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Redefining *Humicola sensu stricto* and related genera in the *Chaetomiaceae*

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Abstract: The traditional concept of the genus *Humicola* includes species that produce pigmented, thick-walled and single-celled spores laterally or terminally on hyphae or minimally differentiated conidiophores. More than 50 species have been described in the genus. Species commonly occur in soil, indoor environments, and compost habitats. The taxonomy of *Humicola* and morphologically similar genera is poorly understood in modern terms. Based on a four-locus phylogeny, the morphological concept of *Humicola* proved to be polyphyletic. The type of *Humicola*, *H. fuscoatra*, belongs to the *Chaetomiaceae*. In the *Chaetomiaceae*, species producing humicola-like thick-walled spores are distributed among four lineages: *Humicola sensu stricto*, *Mycothermus*, *Staphylotrichum,* and *Trichocladium.* In our revised concept of *Humicola*, asexual and sexually reproducing species both occur. The re-defined *Humicola* contains 24 species (seven new and thirteen new combinations), which are described and illustrated in this study. The species in this genus produce conidia that are lateral, intercalary or terminal on/in hyphae, and conidiophores are not formed or are minimally developed (micronematous). The ascospores of sexual *Humicola* species are limoniform to quadrangular in face view and bilaterally flattened with one apical germ pore. Seven species are accepted in *Staphylotrichum* (four new species, one new combination). Thick-walled conidia of *Staphylotrichum* species usually arise either from hyphae (micronematous) or from apically branched, seta-like conidiophores (macronematous). The sexual morph represented by *Staphylotrichum longicolleum* (= *Chaetomium longicolleum*) produces ascomata with long necks composed of a fused basal part of the terminal hairs, and ascospores that are broad limoniform to nearly globose, bilaterally flattened, with an apical germ pore. The *Trichocladium* lineage has a high morphological diversity in both asexual and sexual structures. Phylogenetic analysis revealed four subclades in this lineage. However, these subclades are genetically closely related, and no distinctive phenotypic characters are linked to any of them. Fourteen species are accepted in *Trichocladium,* including one new species, twelve new combinations. The type species of *Gilmaniella*, *G. humicola*, belongs to the polyphyletic family *Lasiosphaeriaceae* (*Sordariales*), but *G. macrospora* phylogenetically belongs to *Trichocladium.* The thermophilic genus *Mycothermus* and the type species *My. thermophilum* are validated, and one new *Mycothermus* species is described. Phylogenetic analyses show that *Remersonia*, another thermophilic genus, is sister to *Mycothermus* and two species are known, including one new species. *Thermomyces verrucosus* produces humicola-like conidia and is transferred to *Botryotrichum* based on phylogenetic affinities. This study is a first attempt to establish an inclusive modern classification of *Humicola* and humicola-like genera of the *Chaetomiaceae*. More research is needed to determine the phylogenetic relationships of "humicola"-like species outside the *Chaetomiaceae*.

Key words: *Humicola*, *Mycothermus*, Phylogeny, *Remersonia*, Sexual morphs, *Staphylotrichum*, *Trichocladium*, 43 Taxonomic novelties.

