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ITS LSU

**DNA Barcodes** 

fungal systematic

www.GeneraofFungi.org

# The Genera of Fungi: fixing the application of type species of generic names

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Abstract: To ensure a stable platform for fungal taxonomy, it is of paramount importance that the genetic application of generic names be based on their DNA sequence data, and wherever possible, not morphology or ecology alone. To facilitate this process, a new database, accessible at www.GeneraofFungi.org (GoF) was established, which will allow deposition of metadata linked to holo-, lecto-, neo- or epitype specimens, cultures and DNA sequence data of the type species of genera. Although there are presently more than 18 000 fungal genera described, we aim to initially focus on the subset of names that have been placed on the "Without-prejudice List of Protected Generic Names of Fungi" (see IMA Fungus 4(2): 381-443, 2013). To enable the global mycological community to keep track of typification events and avoid duplication, special MycoBank Typification identfiers (MBT) will be issued upon deposit of metadata in MycoBank. MycoBank is linked to GoF, thus deposited metadata of generic type species will be displayed in GoF (and vice versa), but will also be linked to Index Fungorum (IF) and the curated RefSeq Targeted Loci (RTL) database in GenBank at the National Center for Biotechnology Information (NCBI). This initial paper focuses on eight genera of appendaged coelomycetes, the type species of which are neo- or epitypified here: Bartalinia (Bartalinia robillardoides; Amphisphaeriaceae, Xylariales), Chaetospermum (Chaetospermum chaetosporum, incertae sedis, Sebacinales), Coniella (Coniella fragariae, Schizoparmaceae, Diaporthales), Crinitospora (Crinitospora pulchra, Melanconidaceae, Diaporthales), Eleutheromyces (Eleutheromyces subulatus, Helotiales), Kellermania (Kellermania yuccigena, Planistromataceae, Botryosphaeriales), Mastigosporium (Mastigosporium album, Helotiales), and Mycotribulus (Mycotribulus mirabilis, Agaricales). Authors interested in contributing accounts of individual genera to larger multi-authored papers to be published in IMA Fungus, should contact the associate editors listed below for the major groups of fungi on the List of Protected Generic Names for Fungi.

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# INTRODUCTION

In The Genera of Fungi, Clements & Shear (1931) summarised the mycological information available at that time to provide a classification of all genera of fungi (kingdom Fungi, including lichen-forming fungi, and also *Oomycota*). In the process, they selected type species for many genera in which no type was designated, thus attempting to fix the application of all generic names. Since that historic publication, mycology as a discipline has advanced rapidly, with the first DNA sequence data becoming available in GenBank around 1991 (Hibbett et al. 2011). Taylor et al. (2000) proposed the Genealogical Concordance Phylogenetic Species Recognition concept to delimit fungal species by utilising characters from multiple

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independent loci. This still provides the benchmark for phylogenetic species delimitation in mycology. However, less precise methods were needed in order to address the challenge to document all life on the planet in a reasonable time frame. The possibility for rapid specimen identification and species discovery by means of their DNA sequence data gained extra momentum when Hebert et al. (2003) introduced the concept of DNA Barcoding. This aims to use a single, variable stretch of DNA (DNA barcode) to identify all eukaryotic life on Earth. Although it soon became clear that the mitochondrial gene CO1 was unsuitable as a universal DNA barcode for fungi, the fungal community addressed the situation by adopting the widely used internal transcribed spacer (ITS) region of the nuclear ribosomal DNA as the Fungal DNA barcode (Schoch et al. 2012). This marker had a 73 % probability to correctly identify a fungal species screened, which was comparable to the two barcode markers for plants. Nevertheless further research to propose additional barcodes that will distinguish species with better resolution are already well advanced (Schmitt et al. 2009, Quaedvlieg et al. 2012, Gryganskyi et al. 2013, Tretter et al. 2013).

Other changes effecting fungal nomenclature include the launching of MycoBank to provide unique identifiers for each taxonomic change and novelty on a global scale (Crous *et al.* 2004), as well as the publication of the "Amsterdam Declaration on Fungal Nomenclature" (Hawksworth *et al.* 2011) to suggest changes to the subsequently renamed *International Code of Nomenclature for algae, fungi, and plants* (ICN). This also signalled the end of dual nomenclature (Hawksworth 2011, Wingfield *et al.* 2012). A further implication of these changes was the option to develop lists of names to obtain "protected status", to be ratified by the next International Botanical Congress (IBCXIX) in China in 2017) (Hawksworth *et al.* 2012).

The Assembling the Fungal Tree of Life project provided a major impetus towards a DNA-based classification of Fungi by applying multigene phylogenetics approaches comparable to the genealogical concordance proposed for species delimitation by Taylor et al. (2000). The focus was on elucidating the older fungal divergences and resulted in a classification that named lineages above ordinal level (Hibbett et al. 2007). However, reliable phylogenetic data for many relationships at genus and family ranks remains wanting. As a consequence of merging a morphology-based taxonomy into this new phylogenetic framework, and the abolishment of dual nomenclature, numerous name changes can be anticipated in some groups of fungi. A major problem encountered by incorporating DNA data was that most species of fungi could only be resolved as species complexes (Crous & Groenewald 2005, Crespo & Lumbsch 2012), while many genera were discovered to be poly- and or paraphyletic (Crous et al. 2009, Aveskamp et al. 2010, Hirooka et al. 2012, Quaedvlieg et al. 2013). Based on these changes, many plant pathogenic, industrial or medically important fungi had to be allocated to different genera, including the Cylindrocarpon complex (Chaverri et al. 2011), the Fusarium complex (Gräfenhan et al. 2011, Schroers et al. 2011), the Alternaria complex (Woudenberg et al. 2013), the Septoria complex (Quaedvlieg et al. 2013), the Botryosphaeria complex (Phillips et al. 2013), and many others.

In order to reduce the dramatic effects of combining phenotypic characters with genotypic characters and obtain a more stable taxonomic system, it is thus of the utmost importance to combine DNA-sequence based comparative methods with the application of generic names. During the past 14 years (2000-2013) the mycological community has described 1 833 fungal genera, for which only 155 (8.4 %) currently have types linked to reliably annotated ITS DNA sequence data in the public databases. In other words, the problems related to the imprecise morphological application of names without DNA data continues to worsen. To alleviate and contain this issue, we herewith launch The Genera of Fungi project, which will aim to sequence, restudy and/or recollect the type species of genera envisaged to be given a protected status at the IBCXIX in 2017. Although there are presently approximately 18 000 generic names of fungi in MycoBank and Index Fungorum, this list will focus on the subset of names that are currently accepted (Kirk et al. 2013).

The aims of this project are to:

- (1) Establish a new website, www.GeneraOfFungi.org, to host a database that will link metadata to other databases such as MycoBank, Index Fungorum, BOLD, UNITE, and associated DNA barcodes (ITS, LSU and other loci as needed) to GenBank and RTL (Schoch *et al.* 2014).
- (2) Source type specimens and cultures of the type species of genera from fungaria and Biological Resource Centres (BRCs), and derive the metadata required as explained below.
- (3) Recollect fresh material of the type species if not already available, and as far as possible derive DNA barcodes and cultures from this material.
- (4) Designate type species, and type specimens of those species, for those genera where this has not been indicated in the original publications.
- (5) Fix the genetic application of the type species of generic names by means of lecto-, neo-, or epitypification as appropriate, and at the same time deposit cultures in at least two Biological Resource Centres (BRCs) from which they would be widely available to the international research community.
- (6) Publish modern descriptions of the type species and relevant typifications in appropriate mycological journals (supplemented by MBT or IF numbers for registration), and also deposit associated metadata in www.GeneraOfFungi.org, which will link metadata to other databases as indicated above.

#### **Generating DNA barcodes**

Although the ITS region is the designated DNA barcode for fungal species identification (Schoch *et al.* 2012), the partial LSU (28S rDNA; spanning at least the first 850 bp – see Material & Methods below) is recommended for phylogenetic analyses to resolve and contextualise genera. Many loci can be employed in phylogenetic analyses but, we recommend that the ITS and LSU loci are generated on a routine basis for this project. To address the circumstances where DNA sequencing facilities are not freely available to many mycologists, the International Mycological Association (IMA), in collaboration with the World Federation for Culture Collections (WFCC), plans to set up a global network of BRCs and fungaria, that will, in exchange for the deposit of cultures (and/or specimens), generate free barcodes for the depositor. These collaborating BRCs will be listed on the Genera of Fungi database website.

# **Publication strategy**

Of primary concern is that metadata related to type species of genera be deposited in the Genera of Fungi database (www. GeneraOfFungi.org), and all new typification events are registered in MycoBank (MBT numbers) or Index Fungorum. Various publication options are possible, and genera could be published as individual articles (or several combined into a single article as done here) in mycological journals of choice. It is recommended that the following issues are addressed in such publications:

- (1) Taxonomic history of the genus.
- (2) Phylogenetic placement of the type species of the genus.
- (3) New nomenclature merging asexual and sexual generic names based on stability.
- (4) Generic and species description, with reference collection (e.g. fungarium), MycoBank or Index Fungorum, and INSDC sequence accession numbers.
- (5) Eventual name changes that result from the new phylogenetic placement.
- (6) Notes discussing the relevance and implications of the phylogeny of the genus.

