono-) to polyblastic conidiogenous cells, and conidial secession rhexolytic leaving large, open scars. The conidia are grayish to grayish black, cheirosporous, with as many as 10-15 divergent branches, each composed of chains of narrowly ellipsoid to slightly barrel-shaped cells, remaining entire at maturity. The phylogenetic relationships of the fungus inferred from partial nuclear small ribosomal subunit (18S) DNA revealed affinities with the Microascales (Ascomycetes).

Brachyconidiella **/ Coprophilous fungi / Microascales / Nepal / taxonomy**

INTRODUCTION

During a survey of coprophilous fungi in the Southern Terai Belt, Nepal, a peculiar conidiomatal fungus was collected on a dung of an unidentified Cervidae and isolated in pure culture. The fungus is mainly characterized by having a sporodochial to synnematal conidiomata, with a narrow, dark brown to black stipe, ending in a slightly fasciculate fertile apex, a holoblastic conidiogenesis, with (mono-) to polyblastic conidiogenous cells, rhexolytic conidial secession leaving large, open scars, and grayish to grayish black, cheirosporous conidia, with as many as 10-15 divergent branches, each composed of non-seceding chains of narrowly ellipsoid to slightly barrel-shaped cells.

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[1] Corresponding authors, decock@mbla.ucl.ac.be
[2] MUCL is a part of the Belgian Coordinated Collections of Micro-organisms, BCCM.

This combination of conidiogenous features is peculiar, and the generic placement of our fungus was problematic. The morphological features prompted us at first to compare it with the monotypic *Brachyconidiella* Castañeda & Kendrick (1990), typified by *B. monilispora* Castañeda & Kendrick (1990), and also characterized by having sporodochial to synnematous conidiomata and cheirosporous conidia. However, differences in several conidiogenous features such as the conidiogenous cells and the mode of conidial secession lead us to question their congenericity.

The phylogenetic relationships of our fungus with *B. monilispora* and other Ascomycetes were inferred from a parsimony analysis of partial SSU (18S) nuclear ribosomal DNA sequences, resulting in the description of *Brachyconidiellopsis fimicola*, *gen. et sp. nov*.

MATERIALS AND METHODS

Cultures were grown on corn meal agar (CMA, Untereiner *et al.* 1998) at 25°C, with a 12/12 hours incident light periodicity (near UV light). Microscopic measurements were made in lactic acid cotton blue. In presenting the size range of several microscopic elements, 5% of the measurements at each end of the range are given in parentheses, when relevant. In the text, the following abbreviations are used: $\bar{x}$ = arithmetic mean; R = ratio of length/width of the conidia; $\bar{x}_R$ = arithmetic mean of the ratio R.

DNA was extracted from freshly collected mycelium grown on liquid malt (Malt 2%) in the dark at 25°C, following the protocol of Lee *et al.* (1988) and purified with Geneclean® III kit (Q-Biogene), following the manufacturer's recommendations. The primer pair NS1-NS8 (White *et al.* 1990) was used to amplify the 18S rDNA. Successful PCR reactions resulted in a single band observed on an 0.8% agarose gel, corresponding to approximately 1700 bp. PCR-products were cleaned using the QIAquick® PCR purification kit (250) (QUIAGEN Inc.), following the manufacturer's protocol. Sequencing reactions were performed using CEQ DTCS Quick Start Kit® (Beckman Coulter), according to the manufacturer's recommendations. Nucleotide sequences were determined with a CEQ 2000 XL capillary automated sequencer (Beckman Coulter). 18S rDNA fragments were sequenced with primers NS1-NS8. Nucleotide sequences were initially automatically aligned with Clustal X for Macintosh (version 1.5b), then manually adjusted as necessary with the editor in PAUP* (version 4.0b10). Phylogenetic analysis of the aligned sequences was performed using the maximum parsimony method of PAUP* version 4.0b10 (Swofford 2002). Gaps were treated as fifth base. Optimal trees were identified using heuristic searches, and further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority-rules in the bootstrap consensus tree.

Accession numbers for sequences obtained from GenBank are included on the list below. Ordinal and supra-ordinal classifications are based on Kirk *et al.* (2001). New sequences were generated and deposited in GenBank for the following fungi: *Brachyconidiellopsis fimicola* Decock *et al.* MUCL 44677 (AY342016), *Brachyconidiella monilispora* Castañeda & Kendrick MUCL 39848 (AY342015), and *Leptoxyphium sp*. MUCL 43740 (AY628692).

