



Conidiobolus stilbeus, a new species with mycelial strand and two types of primary conidiophores

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Abstract

A new entomogenous fungus, *Conidiobolus stilbeus* is described and illustrated from China. The new species differs from other *Conidiobolus* species by forming mycelial strands with 2–6 aerial phototropic hyphae, and by its two types of primary conidiophores: one is shorter and differentiated from aerial hyphae, the other is often longer and inflated and arises from substrate mycelia. Molecular phylogeny inferred from the nuclear large subunit ribosomal DNA supports *C. stilbeus* as a distinct species in the genus, most closely related to *C. lachnodes*, *C. sinensis*, *C. stromoideus* and *C. thromboides*.

Key words – aerial hyphae – *Conidiobolus* –28S rDNA

Introduction

The genus *Conidiobolus* was established by Brefeld in 1884 and includes saprobes, insect pathogens, and facultative human pathogens (Gryganskyi et al. 2012). These fungi, especially *C. coronatus* (Costantin) A. Batko 1964, are widely distributed throughout the world (Nie et al. 2012). In a modern classification system, this genus belongs to *Ancylistaceae*, *Entomophthorales*, *Entomophthoromycetes*, *Entomophthoromycota* (Humber 2012).

Based on the morphological and nutritional data mainly adopted in the studies by Drechsler (1952, 1953, 1955, 1960) and Srinivasan & Thirumalachar (1962, 1968), a total of 27 *Conidobolus* species were accepted by King (1976a, b, 1977). Since then, only six species have been added to the genus: *C. chlapowskii* (Bałazy et al. 1987), *C. iuxtagenitus* (Waters & Callaghan 1989), *C. gustafssonii* (Bałazy 1993), *C. margaritatus* (Huang et al. 2007), *C. thermophilus* (Waingankar et al. 2008) and *C. sinensis* (Nie et al. 2012).

Gryganskyi et al. (2012) suggested that the ancestral fungi of the class *Entomophthoromycetes* might belong to the taxa currently classified in *Conidiobolus*. Then, four loci (LSU, SSU, RPB2, and mtSSU) were used to reconstruct the molecular phylogeny for *Conidiobolus* and separated this genus into at least three groups (Gryganskyi et al 2013) which is consistent with a previous study based only on SSU rDNA data (Jensen et al. 1998). This molecular work supports the secondary conidial types' view of Ben-Ze'ev & Kenneth (1982). In the opinion of

Ben-Ze'ev and Kenneth, the genus *Conidiobolus* was divided into three subgenera: *Delacroixia*, *Capillidium* and *Conidiobolus*. Under this taxonomical scheme, the subgenus *Conidiobolus* includes 16 species.

Since 2008, a survey of *Conidiobolus* species from China has resulted in more than 300 strains, and one new species and four new records have been published (Wang et al. 2010a, b). Higher intra-specific similarity and inter-specific differences of LSU rDNA sequences were shown for *Conidiobolus* species (Nie et al. 2012). Therefore, a novel *Conidiobolus* species is proposed based on morphological features and molecular data of partial LSU rDNA sequences in this study.

Materials & Methods

Isolates and morphology

Soil samples were collected in Shandong Province of China in June 2011. A canopy plating approach similar to that by King (1976a) and Drechsler (1952) was used: The soil samples were placed in sterile polythene bags for transport to the lab. Then in lab, a Petri-dish containing potato dextrose agar (PDA, Difco) was inverted over the soil. The dishes were sealed with parafilm and incubated in dark at 21°C, and examined daily. A dissecting microscope was applied to observe the development of typical compact, glassy colonies of entomophthoralean fungi. When detected on the PDA canopy, discharged conidia were transferred to a new PDA plate for purification and then identified by morphological structures as described by King (1976a). The methods should be adequately detailed or referenced to other work.

DNA extraction, PCR and sequencing

The *Conidiobolus* strains used in this study are shown in Table 1. After incubation on PDA at 21°C for approximately seven days, genomic DNA was extracted with modified CTAB method (Watanabe et al. 2010) from the mycelia scraped from cellophane which were put on the top of the PDA canopy. The extracted DNA was stored in 100 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) at -20°C. The 28S rDNA region was amplified with the primers LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAACTTCG-3') (Vilgalys & Hester 1990).

The PCR reaction used in this study for 28S rDNA amplification has been described by Liu et al. (2005). A 50 µL of PCR reaction mixture contains 200 µM dNTPs each, 1 × Mg-free buffer, 2.5 mM MgCl₂, 0.5 µM primers each, 10-50 ng of genomic DNA and 2 units of Taq polymerase. The thermocycling protocol consists of an initial denaturation at 100°C for 5 min, followed by 95°C for 5 min (during this time, Taq polymerase was added to each tube), then 34 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 2 min, and a final extension step at 72°C for 10 min. The nucleotide sequencing of the PCR products was performed at Shanghai Genecore Biotechnologies Company (Shanghai, China). The generated sequence has been submitted to GenBank (Table 1).