#### Management

A team of associate editors will be appointed for major groups of fungi. It is recommended that mycologists in different countries form a national node, which could focus on trying to recollect the genera described from specific substrates in each country. In the meanwhile, MycoBank and Index Fungorum will try to elucidate which genera were described from different countries and on which substrates. This information is not currently present in our databases for most genera, and is the biggest impediment to this project. Mycologists could contact BRCs to get assistance to generate DNA barcodes of potential neo- or epitype material. The publication strategy followed will depend on the authors, but deposit of data in GoF will be facilitated via MycoBank (there is thus no duplication of work, as these databases are linked). Authors interested in contributing accounts of individual genera as part of larger multi-authored papers published in IMA Fungus, should submit materials to the following associate editors: Pedro Crous (p.crous@cbs. knaw.nl) and David Hawksworth (d.hawksworth@nhm.ac.uk) for ascomycetes and basidiomycetes, Thorsten Lumbsch (tlumbsch@fieldmuseum.org) for lichenized fungi, Kerstin Voigt (Kerstin.Voigt@hki-jena.de) for zygomycetes, and Roger Shivas (Roger.Shivas@daff.qld.gov.au) for rusts and smuts.

# MATERIALS AND METHODS

#### Isolates

Several genera were re-described based on cultures obtained from the CBS-KNAW Fungal Biodiversity centre in Utrecht, The Netherlands (CBS-KNAW) and the working collection of P.W. Crous (CPC), housed at CBS. For fresh collections, leaves and twigs were placed in damp chambers, and incubated at room temperature for 1–2 d. Single conidial colonies were established from sporulating conidiomata in Petri dishes containing 2 % malt extract agar (MEA) as described earlier (Crous *et al.* 1991). Colonies were subcultured onto 2 % potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al.* 2009b), autoclaved pine needles on 2 % tap water agar (PNA) (Smith *et al.* 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains and specimens are maintained at the CBS.

# DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean<sup>™</sup> Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S nrRNA gene, the first internal transcribed spacer (ITS1), the 5.8S nrRNA gene, the second ITS region (ITS2) and approximately 900 bp of the 5' end of the 28S nrRNA gene. The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. Amplification conditions followed Cheewangkoon et al. (2008). The program SegMan v. 7.0.0 (DNASTAR, Madison, WI. USA) was used to obtain consensus sequences of each isolate. Blast searches using ITS and LSU sequences were performed for each isolate and the closest matches were retrieved and included in the phylogenetic analysis. The sequence alignment and subsequent phylogenetic analyses of the LSU data were carried out using methods described by Crous et al. (2006). Gaps were treated as "fifth state" data in the parsimony analysis. Sequence data were deposited in GenBank (Table 1) and the alignment and tree in TreeBASE (http://www.treebase.org). Remaining sequence data are discussed under the species notes below.

# Morphology

Slide preparations were mounted in clear lactic acid, lactophenol cotton blue or Shear's mounting fluid from colonies sporulating on MEA, PDA, PNA, or OA. Sections of conidiomata were made by hand for examination purposes. Observations were made with a Zeiss V20 Discovery stereomicroscope (Zeiss, Oberkochen, Germany), and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and Zen software. Additional photomicrographs were done using a Nikon Eclipse N*i*-U microscope (Nikon, Tokyo, Japan), a Nikon SMZ1500 stereo-microscope, Nikon DS-U3 digital camera and Nis Elements imaging software. Colony

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Species	Culture collection no <sup>1</sup>	Substrate	Location	Collector	GenBank Ac	GenBank Accession no <sup>2</sup>
					LSU	ITS
Bartalinia robillardoides	CBS 122705 (ex-epitype)	Leptoglossus occidentalis	Italy	I	KJ710438	KJ710460
Chaetospermum chaetosporum	CBS 154.59 (ex-neotype)	Submerged dead leaf of <i>Alnus</i> glutinosa	Switzerland: Ticino	A.L. van Beverwijk	KJ710439	KJ710461
	CBS 612.75	Leaf of <i>Cordia myxa</i>	Pakistan: Lahore	S. Ahmed	KJ710440	KJ710462
Coniella cf. fragariae	CBS 110394	Soil of rain forest	Peru: Iquitos	M. Christensen	KJ710441	KJ710463
Coniella fragariae	CBS 167.84 = CPC 3934	Vitis vinifera	Germany: Geisenheim	A. von Tiedemann	EU754149	AY339318
	CBS 172.49 = CPC 3930 (ex-neotype)	Stem base of <i>Fragaria</i>	Belgium: Lint	A. Jaarsveld	AY 339282	AY339317
	CBS 183.52	Tamarix	I	S. de Boer	KJ710442	KJ710464
	CBS 198.82	Soil	France: Chancay	G. Bollen	EU754150	KJ710465
Crinitospora pulchra	CBS 138014 = CPC 22807 (ex-epitype)	Branches of Mangifera indica	Thailand	T. Trakunyingcharoen	KJ710443	KJ710466
Eleutheromyces sp.	CBS 458.88	Lactarius scrobiculatus	Germany	W. Helfer	EU754162	KJ710467
	CBS 139.90 = UAMH 5529	Decaying <i>Russulaceae</i>	Canada: Alberta	L. Sigler	EU754161	KJ710471
Eleutheromyces subulatus	CBS 113.86 (ex-epitype)	Decaying agaric	Sweden	K.A. Seifert	KJ710444	KJ710468
	CBS 126.75	Agaric, blackened and mummified	France: Massif des Cèdres	H.A. van der Aa	KJ710445	KJ710469
	CBS 127.75	Agaric, blackened and mummified	France: Revest de Bion	H.A. van der Aa	KJ710446	KJ710470
	CBS 781.83	Trametes zonata	United Kingdom: Scotland	W. Gams	KJ710447	KJ710472
Kellermania yuccigena	CBS 131727 = AR 3470	dead leaves of <i>Yucca filamentosa?</i>	USA: New Mexico	A.W. Ramaley	KJ710450	KJ710475
	CBS 138015 = CPC 20627 (ex-epitype)	Leaves of <i>Yucca rostrata</i>	USA: California	P.W. Crous	KJ710449	KJ710474
	CPC 20623	Leaves of <i>Yucca rostrata</i>	USA: California	P.W. Crous	KJ710448	KJ710473
Mastigosporium album	CBS 138013 = CPC 22945 (ex-epitype)	Alopecurus pratensis	Netherlands: Utrecht	U. Damm	KJ710451	KJ710476
	CPC 22946	Alopecurus pratensis	Netherlands: Utrecht	U. Damm	KJ710452	KJ710477
Mycotribulus cf. mirabilis	CBS 133172 = CPC 20836	Eucalyptus pellita x brassiana	Indonesia	M.J. Wingfield	KJ710458	KJ710483
Mycotribulus mirabilis	CBS 138016 = CPC 14167 (ex-epitype)	Leaves of Eucalyptus urophylla	China	Cheng Mai & Xudong Zhao	KJ710456	KJ710481
	CPC 13390	Leaves of Eucalyptus camaldulgensis	Venezuela	M.J. Wingfield	KJ710453	KJ710478
	CPC 13391	Leaves of Eucalyptus camaldulgensis	Venezuela	M.J. Wingfield	KJ710454	KJ710479
	CPC 13392	Leaves of Eucalyptus camaldulgensis	Venezuela	M.J. Wingfield	KJ710455	KJ710480
	CPC 14168	Leaves of Eucalyptus urophylla	China	Cheng Mai & Xudong Zhao	KJ710457	KJ710482

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characters and pigment production were noted after 1 mo of growth on MEA and OA (Crous *et al.* 2009b) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

# RESULTS

### Phylogeny

For the type species treated here, amplicons of approximately 1 700 bases were obtained of the partial 18S rRNA, full length ITS and partial 28S rRNA (LSU) genes of the isolates listed in Table 1. The LSU alignment was used to resolve the generic placement of strains (Fig. 1) and the ITS (not shown) for species identification. The manually adjusted LSU alignment contained 86 sequences (including the outgroup sequence) and 798 characters including alignment gaps were used in the phylogenetic analysis; 349 of these were parsimony informative, 42 were variable and parsimony-uninformative, and 407 were constant. The parsimony analysis yielded the maximum setting of 1000 equally most parsimonious trees (TL = 1290 steps; CI = 0.493; RI = 0.928; RC = 0.458), which allowed the genera treated here to be assigned to at least order level (Fig. 1; discussed below in the Taxonomy section). Neighbour-joining analyses using three substitution models on the sequence alignment yielded tree topologies delimiting similar terminal clades to those of the parsimony analysis (data not shown), but with some rearrangements at the deeper nodes.

# THE GENERA

#### Bartalinia Tassi, Bull. Lab. Ort. Bot. Siena 3: 3 (1900).

*Synonymy*: Unconfirmed generic synonyms include *Hyalotia* Guba 1961 (Nag Raj *et al.* 1975) and *Amphiciliella* Höhn. 1919 (Nag Raj & DiCosmo 1984).

Current generic circumscription: Conidiomata stromatic, varying from pycnidioid to indeterminate, subperidermal, intracortical or subepidermal in origin, immersed, uni- to plurilocular, locules occasionally convoluted, dark brown to brown, glabrous, wall of textura angularis or textura globulosa, sometimes of textura prismatica, cells thick-walled and dark brown to brown in the outer layers, becoming thin-walled and paler toward the conidial hymenium. Conidiophores arising from the inner layers lining the conidioma, or at the base and extending part way up the side walls, sparsely septate and irregularly branched, often reduced to conidiogenous cells, hyaline, thin-walled, smooth, with percurrent proliferations, and apical periclinal thickenings (collarettes and regeneration of conidiogenous cells absent). Conidia cylindrical to fusiform with an acute or blunt apex and a truncate base, straight or slightly curved, 3-4-euseptate, apical cell hyaline and devoid of contents, other cells hyaline to pale brown, wall smooth, with or without constrictions at septa, suprabasal cell longer than the rest, apical appendage single, arising as a tubular extension of the apical cell and not separated from it by a septum, invariably trifid with 2-4, narrow, attenuated,

flexuous, divergent branches; basal appendage tubular, single, unbranched, exogenous, filiform, flexuous.