List of sequences obtained from GenBank

A*ureobasidium pullulans* (de Bary) Arnaud (AY030322); *Capnodium citri* Berk. & Desm. (AY016340); *Capronia mansonni* (Schol-Schwarz) E. Müller *et al.* (X79318); *Cenococcum geophilum* Fr.: Fr. (L76617); *Ceratocystis fimbriata* Ellis & Halst. (U43777); *Chaetomium elatum* Kunze: Fr. (M83257); *Cladophialophora arxii* Tintelnot *et al.* (AJ232948); *Coccodinium bartschii* A. Massal. (U7668); *Cyphellophora laciniata* de Vries (AY342010); *Delphinella strobilogena* (Desm.) Sacc. (AY016351); *Dothidea hippophaeos* (Pass.) Fuck. (U42475); *Eupenicillium javanicum* T.H. Beyma) Stolk & D.B. Scott (U21298); *Graphium calicioides* (Fr.) Cooke & Massee (AB007655); *Graphium fructicola* Marchal & Marchal (AB007659); *Graphium tectonae* C. Booth (U43907); *Gymnascella aurantiaca* Peck (AB015772); *Gymnoascus reessii* Baran. (AB015774); *Lomentospora prolificans* Hennebert & Desai (U43910); *Microascus cirrosus* Curzi (M89994); *Morchella esculenta* L.: Fr. (L37537); *Myriangium duriaei* Mont. (AY016347); *Myrothecium verrucaria* (Alb. & Schwein.: Fr.) Ditmar (AJ302003); *Nectria cinnabarina* (Tode: Fr.) Fr. (AB003949); *Nectria pseudotrichia* Berk. & M.A. Curtis (AY342011); *Neolecta vitellina* (Z27393); *Onygena equina* (Will.: Fr.) Pers. (U45442); *Ophiostoma piliferum* (Fr.: Fr.) Syd. & Syd. (AJ243295); *Petriella setifera* (Schmidt) Curzi (U43908); *Phaeotheca fissurella* Sigler *et al.* (A18697); *Pseudallescheria boydii* (Shear) McGinnis *et al.* (M89782); *P. ellipsoidea* (Arx & Fassat.) McGinnis *et al.* (U43911); *Pyronema domesticum* (Sowerby: Fr.) Sacc. (U53385); *Raciborskiomyces longisetosum* (Volkart) M.E. Barr (AY016351); *Ramichloridium anceps* (Sacc. & Ellis) de Hoog (AJ232951); *Rhytidhysteron rufulum* (Spreng.) Speg. (AF201452); *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (V01335); *Scleroconidioma sphagnicola* Tsuneda *et al.* (AY220610); *Sordaria fimicola* (Roberge) Ces. & de Not. (X69851); *Sporothrix schenckii* Hektoen & Perkins (M85053); *Stylodothis puccinoides* (DC : Fr.) Arx & E. Müller (AY016353); *Talaromyces flavus* (Klöcker) Stolk & Samson (M83262); *Taphrina deformans* (Berk.) Tul. (AJ495826).

RESULTS

The sequence of *B. fimicola* has a "normal" length of approximately 1700 bps. However, the sequence of *B. monilispora* MUCL 39848 was longer, reaching approximately 1900 bps, and had, in comparison with other sequences screened at GenBank, three major insertions, each ranging from 52-57 base pairs.

Preliminary indications of the relationships of *B. fimicola* and *B. monilispora* were obtained using the BLAST option at GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). The search for *B. fimicola*, using a 1700 bps fragment, yielded almost exclusively members of the Microascales Luttr. ex Benny & R.K. Benj., such as the ascomycetes genera *Petriella* Curzi and *Pseudallescheria* Negr. & I. Fisch., or the anamorphic genera *Lomentospora* Hennebert & B.G. Desai or *Graphium* Corda. The complete sequence of *B. monilispora* did not match satisfactorily with any of the sequences from GenBank. The search yielded several ascomycetes genera belonging to the large subclass Dothideomycetidae such as *Rhytidhysteron* Speg. (Patellariales) or several anamorphic genera of uncertain taxonomic position such as *Cenococcum* Moug. & Fr. or *Scleroconi-*

dioma Tsuneda *et al.* but all with a very bad score. A second search for *B. monilispora*, deleting the three insertions, yielded some anamorphic fungi of so-called "black yeast" also related to the Dothideomycetidae such as *Aureobasidium* Viala & G. Boyer or *Hormonema* Lagerb. & Melin, or the ascomycete *Discosphaerina* Höhn., the latter having anamorphs related to the two former genera.