Taxonomic novelties: new genus: *Mycothermus* D.O. Natvig *et al.* ex X. Wei Wang, Houbraken & D. O. Natvig; **New species:** *Humicola atrobrunnea* X. Wei Wang, Houbraken, Y.L. Jiang & T.Y. Zhang, *Humicola christensenii* X. Wei Wang & Houbraken, *Humicola degenerans* X. Wei Wang & Houbraken, *Humicola leptodermospora* X. Wei Wang & Houbraken, *Humicola mutabilis* X. Wei Wang & Houbraken, *Humicola pulvericola* X. Wei Wang, Houbraken & Seifert, *Humicola quadrangulata* X. Wei Wang & Houbraken, *Mycothermus thermophiloides* X. Wei Wang & Houbraken, *Remersonia tenuis* X. Wei Wang, Houbraken & Seifert, *Staphylotrichum acaciicola* X. Wei Wang & Houbraken, *Staphylotrichum brevistipitatum* X. Wei Wang & Houbraken, *Staphylotrichum microascosporum* X. Wei Wang & Houbraken, *Staphylotrichum tortipilum* X. Wei Wang & Houbraken, *Trichocladium amorphum* X. Wei Wang & Houbraken; **New combinations:** *Botryotrichum verrucosum* (Pugh, Blakeman & Morgan-Jones) X. Wei Wang & Houbraken, *Humicola ampulliella* (X.Wei Wang) X. Wei Wang & Houbraken, *Humicola cuyabenoensis* (Decock & Hennebert) X. Wei Wang & Houbraken, *Humicola distorta* (L.M. Ames) X. Wei Wang & Houbraken, *Humicola floriformis* (Gené & Guarro) X. Wei Wang & Houbraken, *Humicola homopilata* (Omvik) X. Wei Wang & Houbraken, *Humicola malaysiensis* (D. Hawksw.) X. Wei Wang & Houbraken, *Humicola pinnata* (L.M. Ames) X. Wei Wang & Houbraken, *Humicola seminuda* (L.M. Ames) X. Wei Wang & Houbraken, *Humicola semispiralis* (Udagawa and Cain) X. Wei Wang & Houbraken, *Humicola sphaeralis* (Chivers) X. Wei Wang & Houbraken, *Humicola subspiralis* (Chivers) X. Wei Wang & Houbraken, *Humicola udagawae* (Sergejeva ex Udagawa) X. Wei Wang & Houbraken, *Humicola wallefii* (J.A. Mey. & Lanneau) X. Wei Wang & Houbraken, *Mycothermus thermophilus* (Cooney & R. Emers.) X. Wei Wang, Houbraken & D. O. Natvig, *Staphylotrichum longicolleum* (Krzemien. & Badura) X. Wei Wang & Houbraken, *Trichocladium acropullum* (X.Wei Wang) X. Wei Wang & Houbraken, *Trichocladium antarcticum* (Stchigel & Guarro) X. Wei Wang & Houbraken, *Trichocladium arxii* (Benny) X. Wei Wang & Houbraken, *Trichocladium crispatum* (Fuckel) X. Wei Wang & Houbraken, *Trichocladium griseum* (Traaen) X. Wei Wang & Houbraken, *Trichocladium heterothallicum* (Yu Zhang & L. Cai) X. Wei Wang & Houbraken, *Trichocladium jilongense* (Y.M. Wu & T.Y. Zhang) X. Wei Wang & Houbraken, *Trichocladium nigrospermum* (Schwein.) X. Wei Wang & Houbraken, *Trichocladium seminis-citrulli* (Sergeeva) X. Wei Wang & Houbraken, *Trichocladium uniseriatum* (Yu Zhang & L. Cai) X. Wei Wang & Houbraken; **Nomen novum:** *Trichocladium beniowskiae* (M.D. Mehrotra) X. Wei Wang & Houbraken, *Trichocladium gilmaniellae* (Moustafa) X. Wei Wang & Houbraken.

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INTRODUCTION

Identification and classification of morphologically little-differentiated hyphomycetes have always been challenging. Species producing chlamydospores or similar propagules offer few morphological characters to guide taxonomists. The traditional Saccardoan approach to delimiting genera by the pigmentation and septation of spores and the cells that produce them, and the further refinements offered by studies of conidium ontogeny (including conidial secession) and cultural characteristics, provide additional information. The occurrence of synanamorphs or sexual stages also offers clues.

Phylogenetic studies using DNA provide clarity about relationships and give clues as to what phenotypic characters might be phylogenetically informative, facilitating the integration of morphologically depauperate hyphomycetes among more differentiated, often teleomorph-based taxa. Based on SSU and ITS sequence data, Hambleton *et al.* (2005) attempted to correlate differences in the details of conidium development with phylogenetic relationships among species with chlamydospores or aleuriconidia. Their definitions for chlamydospores and aleurioconidia were based on those from the first Kananaskis conference (Kendrick 1971). Chlamydospores were defined as “thick-walled, thallic, terminal or intercalary spores” formed on vegetative mycelium which “are not dispersed and do not secede until the adjacent hyphal cells dissolve away”, and hence are functionally a kind of “resting spores”. Aleurioconidia were defined as solitary, holothallic or monoblastic conidia produced on somewhat differentiated conidiophores or from conidiogenous cells, which are propagules and can be dispersed. Two relatively large hyphomycete genera that are central to the question of the integration of such fungi into fungal taxonomy are *Humicola* and *Trichocladium.* The spores produced by the species of these genera challenge the definitions separating the terms conidium, aleurioconidium, and chlamydospore, in part because there are so many intermediate forms. In this paper, we use these terms fairly loosely, with conidium used for lateral or terminal propagules that seem likely to secede and be dispersed, and chlamydospores for intercalary spores that seem likely to remain in the substrate and not be dispersed, i.e., resting spores. In these fungi, the occurrence of spore release by rhexolytic secession, necessary for strict use of the term aleurioconidium, is inconsistent and can be difficult to observe; for that reason, we do not use this term in the descriptions in this paper.