Type species: Bartalinia robillardoides Tassi 1900.

# Bartalinia robillardoides Tassi, Bull. Lab. Orto Bot. Siena 3: 3 (1900).

Synonym: Seimatosporium robillardoides (Tassi) Arx, Gen. Fungi Spor. Cult., 3<sup>rd</sup> edn: 224 (1981).

(Fig. 2)

Foliicolous. Conidiomata stromatic, pycnidioid to indeterminate or variable, amphigenous, scattered to gregarious, subepidermal, initially immersed, becoming erumpent, globose or depressed globose to angular, 180-240 µm diam, 80-200 µm high, unilocular, glabrous brown to black, lacking an ostiole; wall to 40 µm thick, of textura angularis, cells thick-walled and brown in the outer layers, becoming thin-walled and paler toward the conidial hymenium. Conidiophores arising all around the cavity of the conidioma from the innermost wall layer, reduced to conidiogenous cells, invested in mucus. Conidiogenous cells ampulliform, hyaline, thin-walled, smooth, 4-8 × 3-4.5 µm. Conidia subcylindrical, 4-septate, smooth, slightly constricted at the septa,  $(19-)21-24(-27) \times 3-4 \mu m$ , bearing appendages; basal cell obconic with a truncate base, hyaline; apical cell conical, hyaline, devoid of contents, forming a tubular, branched appendage; apical appendage branches into three unbranched, attenuated, flexuous, divergent branches, (15–)16–20(–22) µm long; basal appendage single, unbranched, filiform, flexuous, excentric, 4-7 µm long.

*Culture characteristics*: Colonies covering the dish in 2 wk at 25 °C, flat, spreading, with moderate aerial mycelium, and even, lobate margins. On PDA surface greyish at centre and olivaceous black toward the periphery; reverse olivaceous black. On MEA attaining 60 mm diam after 1 mo, surface dirty white to honey, with patches of pale olivaceous grey, reverse olivaceous grey with patches of dirty white. On OA surface umber with patches of pale olivaceous grey and buff, reverse dark brown at centre and sepia toward the periphery.

Specimens examined: Italy: Siena, Botanical Gardens, on *Callistemon speciosum*, Jan. 1900 (SIENA – holotype in Siena lost in transit; IMI 93089 (slide ex-holotype) – lectotype designated here, MBT178268). – Italy: on *Leptoglossus occidentalis*, collector unknown (CBS H-21728 – epitype designated here, MBT178279; culture ex-epitype CBS 122705). – The Netherlands: Raalte, on *Poa* sp., 2013, *W. Quaedvlieg* (CBS 136768).

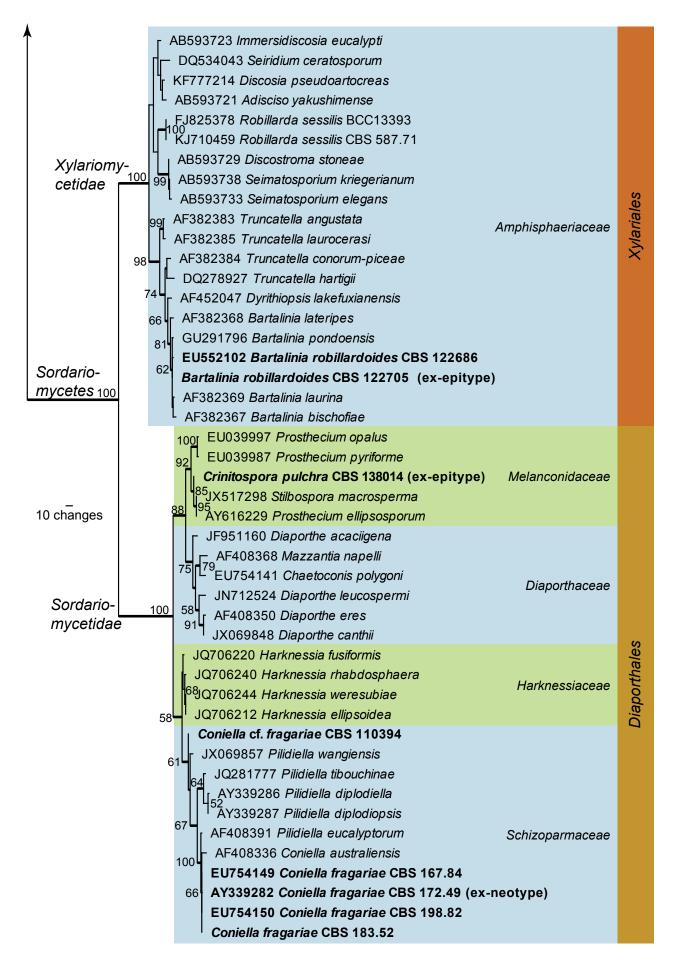
Notes: The genus Bartalinia (Amphisphaeriaceae, Xylariales), has no known sexual morph, and presently contains around 22 names representing about 18 taxa, six of which were treated by Nag Raj (1993). Although von Arx (1981) regarded Bartalinia as synonym of Seimatosporium, this was not accepted by Nag Raj (1993) because of differences in their conidial appendages. Both are now recognised as genera in their own right (Tanaka *et al.* 2011). Nag Raj (1979) and Sutton (1993) transferred Bartalinia nolinae and B. themedae to the genera Libartania and Kellermania, respectively. Later Nag Raj (1993) included in Bartalinia some species of



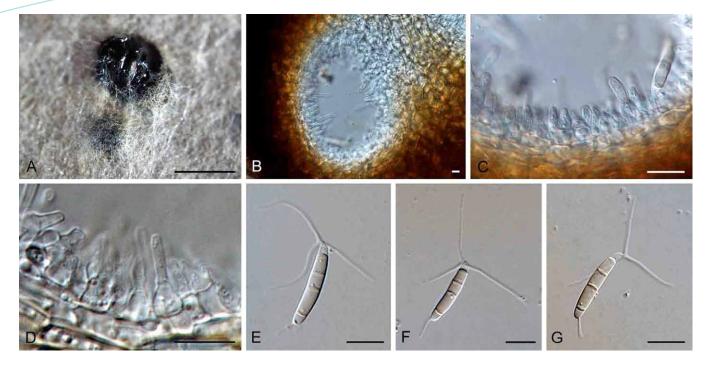
Z73326 Saccharomyces cerevisiae EF589735 Chaetospermum artocarpi Incertae sedis Sebacinales DQ520103 Craterocolla cerasi Sebacinaceae 100 JQ794488 Chaetospermum camelliae Incertae sedis Basidiomycota: Chaetospermum chaetosporum CBS 154.59 (ex-neotype) a. Agaricomycotina 100 Chaetospermum chaetosporum CBS 612.75 JF297608 Rhizomarasmius epidryas HM005091 Strobilurus conigenoides 100 AY639883 Flammulina velutipes Physalacriaceae KC179743 Flammulina rossica KC179750 Rhodotus palmatus Agaricales JN585132 Marasmius thwaitesii Marasmiaceae EF589740 Mycotribulus cf. mirabilis 100 84 Mycotribulus cf. mirabilis CPC 20836 Mycotribulus mirabilis CPC 13390 100 Mycotribulus mirabilis CPC 13391 Incertae sedis Mycotribulus mirabilis CPC 13392 94 L Mycotribulus mirabilis CBS 138016 (ex-epitype) Mycotribulus mirabilis CPC 14168 EU754162 Eleutheromyces sp. CBS 458.88 Incertae 100 GU727556 "Mollisia incrustata" sedis Ascomycota: Pezizomycotina EU754161 Eleutheromyces sp. CBS 139.90 76 Incertae sedis Eleutheromyces subulatus CBS 113.86 (ex-epitype) Eleutheromyces subulatus CBS 126.75 Eleutheromyces subulatus CBS 781.83 76 Eleutheromyces subulatus CBS 127.75 KC005811 Collembolispora aristata Helotiales Leotio- 94 mycetes KC005812 Collembolispora barbata 100 Mastigosporium album CBS 138013 (ex-epitype) 94 Mastigosporium album CPC 22946 porales EU754203 Pseudorobillarda phragmatis 99 Pleo-95 FJ825375 Pseudorobillarda siamensis Incertae sedis 99 Dothideo-- FJ825376 Pseudorobillarda sojae 52 mycetes KC315869 Cryomyces minteri Incertae sedis DQ377882 Saccharata proteae Saccharataceae 79 JX681071 Aplosporella prunicola 99 Aplosporellaceae KF766365 Melanops tulasnei Melanopsaceae Botryosphaeriales 61 KF766319 Botryosphaeria dothidea Botryosphaeriaceae 100L JX646808 Botryosphaeria agaves 67 KF766354 Kellermania uniseptata KF766343 Kellermania anomala 100 Kellermania yuccigena CBS 131727 Planistromellaceae Kellermania yuccigena CBS 138015 (ex-epitype) 67 Kellermania yuccigena CPC 20623 10 changes KF766355 Kellermania yuccifoliorum

**Fig. 1.** The first of 1000 equally most parsimonious trees resulting from a parsimony analysis of the LSU sequence alignment. The bootstrap support values are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Orders are indicated in coloured blocks and species names in black text. GenBank accession numbers for downloaded sequences are shown before species names and culture collection numbers after species names. The tree was rooted to *Saccharomyces cerevisiae* (GenBank Z73326).