Subsequently, a 18S data matrix containing 1670 characters (including gaps) for 42 taxa of ascomycetous and related anamorphic fungi (c. list) was set up. However, in the analysis, the three insertions of *B. monilispora* were each considered as a unique event, and recoded as a single nucleotide position, what resulted in a final data matrix of 1508 characters, gaps included.

A heuristic search with 10.000 random additions sequences yielded 22 equally parsimonious trees (EPTs), 1254 steps in length (CI = 0.588, RI = 0.767). The tree topologies of the 22 MPTs were identical with regard to the definition of several clades such as the Chaetothyriales, Onygenales-Eurotiales, Ophiostomatales-Sordariales, Microascales-Hypocreales, and Dothideales, and conforms to some previously published trees (Sterflinger *et al.*, 1999, Sugiyama 1998). The variations within the 22 MPTs concerned mainly the relative position of some terminal branches within the different clades (orders) and the position of *B. monilispora*. One of the 22 MPTs is represented at fig. 8.

In this analysis, *B. fimicola* clustered with members of the Microascales, with a strong bootstrap support (86%). However, the taxonomic relationships of *B. monilispora* remained unresolved.

TAXONOMY

Brachyconidiellopsis Decock, Castañeda, et Adhikari, ***anam. gen. nov***. Figs 1-6.

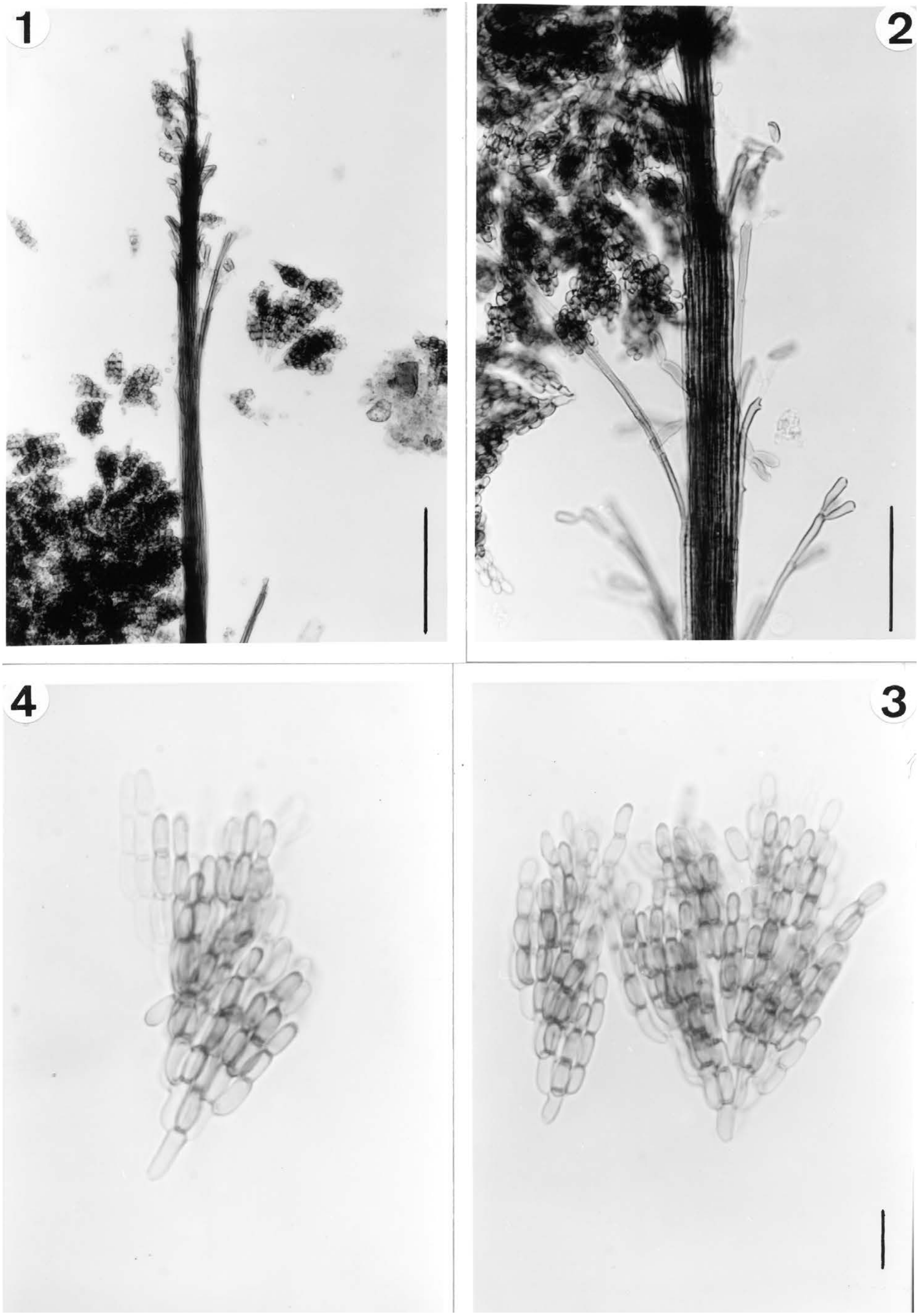
Ad fungos conidiales, Hyphomycetes pertinens. Coloniae in substrato naturali effusae, brunneae. Mycelium plerumque in substrato immersum. Conidiomata sporodochia vel synnemata, indeterminata vel determinata, dissita, griseo-brunnea usque ad brunnea. Conidiophora (filamentis) inconspicua vel conspicua, septata, levia vel verrucosa, pallide brunnea usque ad brunnea, in textura porrecta disposita. Cellulae conidiogenae monoblasticae, polyblasticae ad determinatae vel indeterminatae, sympodiales. Secedentia conidiorum schizolytica. Conidia holoblastica, cheiroidea, moniliformia, penicillata usque ad irregularia, acrogena vel acropleurogena, pigmentata, repetite ramosa, ex cellulis ellipsoideis, oblongis usque ad sphaericis, blastocatenatis, levibus vel verrucosis, composita.