The hyphomycete genus *Humicola* was introduced by Traaen (1914) for two species, *H. fuscoatra* (the type) and *H. grisea*. Both species produce thick-walled, pigmented and usually single-celled spores. These spores are formed laterally or terminally on hyphae or minimally differentiated conidiophores. Although, in earlier studies, they have been called chlamydospores (Traaen 1914, de Bertoldi 1976) or aleurioconidia (Hambleton *et al.* 2005), based on the definitions discussed above, we call these propagules conidia. This basic morphology, a round spore in a hypha or on a stalk, is about as simple a sporulating morphology can be. Up to now, more than 50 species were described in *Humicola* (e.g. Nicoli & Russo 1974, de Bertoldi 1976, Jiang *et al.* 2016, Wang *et al.* 2016a,b), but the genus has never been comprehensively revised, nor has a thorough phylogenetic analysis been undertaken.

Trichocladium (Harz 1871) is a phragmoconidial counterpart of *Humicola,* differentiated by the production of darkly pigmented, septate conidia. As reviewed by Goh & Hyde (1999), it includes 18 species that, despite their simple morphology, encompass a significant heterogeneity in conidial shape, pigmentation, and development. Hambleton *et al.* (2005) were surprised that the ITS sequences of *H. grisea* and the type species of *Trichocladium*, *T. asperum*, were identical, suggesting that the two species at least belong to the same genus. Some of the species of both genera also produce an inconspicuous acremonium-like synanamorph (Gams 1971), and the conidia may also have pale or thin-walled pore-like areas that might represent germ pores (Hambleton *et al.* 2005).

Both *Humicola* and *Trichocladium* have been associated with *Chaetomiaceae* (Seifert *et al.* 2011). As defined by its type species, *Humicola* is commonly considered an asexual genus in the *Chaetomiaceae* (Gams 1971, Kirk *et al.* 2008). However, there is evidence of a close relationship between asexual *Humicola* species and ascoma-producing species in the *Chaetomiaceae* (Zhang *et al.* 2017b). Several species within *Chaetomiaceae* produce humicola-like conidia together with ascomata and ascospores (von Arx *et al.* 1986, Decock & Hennebert 1997, Wang & Zheng 2005a,b). Among those, three species were classified in the genus *Farrowia*: *F. longicollea* (type species = *Chaetomium longicolleum*), *F. seminuda* (= *Chaetomium seminudum*) and *F. malaysiensis* (= *Chaetomium malaysiense*). *Farrowia* was characterized by ascomata with synchronously produced terminal hairs arising from elongated cells that fused to form a “distinct neck-like structure” below the perithecial apex (Hawksworth 1975). However, *Farrowia* was not accepted in subsequent monographs (Carter 1982, von Arx *et al.* 1986), although Untereiner *et al.* (2001) accepted the genus on the basis of the phylogenetic analysis of LSU sequences. In that study, the three *Farrowia* species clustered closely with *C. homopilatum*, *C. floriforme,* and *C. sphaerale*, which produce a humicola-like morph and ascomata lacking conspicuous necks. Detailed multi-locus molecular studies on the relationship and taxonomy of species producing chaetomium-like ascomata and/or humicola-like asexual structures are lacking.

Other species with similar thick-walled conidia, classified in various hyphomycete genera, have a similar ecology and may also be associated with *Chaetomiaceae*. A few genera with more developed conidiophores, but similar conidia and conidiogenesis to *Humicola,* are also relevant to the phylogenetic reevaluation of asexual members of *Chaetomiaceae* presented in this paper. *Botryotrichum,* with its setose, macronematous conidiophores bearing clusters of thick-walled conidia, has long been associated with *Chaetomium,* following the association of the type species *B. piluliferum* with its sexual state (Daniels 1961). Two other genera were not previously associated with *Chaetomium* sexual states but were revealed to partly belong to *Chaetomiaceae* by our phylogenetic studies. *Gilmaniella* was separated from *Humicola* and *Botryotrichum* by its branched conidiophores and the conidia with a conspicuous germ pore (Barron 1964). The conidiophores of *Staphylotrichum* species are also similar to those of *Botryotrichum* but do not terminate in setae (Nonaka *et al.* 2012).

Just because a species is morphologically simple, it does not mean it is biologically or economically unimportant. Most *Humicola* species are isolated from soil, while some are from compost, rotting plant materials, indoor environments or even fur of cats (Cooney & Emerson 1964, Tiscornia *et al.* 2009, Betancourt *et al.* 2013, Wang *et al.* 2016b). Some *Humicola*