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**Fig. 2.** *Bartalinia robillardoides* (CBS 122705). **A.** Colony sporulating on PDA. **B–D.** Section through conidiomatal wall, showing conidiogenous cells. **E–G.** Conidia. Bars: A = 250 μm, all others = 10 μm.

Pestalotia and Hyalotia, accepting six species in Bartalinia and questioned the status of four of them i.e. B. bella, B. terricola, B. begoniae and B. bombacicola. More recently, six new species have been added to the genus. Andrianova & Minter (2007) introduced the new species B. goniolimonis from leaf spots of Goniolimon speciosum and provided a taxonomic key to the genus. The most recent species is B. pondoensis, isolated from leaves of Maytenus abbottii in South Africa; which is similar on ITS to B. laurina, but morphologically different (Marincowitz et al. 2010). In spite of Bartalinia being a relatively unknown genus, cultures of B. robillardoides were reported to produce the anticancer drug taxol (Gangadevi & Muthumary 2008).

There are currently four ITS sequences listed as "Bartalinia robillardoides" in the NCBI GenBank nucleotide database. Of these, one (GenBank EU552102 derived from CBS 122686; from Leucadendron sp., South Africa) differs with 1 nucleotide from our ex-epitype strain, and the second (GenBank KF656706 derived from TCM-50; host and country not clearly specified) has some mismatches at the beginning and end of the sequences that could be the result of sequence annotation. The remaining two sequences (GenBank HM802301 derived from SKJM1096; host and country not specified; and GenBank AF405301 derived from BRIP 14180; from Macrotyloma daltonii, Australia) are dissimilar to our sequence and a megablast analysis using these sequences reveal that they are most likely Bartalinia pondoensis, a species described in 2010 and of which the sequence was released after the release date of these two GenBank sequences. Only two LSU sequences are currently present in GenBank; the first (GenBank EU552102 derived from CBS 122686; from Leucadendron sp., South Africa) differs in two nucleotides from our ex-epitype strain, and the second (GenBank AF382366 derived from BRIP 14180; from

*Macrotyloma daltonii*, Australia) also differs in two nucleotides from our sequence. Although GenBank AF382366 is from the same strain that could be *Bartalinia pondoensis*, a blast search only confirmed the affiliation of the sequence to the genus but not to the species.

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Chaetospermum Sacc., Syll. Fung. 10: 706 (1892). Synonym: Efibulobasidium K. Wells, Mycologia 67: 148 (1975).

*Synonymy*: Unconfirmed generic synonyms include *Entomopatella* Petr. 1934, *Ciliospora* Zimm. 1902 and *Chaetospermella* Naumov 1929 (Sutton 1977).

Current generic circumscription: Conidiomata stromatic, pycnidial, innate-erumpent, initially closed, ultimately opening by an irregular split in the apical wall, gelatinous, off white or pearl white when moist, unilocular, with the locule occasionally irregularly divided or convulated, glabrous; wall heavily gelatinised, of textura intricata to textura oblita. Conidiophores lining the base and part way up the side walls and arising from the innermost elements of the wall, loosely aggregated, sparingly branched and septate at the base, hyaline, smooth, invested in mucus. Conidiogenous cells discrete, cylindrical to subcylindrical or irregular, hyaline, smooth, bearing a single terminal conidium or an apical cluster of up to four conidia; conidiogenous cell with several percurrent annellations with periclinal thickening, but collarettes absent. Conidia broadly ellipsoidal to cylindrical with obtuse ends, unicellular, hyaline, smooth; appendages tubular, not separated from the conidium body by septa, polar

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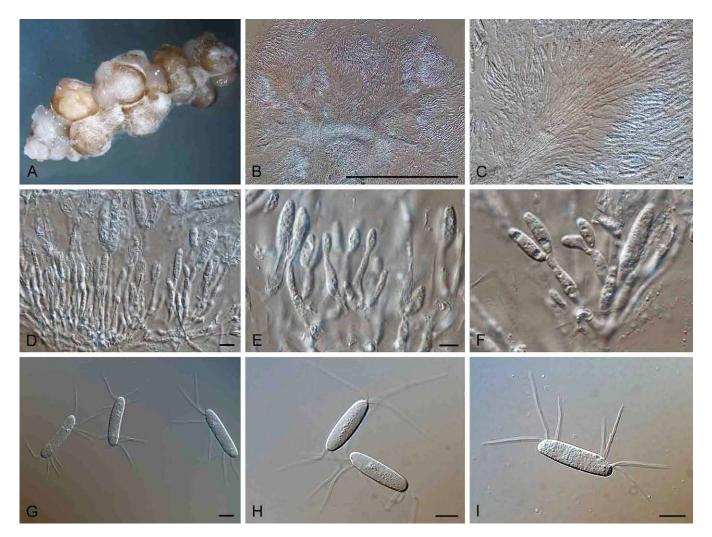


Fig. 3. Chaetospermum chaetosporum (CBS 154.59). A. Colony sporulating on SNA. B–F. Sections through conidiomata, showing conidiogenous cells. G–I. Conidia. Bars: B = 500 µm, all others = 10 µm.

or subpolar, occasionally lateral as well, unbranched, filiform or narrow and attenuated, flexuous, often collapsing and ribbon-like with age.

*Type species: Chaetospermum chaetosporum* (Pat.) A.L. Sm. & Ramsb. 1914.

# Chaetospermum chaetosporum (Pat.) A.L. Sm. & Ramsb., *Trans. Brit. mycol. Soc.* **4**: 328 (1914).

Basionym: Tubercularia chaetospora Pat., Bull. Soc. Mycol. Fr. 4: 39 (1888).

Synonym: Chaetospermum chaetosporum (Pat.) Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien. 1: 87 (1924).

(Fig. 3)

Caulicolous or foliicolous. *Conidiomata* stromatic, pycnidioid, scattered to gregarious and confluent, subepidermal or subperidermal in origin, innate erumpent, globose to subglobose or hemispherical in sectional view, 400–500 µm diam, closed but dehiscing by an irregular split in the apical wall, pearl white and gelatinous when moist, yellowish brown and waxy when dry, glabrous; wall to 50 µm thick, of *textura intricata* to *textura oblita*. *Conidiophores* arising from the inner layer of the cavity, loosely aggregated, sparingly branched

and septate at the base, hyaline, smooth, invested in mucus. *Conidiogenous cells* discrete, cylindrical to subcylindrical or irregular, hyaline, smooth, bearing a single terminal conidium,  $(10-)12-21(-27) \times 2-4 \mu m$ , without holoblastic-sympodial proliferations. *Conidia* broadly ellipsoidal to cylindrical with obtuse ends, hyaline, smooth,  $(24-)28-34(-36) \times (5-)6-9(-10) \mu m$ ; appendages 5–10 at each end, tubular, not separated from the conidium body by septa, circumpolar to subpolar, unbranched, filiform, flexuous, often collapsing and ribbon-like with age,  $(20-)28-43(-53) \mu m \log$ .

*Culture characteristics*: Colonies spreading, flat, covering the dish in 2 wk at 25 °C, with sparse aerial mycelium, and even, smooth margins. On MEA and PDA surface dirty white with pale vinaceous pycnidia. On OA surface whitish or pale luteus covered with rosy buff pycnidia; reverse pale luteus.

Specimens examined: France: Lons-ie-Saulnier (Jura), on roots of *Poaceae*, 1888?, holotype presumed lost. – Switzerland: Ticino, affluent to Lago di Origlio, from submerged dead leaf of *Alnus glutinosa*, July 1958, *A. L. van Beverwijk* (CBS H-10131, – neotype designated here, MBT178269; culture ex-neotype CBS 154.59). – Pakistan: Lahore, from leaf of *Cordia myxa*, *S. Ahmed* (CBS H-10132, culture CBS 612.75).

Notes: Chaetospermum was introduced by Saccardo (1892) to accommodate Tubercularia chaetospora, a species described previously by Patouillard (1888) from decaying grass, using the name C. tubercularioides, which was changed to C. chaetosporum by Smith & Ramsbottom (1914) following the International rules of nomenclature, which is currently used for the type species of the genus. Pestalozziella ambigua from stems of Artemisa was described by von Höhnel (1907), with similar conidia to those of Tubercularia chaetospora. Later the same author considered both species as co-generic (von Höhnel 1924). Nag Raj (1993) reviewed the genus considering C. gelatinosum a synonym of Mastigonema gelatinosum and C. carneum a nomem dubium. Rajeshkumar et al. (2010) proposed the new species Chaetospermum setosum, isolated from leaves of Mangifera indica in India, and considered C. indicum as a synonym of C. chaetospermum. They also provided a taxonomy key for the genus. The genus Chaetospermum (incertae sedis, Sebacinales) presently contains eight species. Other than Chaetospermum chaetosporum frequently being isolated from leaf litter of diverse substrate, not much is known of this species, or the genus Chaetospermum. A conidiomatal developmental study of C. chaetospermum was published by Fonseka (1960), while Sutton (1977) treated several generic synonyms, and Nag Raj (1993) provided a key to four species.