Teleomorphosis ignota.

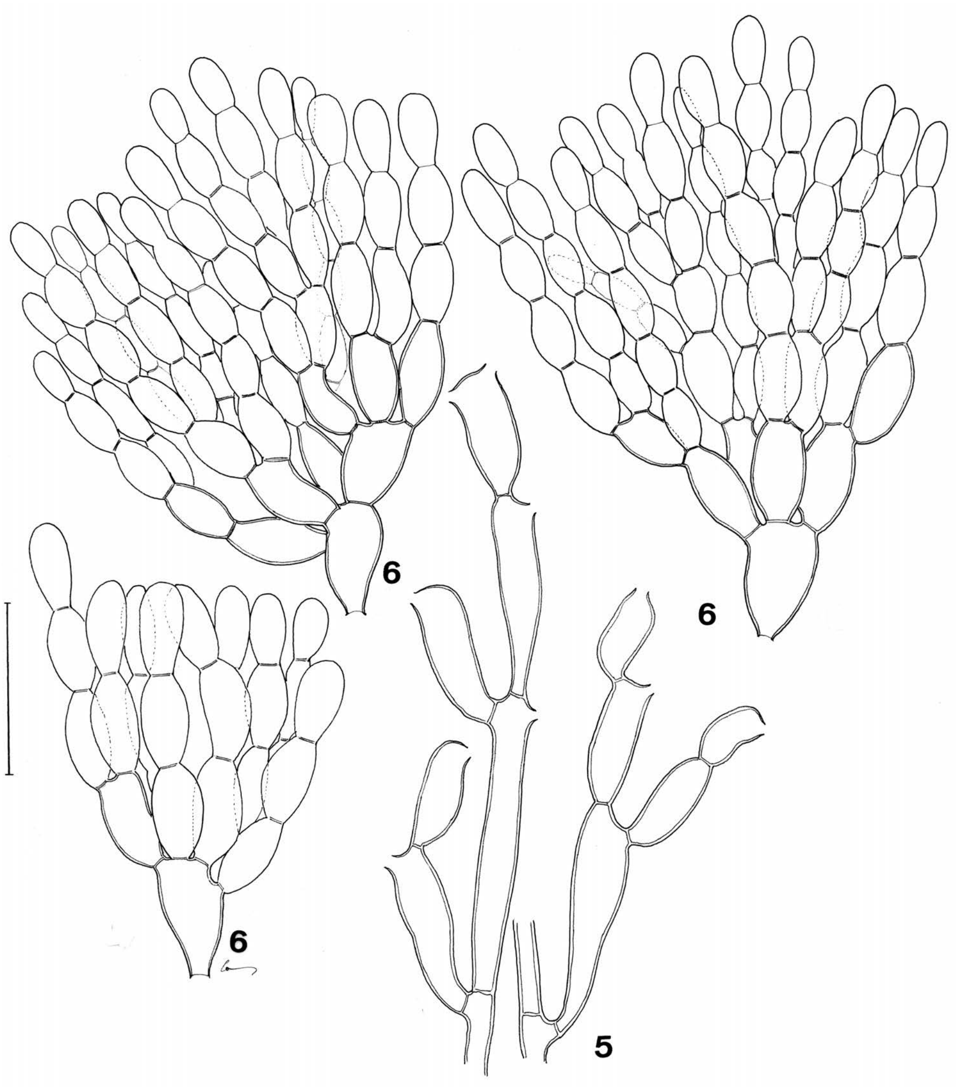
Species typica: Brachyconidiellopsis fimicola Decock, Castañeda, et Adhikari.

Conidial fungi, hyphomycetes. *Colonies* on the natural substrate effuse, brown. *Mycelium* mostly immersed. *Conidiomata* sporodochial or synnematal. *Sporodochia* scattered, cushion shaped. *Synnemata* scattered, determinate, gray-brown to brownish black, stipe with a *textura porrecta*, slightly apically fasciculate. *Conidiogenous cells* mono- to polyblastic, determinate or indeterminate, sympodial. *Conidial secession* rhexolytic. *Conidia* holoblastic, grayish brown to grayish black, cheiroid, repeatedly branched, each branch composed of ellipsoid to oblong, smooth cells in non-seceding chains.

Teleomorph: unknown.



Figs 1-4. *Brachyconidiellopsis fimicola*. Fig. 1. Part of the synnemata (scale bar = 50 µm). Fig. 2. Part of the synnemata (scale bar = 25 µm). Figs 3-4. Conidia (scale bar = 10 µm).



Figs 5-6. *Brachyconidiellopsis fimicola.* Fig. 5. Conidiogenous cells. Fig. 6. Conidia (scale bar = 10 µm).

Brachyconidiellopsis fimicola Decock, Castañeda, et Adhikari, ***anam. sp. nov.***

Coloniae in substrato naturali effusae, brunneae. Mycelium plerumque in substratum immersum, ex hyphis septatis, ramosis, levibus, brunneis, 2-3 µm latis, compositum. Conidiomata sporodochia vel synnemata. Sporodochia pulvinata, cinereo-brunnea ad brunnea, usque ad 150 µm diam. lata. Synnemata plus minusve cylindrica, ad apicem attenuata, dissita, indeterminata, usque ad 700 µm alta, ad basim 25-35 µm lata et ad apicem 15-25 µm lata, atrobrunnea usque ad brunnea ver-

sus pallide brunnea ad apicem, ex conidiophoris (filamentis) paralellis, compactis, incrassatis, septatis, 1.5-2.5 µm, ex textura porrecta conformata. Cellulae conidiogenae hologenosae (holoblasticae), mono- et polyblasticae, 1-3- protuberantibus conidiogenis locis; sympodiales, indeterminatae, terminales et intercalares, cylindricae, clavatae usque ad ellipsoideae, incrassatae, pallide brunneae usque ad subhyalinae, 11-16(-26) × 3.5-5.0 µm. Secedentia conidiorum schizolytica. Conidia holoblastica, cheiroidea, moniliformia, penicillata, acrogena vel acropleurogena, (5-)10-15 ramis repetite ramosa; conidia tota 35-65 µm alta × 20-60 µm lata, ex cellulis basalibus cylindricis usque ad clavatis, vel triangularibus, parietibus tenuis usque ad incrassatis, hyalinis vel pallide flavidis, 6.5-8.0 × 2.5-4.5 µm composita et ramis cum cellulis alter cellulis ellipsoideis usque ad oblongis, incrassis, levibus, blastocatenatis, hyalinis usque ad pallide brunneis, 4.5-6.0 × 2.5-3.0(-3.3) µm, munita.

Teleomorphosis ignota.