The phylogeny of Chaetospermum is poorly known. Rungjindamai et al. (2008) based on LSU and SSU sequences suggested that Chaetospermum could be located in basidiomycetes, since two species of the genus, C. camelliae and C. artocarpi, were phylogenetically related with members of Sebacinaceae (Sebacinales, Agaricomycetes). Unfortunately, the type species of Chaetospermum was not included in that study. Our study revealed that the type species of the genus is a member of Sebacinales, which agrees with Rungjindamai et al. (2008), Wells & Bandoni (2001) and Kirschner & Oberwinkler (2009). Further evidences are the presence of a Chaetospermum morph in cultures of the basidiomycete Efibulobasidium albescens (Wells & Bandoni 2001), and of conidia of Chaetospermum gossypinum together with basidiospores of Efibulobasidium albescens in the same specimens (Kirschner & Oberwinkler 2009). Additionally, they observed morphological characteristics typical of Sebacinales such as dolipore septa with continuous parenthesomes in specimens of C. chaetosporum.

The ITS sequence data of *E. albescens* (type species of the genus *Efibulobasidium*, AF384860) shows 98.9 % similarity with CBS 154.59 (neotype of *C. chaetosporum*), suggesting that they are congeneric, and that *Chaetospermum* (1892) should have preference over *Efibulobasidium* (1975) (Wells 1975).

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**Coniella** Höhn., *Ber. dt. bot. Ges.* **36**: 316 (1918). *Synomym: Cyclodomella* Mathur *et al., Sydowia* **13**: 144 (1959).

*Current generic circumscription: Conidiomata* pycnidial, immersed to semi-immersed, unilocular, glabrous, ostiolate,

brown to dark brown or black wall of thin, pale brown textura angularis on the exterior, and hyaline, thin-walled, textura prismatica in the inner layers except at the base which has a convex, pulvinate tissue of hyaline textura angularis giving rise to conidiophores or conidiogenous cells; ostiole central, circular or oval, often situated in a conical or rostrate neck. Conidiophores mostly reduced to conidiogenous cells, occasionally septate and branched at the base, invested in mucus. Conidiogenous cells discrete, cylindrical, subcylindrical, obclavate or lageniform, hyaline, smoothwalled, proliferating percurrently, or with visible periclinal thickening. Conidia ellipsoid, globose, napiform, fusiform or naviculate with a truncate base and an obtuse to apiculate apex, unicellular, thin- or thick-walled, smooth, olivaceous brown to brown, sometimes with a longitudinal germ-slit, with or without a mucoid appendage extending from the apex to base on one side of the conidium. Spermatophores formed in same conidioma, hyaline, smooth, 1-septate with several apical conidiogenous cells, or reduced to conidiogenous cells. Spermatogenous cells cells hyaline, smooth, lageniform to subcylindrical, with visible apical periclinal thickening. Spermatia hyaline, smooth, red-shaped with rounded ends.

*Type species: Coniella fragariae* (Oudem.) B. Sutton 1977 (syn. *Coniella pulchella* Höhn. 1918).

Coniella fragariae (Oudem.) B. Sutton, *Mycol. Pap.* **141**: 47 (1977).

Basionym: Coniothyrium fragariae Oudem., Versl. Meded. Ned. K. Akad. Wet., ser. 2, **18**: 37 (1883).

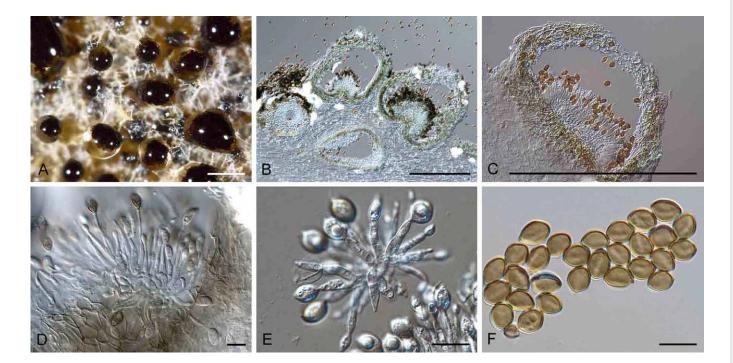
Synonyms: Clisosporium fragariae (Oudem.) Kuntze, Rev. Gen. Pl. 3: 458 (1898).

Coniella pulchella Höhn., Ber. dt. bot. Ges. 36: 316 (1918).

Cyclodomella nigra P.N. Mathur et al., Sydowia 13: 145 (1959).

(Fig. 4)

Conidiomata pycnidial, globose to depressed, 250-500 µm wide, initially appearing hyaline with a dark brown, internal conidial mass, becoming brown with age; ostiole central, 10-50 µm wide; wall 20–30 µm thick, consisting of 3–6 layers of pale to medium brown textura angularis; conidiomata containing a basal, central cushion of hyaline cells that give rise to hyaline conidiophores. Conidiophores densely aggregated, slender, subulate, simple, frequently branched above, reduced to conidiogenous cells, or with 1-2 supporting cells,  $15-30 \times (2-)$ 3-4 µm. Conidiogenous cells simple, tapering, hyaline, smooth, (12–)14–18(–20) × 3–4 µm, 1–1.5 µm wide at apex, surrounded by a gelatinous coating, apex with visible periclinal thickening, rarely with percurrent proliferation. Conidia ellipsoid, apices tapering, subobtusely rounded, tapering from middle towards a narrowly truncate base, medium brown, multi-guttulate when immature, becoming 1-2 guttulate when mature, wall darker brown than medium brown body of conidium, frequently with a lighter band of pigment extending over conidium, with a germ slit visible in older conidia, and mucous appendages also visible in lactic acid; appendages mostly basal, but also lateral along the length of the conidium, 7–12.5 × (4–)6–8(–10)  $\mu$ m. Spermatia also observed in some cultures, cylindrical, hyaline, straight with obtuse ends,  $4-5 \times 1-1.5 \mu m$ .



**Fig. 4.** *Coniella fragariae* (CBS 172.49). **A.** Colony sporulating on PDA. **B–C.** Sections through conidiomata. **D–E.** Conidiogenous cells. **F.** Conidia. Bars: A–B = 500 μm, all others = 10 μm.

*Culture characteristics*: Colonies on MEA flat, white on the surface, and pale luteous in reverse, reaching 60 mm after 2 wk at 25 °C, with black conidiomata evenly distributed on surface on the plate, same on PDA and OA.

Specimens examined: Belgium: Lint near Antwerpen, from stem base of *Fragaria* sp., Apr. 1949, *A. Jaarsveld* (CBS H-10697, neotype designated here for *Coniothyrium fragariae*, MBT178270; culture ex-neotype CBS 172.49 = CPC 3930); from *Tamarix* sp., *S. de Boer* (CBS H-10936, culture CBS 183.52). – France: Chancay, S-W of Tours, from soil sample, 1981, *G. Bollen* (CBS H-10723, culture CBS 198.82). – Germany: Sachsen, Königstein, on leaves of *Paeonia officinalis*, Sept. 1916, *W. Krieger* (FH – holotype of *Coniella pulchella*; slide ex-type WINF(M) 2408); Geisenheim, from *Vitis vinifera*, 1983, *A. von Tiedemann* (CBS H-10699, culture CBS 167.84). – Peru: Iquitos, from soil of rain forest, *M. Christensen* (CBS 110394).

*Notes*: We have been unable to trace any original material of *Coniothyrium fragariae* (The Netherlands, on *Fragaria vesca, 1883*, C.A.J. Oudemans), and hence a neotype is designated here to fix the application of the name. The concept of *C. fragariae* has not changed since the first molecular phylogeny was published on the genus (van Niekerk *et al.* 2004).

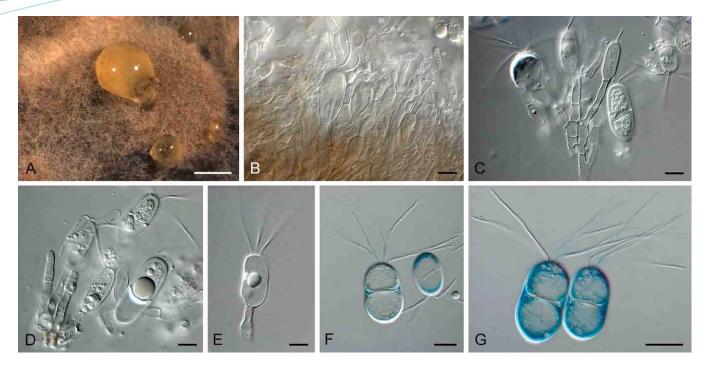
The genus *Coniella* currently includes about 30 species (*Schizoparmaceae, Diaporthales*), many of which are soilborne, and well-known as leaf, stem, and root pathogens of a diverse range of hosts such as *Fragaria* spp., *Ananas comosus, Pinus patula, Rosa* spp., *Pisum* spp. (Sutton 1980, van Niekerk *et al.* 2004, Miranda *et al.* 2012). The genus was proposed by von Höhnel (1918) with a single species, *C. pulchella,* described from *Paeonia officinalis*. Petrak & Sydow (1927) split *Coniella* into two subgenera: *Euconiella* 

and Pseudoconiella. The former included C. pulchella and C. diplodiella and the latter comprised C. granati (Sutton 1969). The genera Anthasthoopa and Cyclodomella were proposed for A. simba and C. nigra, respectively; the first species occurring on pods of Caesalpinia pulcherrima and the second one isolated from soil (Subramanian & Ramakrishnan 1956, Mathur & Thirumalachar 1959). Petrak (1960) did not agree with this proposal, and concluded that Cyclodomella nigra is a cultural variant of Coniella diplodiella. Later, Sutton (1969) considered both genera, Anthasthoopa and Cyclodomella, as synonymys of Coniella. Coniella pulchella was considered a synonym of C. fragariae by Sutton (1980), who as well as Nag Raj (1993), who treated the genus Pilidiella as a synonym of Coniella. However, van der Aa (in von Arx 1973) and von Arx (1981) treated Coniella and Pilidiella as separate genera, the former characterised by dark brown conidia and Pilidiella by hyaline conidia becoming pale brown with age. Molecular studies have confirmed the criteria of van der Aa (in von Arx 1973) and von Arx (1981) demonstrating that both genera are different. Pilidiella presently contains species with pigmented, as well as hyaline conidia, and Schizoparme sexual morphs, while Coniella comprises species with dark brown conidia. Rossman et al. (2007) introduced the family name Schizoparmaceae (Diaporthales) to accommodate these genera.