Mycelium mostly immersed in the substrate, composed of septate, branched, smooth-walled, brown hyphae, 2-3 µm wide. *Conidiomata* as loose sporodochial tufts of conidiogenous hyphae, scattered, cushion-shaped, up to 150 µm across, grayish brown to brown, or by elongation, synnematal. *Synnemata* scattered, of indeterminate growth, more or less cylindrical or tapered slightly towards the apex, dark brown to brown and 25-35 µm wide at the base, progressively turning light brown and tapering to 15-25 µm wide at the apex, up to 700 µm tall. *Synnemata* of *textura porrecta*, composed of parallel, moderately packed, thick-walled, septate, brown to pale brown hyphae, 1.5-2.5 µm wide, giving birth in the upper half to lateral conidiogenous cells. *Conidiophores* indistinct from the hyphae. *Conidiogenesis* holoblastic. *Conidiogenous cells* in the upper half, intercalate or terminal, cylindrical, clavate to ellipsoid, thick-walled, pale brown to sub-hyaline, mono- to polyblastic, with 1-3 large conidiogenous loci, 11-16(-26) × 3.5-5.0 µm, leaving a large scar after the conidial secession. *Conidia* cheirosporous, penicilliform, repeatedly branched, ultimately giving rise to (5-)10-15 branches, all the conidia measuring 35-65 µm in height × 20-60 µm in width. *Basal cell* of the conidia cylindrical to clavate up to triangular, hyaline to pale brownish, thin- to thick-walled, 6.5-8.0 × 2.5-4.5 µm. Each branch composed of a non-seceding, short chain of (2-)4-7(-8), ellipsoid to almost oblong, thin- but soon becoming thick-walled, hyaline to pale brownish cells, each individual cells measuring 4.5-6.0 × 2.5-3.0(-3.3) µm, R = 1.7-2.3 ($\bar{x}$ = 5.3 × 2.7 µm, $\bar{x}_R$ = 1.9). Teleomorph unknown.

Colonies on CMA at 25°C, under alternate 12h-12h. dark / light reaching 10 mm in one week. After 4 weeks, colonies grayish black. *Mycelium* immersed or superficial, rampant, septate, branched, 2.5-3.5 µm. *Conidiogenesis* as in the natural substrate. *Conidiomata* either absent, with the conidiogenous cells borne directly on hyphae, or as loose tufts of hyphae, loosely sporodochial, rarely as small synnemata; *sporodochia* appearing quickly, scattered or aggregated, cushion-shaped, grayish black, dark mouse gray. *Synnemata* appearing later, scattered, isolated or in tuft of 2-4, first pale gray, when maturing turning dark gray to black. *Conidiogenesis* is identical as in the natural substrate. *Conidia* are identical as in the natural substrate or slightly longer, some of the newest cells of the branches differentiating occasionally into *Conidiogenous* cell to form secondary conidia.

Holotype: **Nepal**, Terai, Royal Chitwan National Park, Kosara, near the Park Headquarters, on dung of an unidentified cervidae, on the ground in the forest, May 2001, collected by O. Laurence and M. K. Adhikari, isolated by G. Delgado & C. Decock at MUCL, MUCL 44677 (and as dried culture ex- holotype: MUCL 44677). Culture ex-holotype: MUCL 44677.