One strain that was previously identified as *Coniella fragariae* (CBS 110394), was revealed to belong to a different species based on the LSU sequence (Fig. 1).

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**Fig. 5.** *Crinitospora pulchra* (CBS 138014). **A.** Colony sporulating on MEA. **B–E.** Conidiogenous cells giving rise to conidia. **F–G.** Conidia. Bars: A = 300 μm, all others = 10 μm.

#### Crinitospora B. Sutton & Alcorn, *Trans. Brit. mycol.* Soc. 84: 439 (1985).

*Current generic circumscription: Conidiomata* stromatic, acervuloid, epidermal, immersed to semi-immersed, brown; basal stroma of *textura angularis* to *textura globulosa. Conidiophores* arising from the uppermost cells of basal and parietal tissue, unbranched, septate at only the base, hyaline, smooth, invested in mucus. *Conidiogenous cells* discrete or integrated, cylindrical to lageniform, hyaline, smooth-walled; proliferating several times percurrently at apex. *Conidia* ellipsoid with an obtuse apex and broad truncate base, euseptate, hyaline, thick-walled, smooth, with several appendages that are tubular, unbranched, filiform, flexuous, arising from the apex.

Type species: Crinitospora pulchra B. Sutton & Alcorn 1985.

Crinitospora pulchra B. Sutton & Alcorn, *Trans. Br.* mycol. Soc. 84: 439 (1985).

(Fig. 5)

Caulicolous. Conidiomata stromatic, acervuloid separate, immersed to erumpent, 200–300  $\mu$ m high, 300–500  $\mu$ m wide, brown, opening by irregular rupture with yellow conidial cirrhus, that turns brown with age; wall of several layers of pale brown *textura angularis* to *globulosa*. Conidiophores lining the inner cavity, hyaline, smooth, 1–2-septate, unbranched, subcylindrical, to 50  $\mu$ m long. Conidiogenous cells subcylindrical to lageniform, hyaline, smooth, 8–25 × 3–6  $\mu$ m. Conidia hyaline, smooth, guttulate, ellipsoid, with obtuse apex and broadly truncate base (3–5  $\mu$ m diam), medianly 1–septate, rarely 0–2-septate, (20–)30–35(–40) × (10–)15–17(–20)  $\mu$ m, with 4–10 apical appendages, tubular,

unbranched, filiform, divergent, flexuous, to 50  $\mu m$  long; conidia turn brown at germination in culture.

*Culture characteristics*: Colonies reaching 40 mm diam after 2 wk at 25 °C, flat, spreading, with sparse aerial mycelium, and lobate, feathery margins. On MEA surface olivaceous grey, with patches of buff, reverse reverse olivaceous grey in centre, dirty white in outer region. On OA surface olivaceous grey. On PDA surface dirty white, mostly with submerged mycelium, developing yellow concentric rings in older cultures.

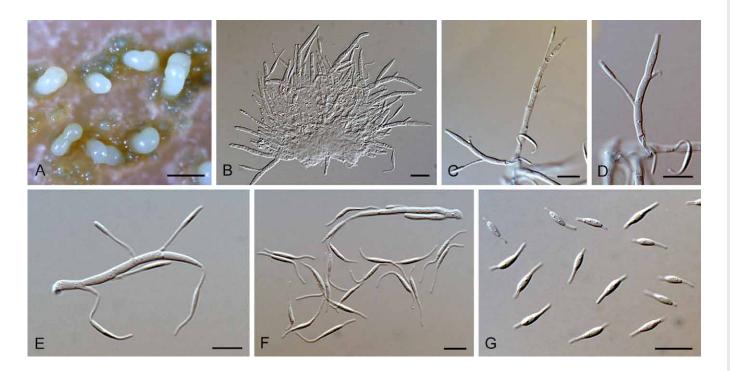
Specimen examined: Australia: Queensland, Bowen, on branches of Mangifera indica, 18 Dec. 1980, *I.F. Muirhead* (IMI 259110 – holotype). – Thailand: on branches of *M. indica*, 2012, *T. Trakunyingcharoen* (CBS H-21729 – epitype designated here, MBT178271; CPC 22807 = CBS 138014 – culture ex-epitype).

Notes: The genus *Crinitospora* (*Melanconidaceae, Diaporthales*) is monotypic and no sexual morph has thus far been linked to it. The fungus was initially collected from twigs of *Mangifera indica* in Australia (Sutton & Alcorn 1985); the collection from Thailand studied here, also on *M. indica*, represents the second report of this fungus. Although it is caulicolous on *M. indica*, not much is known about its ecology or pathology.

#### Authors: P.W. Crous and T. Trakunyingcharoen

# Eleutheromyces Fuckel, Jahrb. Nassau Ver. Naturk. 23–24: 183 (1870) ["1869"].

*Synonymy*: Unconfirmed generic synonyms include *Eleutheromycella* Höhn. 1908, and *Eleutheris* Clem. & Shear 1931 (Nag Raj 1993).



**Fig. 6.** *Eleutheromyces subulatus* (CBS 113.86). **A.** Colony sporulating on OA. **B–F.** Conidiophores giving rise to conidia. **G.** Smaller conidia of unidentified *Eleutheromyces* sp. (CBS 139.90). Bars: A = 250 μm, all others = 10 μm.

*Current generic circumscription: Conidiomata* pycnidial, conical to cornute, gelatinous, translucent yellowish or yellowish brown to dark brown, unilocular, glabrous, ostiolate, wall of *textura angularis;* ostiole central, circular. *Conidiophores* arising all around the cavity of the conidioma, cylindrical, branched mostly at the base, septate, hyaline, smooth, invested in mucus. *Conidiogenous cells* integrated with the conidiogenous loci immediately below the septa, hyaline, smooth-walled, with visible periclinal thickening at apex. *Conidia* aseptate, lenticular to fusiform, hyaline, smooth-walled; apical and basal appendages cellular, delimited from the conidium body by septa; basal appendage developing before the conidium body.

Type species: Eleutheromyces subulatus (Tode) Fuckel 1870.

- Eleutheromyces subulatus (Tode) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 183 (1870) ["1869"].
- Basionym: Sphaeronaema subulatum Tode, Fung. mecklenb. sel. 2: 44 (1790) : Fr., Syst. mycol. 2: 536 (1823). (Fig. 6)

Fungicolous. *Conidiomata* pycnidial, scattered to densely gregarious, seemingly superficial but innate erumpent, oval, long conical or cornute, 100–250  $\mu$ m diam, 150–500  $\mu$ m high, unilocular, glabrous, gelatinous, translucent, yellowish brown when dry, paler coloured when moist; wall up to 45  $\mu$ m thick, of *textura angularis*, cells thick-walled, pale brown to pale yellow; ostiole central, circular. *Conidiophores* lining the cavity of the conidioma, cylindrical, branched mostly at the base, septate, often variously curved, hyaline, smooth, to 60  $\mu$ m long, invested in mucus. *Conidiogenous cells* cylindrical, integrated, hyaline, smooth, 5.5–13 × 2.5–4  $\mu$ m. *Conidia* 

ellipsoidal or lenticular, aseptate, hyaline, 4.5–7 × 2–4  $\mu$ m (av. 6 × 2  $\mu$ m), one appendage at each end delimited by a septum; appendages tubular, attenuated; apical appendage 2–5  $\mu$ m long; basal appendage 1–3  $\mu$ m long.

*Culture characteristics*: Colonies flat, spreading, reaching 40 mm diam after 2 wk at 25 °C, with sparse aerial mycelium, and smooth margins. On MEA reaching 11–33 mm after 1 mo, surface and reverse dirty white to peach or coral with honey regions. On OA attaining 40–45 mm after 1 mo; surface buff, ochraceous or flesh, pycnidia with spores beige in mass.