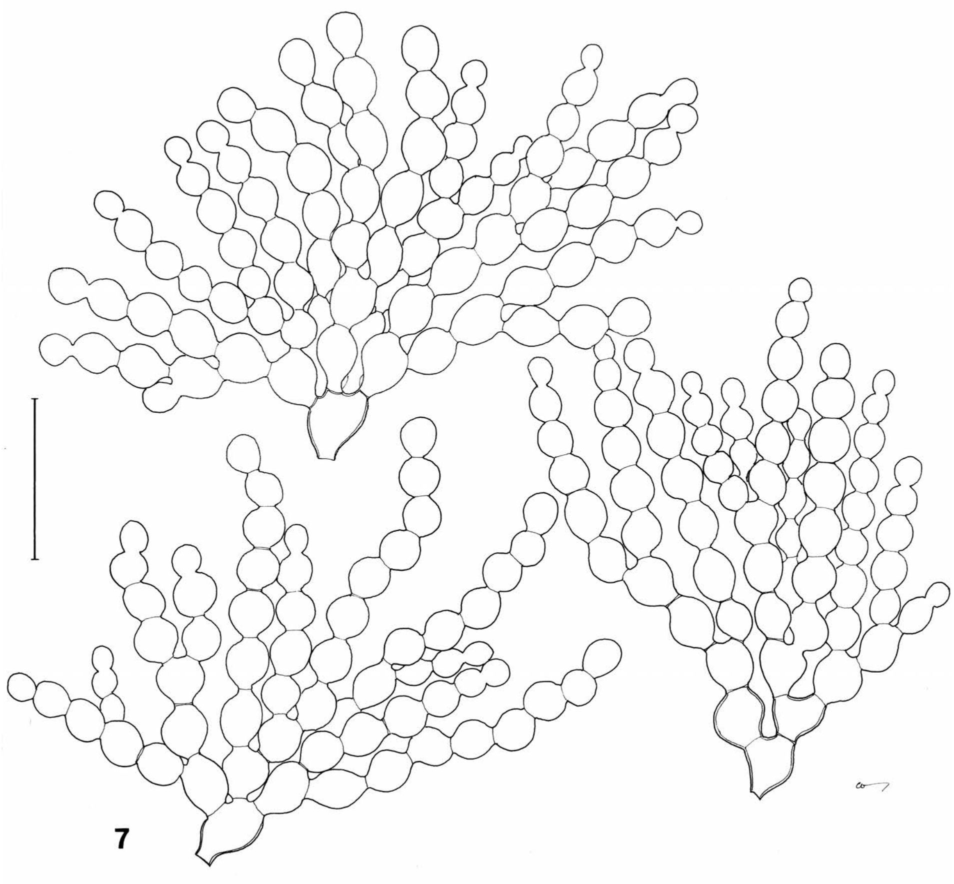


Fig. 7. *Brachyconidiella monilispora* MUCL 39848. Conidia (scale bar = 10 µm).

Remarks. *Brachyconidiellopsis fimicola* is well characterized by its grayish to grayish black cheirosporous conidia with numerous, compact branches, composed of ellipsoid to oblong cells, 4.5-6 × 2.5-3.0 µm ($\bar{x}$ = 5.3 × 2.7 µm, R = 1.7-2.3), born from mono- to polyblastic conidiogenous cells, arranged in a loose sporodochial structure or a short synnemata.

The occurrence of cheirosporous conidia is a rare feature in sporodochial or synnematous hyphomycetes. At first glance, the conidiomata and conidial features of *Brachyconidiellopsis* prompted us to compare it with *Brachyconidiella* Castañeda & Kendrick (1990), a genus so far monotypic, with *B. monilispora* Castañeda & Kendrick as type. *Brachyconidiella* is known only from decayed leaves in Cuba.

Both *Brachyconidiellopsis* and *Brachyconidiella* share a sporodochial to short synnematous conidiomata, a holoblastic conidiogenesis, and cheirosporous, penicilliform conidia, the branches of which appear as acropetal, moniliform, non-seceding chains (fig. 7). However, they differ in some conidiogenesis features,

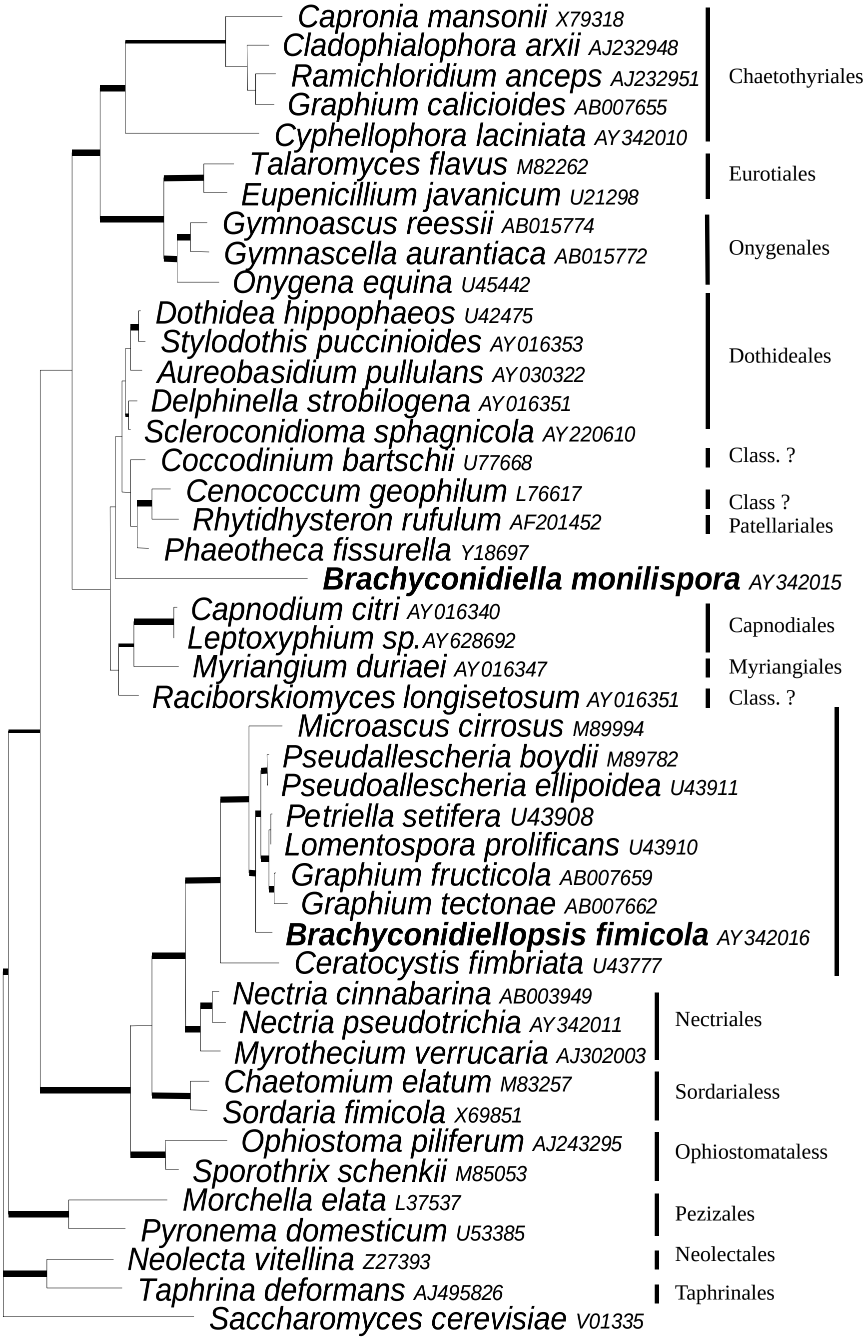


Fig. 8. One of the 22 equally parsimonious tree for a heuristic search (cf. text for explanation). The branches in bold are those with a bootstrap value between 70-100%. Class. ? = uncertain classification.

especially the conidiogenous cells and the mode of secession of the conidia. In *Brachyconidiellopsis*, the conidiogenous cells are very commonly polyblastic and the conidial secession rhexolytic, leaving open scars. In *Brachyconidiella*, the conidiogenous cells are always monoblastic and with an schizolytic secession, leaving a plane, closed scar. The preliminary phylogenetic analysis inferred from rDNA 18S DNA sequences (fig. 8) confirmed that the two fungi, notwithstanding their superficial similarities in conidiomata and conidia, belong in fact to two unrelated genera.

The taxonomic relatives of *B. fimicola*, as revealed by the phylogenetic analysis (fig. 8), are among the Microascales. More precisely, *Brachyconidiellopsis* belongs probably to the Microascaceae Luttr. ex Malloch. However, more detailed molecular studies, based on other ribosomal or protein-coding gene sequences would be necessary to infer more precisely the affinities of the genus within the order.

Many species of Microascales have an anamorphic state, either momonematous (micro, macronematous) or synnematous, most of them with a phialidic or annellidic conidiogenesis, or solitary aleurioconidia. So far, the conidiogenous and conidial features of *Brachyconidiellopsis* were unknown in the order.

The precise affinities of *Brachyconidiella* remained uncertain and its taxonomic relationships were not resolved by our preliminary phylogenetic analysis. The relative position of *Brachyconidiella* was variable within the 22 EPTs but, mainly, the genus clustered within the Dothideomycetidae, a large subclass which internal phylogenetic relationships remain still largely unknown. The conidiogenesis and the conidial morphology of *Brachyconidiella* in some respects, show some resemblance to the anamorph of some species of *Sereutia* Pat. (Seuratiaceae Vuill. ex M.E. Barr[3]), as for instance *S. millardetii* (Racib.) Meeker (Meeker 1975). The latter taxon has branched conidia composed of moniloid chains of spheroid cells, borne in sporodochia, in which features it differs from *Brachyconidiella*. No sequence of *Seuratia* was available for inferring its phylogenetic relationships with *Brachyconidiella*. However, *Brachyconidiella* did not showed any clear phylogenetic affinities with *Myriangium duriaei* Mont., a member of the Myriangiales.

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[3] The ordinal placement of this family is uncertain but Meeker (1975) placed it in the Myriangiales Starbäck

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