Specimens examined: Canada: Alberta: Rocky-Clearwater Forest, from decaying *Russulaceae*, Sep. 1986, *L. Sigler* (culture CBS 139.90 = UAMH 5529). – France: Massif des Cèdres, MTG. du Lubéron, from agaric, blackened and mummified, Oct. 1974, *H.A. van der Aa* (CBS 126.75); Revest de Bion, Vaucluse, from agaric, blackened and mummified, Oct. 1974, *H.A. van der Aa* (CBS H-12348, H-12349, H-12350, H-12351and H-12352; culture CBS 127.75). – Sweden: on decaying agaric [Fries, Scleromyceti Sueciae no. 325] (UPS – lectotype designated here, MBT178272); near Lund, from decaying agaric, Sep. 1985, *K.A. Seifert* (CBS H-12355 – epitype designated here, MBT178280; CBS 113.86 = UAMH 5671 – culture ex-epitype). – UK: Argyllshire: Struan Wood, from *Trametes zonata*, Sept. 1983, *W. Gams* (culture CBS 781.83).

Notes: The genus Eleutheromyces (incertae sedis, Helotiales) presently contains two species that are fungicolous, growing on agarics. The genus has been reported from North America and Europe. A *Hyphozyma* synasexual morph was reported for *E. subulatus* by Sigler (1990), while Tsuneda *et al.* (1997) again linked this morph to black spot disease of *Lentinula edodes*. Two cultures listed in the CBS collection as *E. subulatus* (CBS 458.88 and CBS 139.90) were found to be

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phylogenetically and morphologically distinct (Figs 1 & 6, respectively) and represent different taxa. Highest similarity of the LSU sequences was found with "*Mollisia incrustata*" GenBank GU727556; however, this sequence does not appear to be congeneric with other *Mollisia* sequences on GenBank (data not shown) and thus the application of the supposed taxonomic lineage of *Mollisia* (*Leotiomycetes*; *Helotiales*; *Dermateaceae*) would not be confirmed here. Examination of the blast results also did not result in a clear affinity to any of the classes, with a more or less equal similarity to both *Leotiomycetes* and *Sordariomycetes* and therefore this genus is treated here as *incertae sedis*. It is quite possible that the genus belongs to a class that is currently not represented in the NCBI GenBank nucleotide database.

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Kellermania Ellis & Everh., J. Mycol. 1: 153 (1885).

- Synonyms: Piptarthron Mont. ex Höhn., Hedwigia: **60**: 203 (1918).
- Alpakesa Subram. & K. Ramakr., J. Indian Bot. Soc. 33: 204 (1954).

Planistroma A.W. Ramaley, Mycotaxon 42: 69 (1991). Planistromella A.W. Ramaley, Mycotaxon 47: 260 (1993).

Current generic circumscription: Conidiomata pycnidial, immersed, glabrous, ostiolate; wall thick, of textura angularis, cells thick-walled, dark brown to brown in the outer layers, and of *textura prismatica*, cells thin-walled, hyaline in the inners layers, with columnar, thin-walled, colourless cells surrounding the ostiole; ostiole circular or oval, nonpapillate. Conidiophores lining the cavity of the conidioma, reduced to conidiogenous cells, invested in mucus. Conidiogenous cells discrete, often of two kinds: those producing macroconidia, lining most of the conidiomatal cavity, cylindrical to subcylindrical, hyaline, smooth; those producing microconidia confined to the area of inner wall around the ostiole, ampulliform to broadly ampulliform, hyaline, smooth. Conidiogenesis ontogeny holoblastic by apical wall-building in the first conidium and by replacement wall-building in subsequent conidia; maturation by moderate diffuse wall- building synchronous with conidium ontogeny; delimitation by a transverse septum; secession schizolytic; proliferation usually absent, when present enteroblasticpercurrent to produce an additional conidium at a higher level; periclinal thickenings absent, but occasional annellation may be present; regeneration of conidiogenous cells absents. Macroconidia cylindrical to narrowly clavate with a truncate base, unicellular or euseptate, hyaline, thick-walled, smooth; appendages arising initially as tubular extensions of the conidium body, single, stout and unbranched, or up to six, attenuated, flexuous. Microconidia cylindrical to ellipsoidal or irregular with a rounded or blunt apex and a truncate base bearing minute marginal frills, unicellular, hyaline, smooth.

Type species: Kellermania yuccigena Ellis & Everh. 1885.

# Kellermania yuccigena Ellis & Everh., *J. Mycol.* **1**(12): 154 (1885); as '*yuccaegena*'.

Synonym: Planistromella uniseptata A.W. Ramaley, Mycotaxon **47**: 267 (1993).

(Fig. 7)

Foliicolous. Description based on colonies sporulating on OA. Conidiomata pycnidial, black, solitary, immersed, globose, unilocular, to 300 µm diam; wall of 8–10 cells of brown textura angularis; ostiole central, non-papillate, to 20 µm diam, exuding a hyaline conidial mass. Conidiophores lining the inner cavity, reduced to conidiogenous cells, hyaline, smooth, subcylindrical to ampulliform, (10-15  $(-25) \times 5-7(-25) \mu m$  (shorter on host material, to 12  $\mu m$ in length, and 6  $\mu m$  in width), proliferating percurrently at apex (much more prominent in culture), invested in mucus. Conidia hyaline, smooth, guttulate, cylindrical, 1-septate (submedian),  $(35-)40-50(-62) \times (9-)10-12(-14) \mu m$ (slightly wider on OA than on host tissue); apex giving rise to a simple setulate, unbranched appendage (but on OA at times bifurcate), 22-32 µm long; conidial base truncate, with a minute marginal frill, 1 µm long. Microconidia observed in culture, forming in same conidioma, hyaline, smooth, guttulate, subcylindrical, aseptate, apex obtuse, base truncate,  $6-20 \times 4-6 \mu m$ .

*Culture characteristics*: Colonies spreading, flat, reaching 50 mm diam after 2 wk at 25 °C, with moderate, fluffy aerial mycelium and feathery, lobate margins. On MEA surface dirty white, reverse buff. OA surface dirty white to cream; on PDA surface and reverse dirty white.

Specimens examined: **USA**: Kansas: Riley Co., Manhattan, on leaves of Yucca angustifolia, 5 June 1885, *W.A. Kellerman* 753 (NY – holotype of *K. yuccigena*; BPI 374463 – isotype; *New Mexico*: Chaves Co., mile 302.05 on US Highway 380, on dead leaves of Yucca filamentosa?, 24 Oct. 1993, *A.W. Ramaley* (AR 3470 = CBS 131727; isolated by Ramaley from AWR 9325, BPI 882828 –dried culture on PDA); *New Mexico*: Soccoro County, west side of US Highway 25, mile 105.4, on leaves of Yucca elata, 12 Apr. 1992, *A.W. Ramaley 9217* (UC 1475102 – holotype of *P. uniseptata*); *California*: Walnut Creek, Ruth Bancroft Garden, 1552 Bancroft Road, on leaves of *Yucca rostrata*, 20 Mar. 2012, *P.W. Crous* (CBS H-21730 – **epitype designated here** of *K. yuccigena*, MBT178281; cultures ex-epitype CPC 20627 = CBS 138015, CPC 20623).

Notes: The genus Kellermania (Planistromataceae, Botryosphaeriales; Slippers et al. 2013) presently includes around 40 species, many of which were recently included in phylogenetic studies (Minnis et al. 2012, Crous et al. 2013). Although Sutton (1980) retained Alpakesa, Kellermania, and Piptarthron as separate genera, Nag Raj (1993) reduced Alpakesa to synonymy under Kellermania. Minnis et al. (2012) published the first phylogenetic revision of the group, and reduced all these genera to synonymy under Kellermania, supporting the view of Crous et al. (2012) that conidial appendages as single characters have insufficient value to separate genera in coelomycetes, and should rather be seen as species-specific characters. Names formerly described in genera typified by sexual morphs (Planistroma,



**Fig. 7.** *Kellermania yuccigena* (A–D, I = CBS 131727, others = CBS 138015). **A.** Colony sporulating on OA. **B.** Section through conidioma showing conidiogenous cells. **C–F.** Conidiogenous cells giving rise to conidia. **G–J.** Conidia. Bars: A–B = 300 μm, all others = 10 μm.

*Planistromella*), were combined into *Kellermania* by Minnis *et al.* (2012).

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#### Mastigosporium Riess, Beitr. Mykol. 2: 56 (1852).

*Synonymy*: Unconfirmed generic synonyms include *Amastigosporium* Bond.-Mont. and *Amastigis* Clem. & Shear 1931 (Braun 1995).

*Current generic circumscription*: Graminicolous, causing leaf spots. *Colonies* amphigenous, whitish, subeffuse to dense. Mycelium internal, hyphae inter- and intracellular, hyaline, septate, sparsely branched, narrow. *Conidiophores* usually reduced to a single conidiogenous cell, solitary or loosely grouped, occasionally subfasciculate, arising from internal hyphae by the formation of a narrow penetration hypha which perforates the outer epidermal wall and cuticle and develops into a more or less cylindrical, colourless superficial conidiogenous cell. *Conidiogenesis* monoblastic, determinate to polyblastic, proliferation percurrent, inconspicuously annellated; conidial scars unthickened, not darkened, more

or less flat, truncate to somewhat convex. *Conidia* solitary, subcylindric, broadly ellipsoid-fusiform, euseptate, hyaline, smooth, without or with filiform appendages, hila unthickened, not darkened, but conidia sometimes with a small cingulum-like ring at the base; conidial secession schizolytic.

Sexual morph: unknown

Type species: Mastigosporium album Riess 1852.

Mastigosporium album Riess, *Beitr. Mykol.* **2**: 56 (1852).

(Fig. 8)

*Leaf spots* amphigenous, pale brown, subcircular, up to 5 mm diam, containing creamy sporodochia. *Mycelium* consisting of hyaline, smooth, thin-walled, branched, septate, 2–3  $\mu$ m diam hyphae. *Conidiophores* smooth, hyaline, subcylindrical, 1–3-septate, mostly unbranched, flexuous, arising from a brown stroma, 20–70 × 5–7  $\mu$ m. *Conidiogenous cells* terminal, integrated, subcylindrical, smooth, hyaline, proliferating sympodially and percurrently at apex, 15–25 × 5–7  $\mu$ m. *Conidia* solitary, obclavate to fusoid-ellipsoid, hyaline, guttulate, straight, 3–5 transversely euseptate, constricted at septa, hyaline but

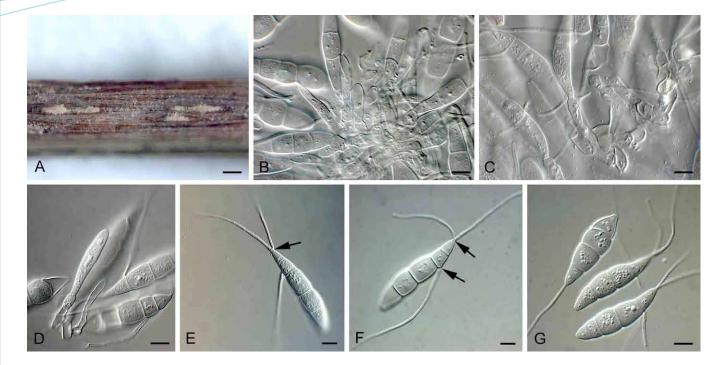


Fig. 8. *Mastigosporium album* (CPC 22946). A. Conidiomata forming on PNA. B–D. Conidiogenous cells giving rise to conidia. E–G. Conidia with lateral and apical appendages (arrows). Bars: A = 300 µm, all others = 10 µm.

appearing olivaceous with age, widest in second cell from base, hilum truncate,  $3-7 \mu m$  diam, with minute marginal frill, (48–)  $55-65(-70) \times (10-)12-15(-17) \mu m$ . Conidia containing several cellular appendages that are hyaline, smooth, subcylindrical, branched or not, septate. Apical appendage arising from terminal end,  $20-120 \times 2-3 \mu m$ , with 1–3 lateral branches, or branching dichotomously, flexuous, or apex giving rise to two appendages; apical appendage bluntly rounded, rarely with clavate apex. Lateral appendages (1–2) arising from apical cell or second or even third cell from apex,  $40-100 \mu m \log$ , 0-3-septate.

*Culture characteristics*: Colonies slow-growing, reaching 10 mm diam after 2 wk at 25 °C, erumpent, with sparse aerial mycelium, and uneven, lobate margins. On MEA and PDA surface dirty white to buff. On OA surface umber with ochreous, diffuse pigment.

Specimens examined: Germany: near Kassel, on Alopecurus pratensis, 1852, Riess [Klotzsch, Herb. Viv. mycol. no. 1758] (HAL – lectotype, designated in Braun 1995: 261). – The Netherlands: Utrecht, Rhijnauwen, on *A. pratensis*, May 2013, *U. Damm* (CBS H-21731 – epitype designated here, MBT178282; cultures exepitype CPC 22945 = CBS 138013, CPC 22946).

*Notes*: The genus *Mastigosporium* (*Helotiales*) has around 10 species and five varieties, and is known to have a high degree of host specialisation. Species of the genus are commonly associated with leaf spot diseases of *Poaceae*. The type species, *M. album*, is known from several grass species, occurring commonly in temperate regions, but also known from the Arctic. It is especially common on species of *Alopecurus* (Braun 1995).

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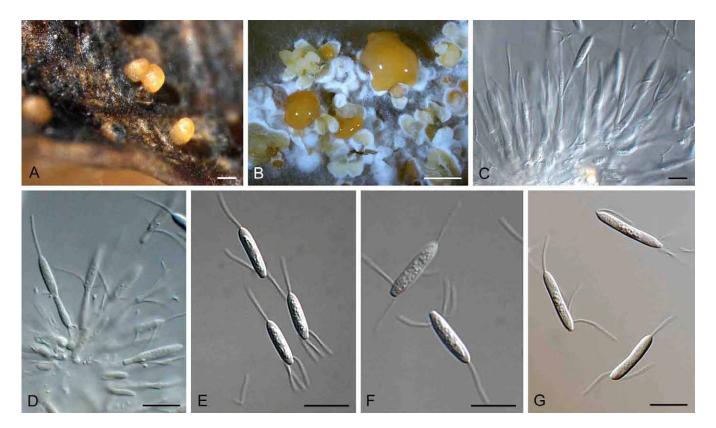
#### Mycotribulus Nag Raj & W.B. Kendr., *Canad. J. Bot.* 48: 2219 (1970).

Current generic circumscription: Conidiomata pycnidioid, immersed at first and then becoming partly erumpent, unilocular, glabrous, brown, lacking an ostiole but dehiscing by an irregular rupture in the apical wall and overlying host tissue; wall of textura angularis, cells thick-walled and brown in the outer layers, thin walled and colourless in the inner layers. Conidiophores intermingled with paraphyses, arising from the inner layer of cells of the wall all around the cavity of the conidioma, branched or unbranched and septate at the base, colourless, smooth, invested in mucus. Paraphyses filamentous, branched or unbranched, septate, colourless, smooth, narrow at the base, broad and deeply lobed or irregular at the apex. Conidiogenous cells discrete, subcylindrical to obclavate, colourless, smooth. Conidiogenesis holoblastic, maturation by diffuse wallbuilding synchronous with conidium ontogeny; delimitation by a transverse septum; secession schizolytic, annellations, periclinal thickenings and regeneration of conidiogenous cells absent. Conidia naviculate to fusiform with a truncate base and an acute apex, unicellular, colourless, guttulate; bearing appendages at both ends; appendages tubular, filiform, flexuous, unbranched, apical appendage single; basal appendages 2-4; inserted laterally, slightly above conidium base.

*Type species: Mycotribulus mirabilis* Nag Raj & W.B. Kendr. 1970.

**Mycotribulus mirabilis** Nag Raj & W.B. Kendr., *Canad. J. Bot.* **48**: 2219 (1970).

(Fig. 9)



**Fig. 9.** *Mycotribulus mirabilis* (CBS 138016). **A.** Conidiomata on *Eucalyptus* leaf *in vivo.* **B.** Conidiomata forming on PDA. **C–D.** Conidiogenous cells giving rise to conidia. **E–G.** Conidia. Bars: A = 300 μm, B = 250 μm, all others = 10 μm.

Associated with leaf litter of Eucalyptus spp. Conidiomata pycnidioid, separate, subepidermal, exuding a pale yellow conidial cirrus; subglobose, to 250 µm wide, and 300 µm high, unilocular, often irregularly lobed, opening by irregular rupture of apical wall, 20-30 µm thick, of brown textura angularis, becoming hyaline towards centrum. Paraphyses hyaline, smooth, branched or not, septate,  $15-100 \times 1-3 \mu m$ ; apex irregularly curved to lobed. Conidiophores 0-2-septate, unbranched or branched below, 10-20 × 3-4 µm, hyaline, smooth, subcylindrical. Conidiogenous cells subcylindrical, terminal and lateral, hyaline, smooth, 8-17 × 1.5-2.5 µm. Conidia naviculate to fusiform, tapering to acutely rounded apex, and truncate base, aseptate, smooth, guttulate, (9-)  $13-15(-18) \times (2.5-)3(-3.5) \mu m$ , bearing a single tubular, filiform, flexuous apical appendage, 7-12 µm long; basal appendages (2-5) lateral, slightly above truncate base, unbranched, divergent, straight to flexuous, 8–12 µm long.

*Culture characteristics*: Colonies spreading, flat, reaching 55 mm diam after 2 wk at 25 °C, with sparse aerial mycelium and feathery margins. On OA, MEA and PDA surface and reverse buff to dirty white.

Specimens examined: India: Karnataka State: Balehonnur, Coffee Research Station, on rotting leaves of *Eucalyptus* sp., 12 Nov. 1963, *T.R. Nag Raj* (K (M) IMI 128041 – holotype; DAOM 124817 – isotype). – Venezuela: Caracas: on leaves of *Eucalyptus camaldulgensis*, 4 Oct. 2006, *M.J. Wingfield* (cultures CPC 13390–13392). – China: *Guangdong*: on leaves of *Eucalyptus urophylla*, 18 Jun. 2007, *C. Mai* & *X. Zhao* (CBS H-21732 – epitype designated here, MBT178283; cultures ex-epitype CPC 14167 = CBS 138016, CPC 14168). Notes: The genus *Mycotribulus* is one of the few coelomycete genera confirmed within the *Basidiomycota* (*Physalacriaceae, Agaricales* according to Rungjindamai *et al.* 2008) and is presently monotypic. Isolates are commonly associated with *Eucalyptus*, but the species can also occur on other hosts such as *Apodytes abbottii, Mangifera indica* and *Syzygium cordatum* (Crous 1993, Marincowitz *et al.* 2010). The LSU sequence of two strains of *Mycotribulus* from *Eucalyptus pellita* × *brassiana* in Indonesia (this study) and *E. camaldulensis* in Thailand (BCC13341, GenBank accession EF589740, Rungjindamai *et al.* 2008), respectively, differed in their LSU sequence from *M. mirabilis* (Fig. 1) and might represent a second *Mycotribulus* species. Unfortunately no ITS sequence of BCC13341 was available for comparison.

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