Phylogeny reconstruction

Multiple sequence alignments for partial 28S rDNA were conducted using BioEdit (Hall 1999) and Clustal X (Thompson et al. 1997). This alignment was deposited to TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S19863>). Three entomogenous fungi (*Batkoa apiculata*, *Entomophthora muscae* and *Erynia conica*) were used as outgroups. Maximum parsimony (MP) was applied to the partial 28S rDNA dataset. PAUP* 4.0b10 (Swofford 2003) was used to perform the MP analysis. All characters were weighted, and gaps were treated as missing data. Branch swapping algorithm by tree bisection-reconnection (TBR) and MulTrees were used. Branch support was estimated by bootstrapping with 1,000 replicates (Felsenstein 1985). Bayesian inference of molecular data sets was conducted with an online version of MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). A general model of DNA substitution with gamma-distributed rate variation across sites (GTR+G) was adopted. Four simultaneous Markov chains (three cold, one

Table 1 Cultures and their corresponding GenBank numbers used in phylogenetic analyses*.

Species	Strain #	28S rDNA
<i>Conidiobolus adiaeretus</i> Drechsler	ARSEF451 (T)	KC461182
<i>C. antarcticus</i> S. Tosi, Caretta & Humber	ARSEF6913 (T)	DQ364207
<i>C. bangalorensis</i> Sriniv. & Thirum.	ARSEF449 (T)	DQ364204
<i>C. brefeldianus</i> Couch	ARSEF452 (T)	EF392382
<i>C. chlamydosporus</i> Drechsler	ATCC12242 (T)	JF816212
<i>C. coronatus</i> Batko	AFTOL137	AY546691
	ARSEF525	DQ364205
	RCEF4518	JN131537
<i>C. couchii</i> Sriniv. & Thirum.	ATCC18152 (T)	JN131538
<i>C. firmipilleus</i> Drechsler	ARSEF6384	JX242592
<i>C. heterosporus</i> Drechsler	RCEF4430	JF816225
<i>C. humicolus</i> Sriniv. & Thirum.	ATCC28849 (T)	JF816220
<i>C. iuxtagenitus</i> S.D. Waters & Callaghan	ARSEF6378 (T)	KC788410
	RCEF4445	JX946695
<i>C. khandalensis</i> Srin. & Thirum.	ATCC15162 (T)	KX686994
<i>C. lachnodes</i> Drechsler	ARSEF700	KC788408
<i>C. lichenicolus</i> Srin. & Thirum.	ATCC16200 (T)	JF816216
<i>C. lobatus</i> Sriniv. & Thirum.	ATCC18153 (T)	JF816218
<i>C. mycophilus</i> Srin. & Thirum.	ATCC16199 (T)	KX686995
<i>C. nodosus</i> Srin. & Thirum.	ATCC16577 (T)	JF816217
<i>C. osmodes</i> Drechsler	ARSEF79	EF392371
	RCEF4447	JN131539
<i>C. parvus</i> Drechsler	ATCC14634 (T)	KX752051
<i>C. paulus</i> Drechsler	ARSEF450 (T)	KC788409
<i>C. polytocus</i> Drechsler	ATCC12244 (T)	JF816213
<i>C. pumilus</i> Drechsler	ARSEF453 (T)	EF392383
<i>C. rhyosporus</i> Drechsler	ATCC12588 (T)	JN131540
<i>C. sinensis</i> Y. Nie, X.Y. Liu & B. Huang	RCEF4952 (T)	JF816224
<i>C. stilbeus</i>	RCEF5584 (T)	KP218521
<i>C. stromoideus</i> Sriniv. & Thirum.	ATCC15430 (T)	JF816219
<i>C. terrestris</i> Srin. & Thirum.	ATCC16198 (T)	KX752050
<i>C. thromboides</i> Drechsler	ATCC12587 (T)	JF816214
	RCEF4492	JF816223
<i>Batkooa apiculata</i> (Thaxt.) Humber	ARSEF3130	EF392404
<i>Entomophthora muscae</i> (Cohn) Fresen.	ARSEF3074	DQ273772
<i>Erynia conica</i> (Nowak.) Remaud. & Hennebert	ARSEF1439	EF392396

*The taxonomy refers to the scheme of King (1976a, b; 1977). ARSEF = ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC = American Type Culture Collection (Manassas, U.S.A.). RCEF = Research Center for Entomogenous Fungi (Hefei, China). AFTOL = Assembling the Fungal Tree of Life. T = ex type.

heated) were run for a total of 500,000 Markov Chain Monte Carlo (MCMC) generations. The first 25% of trees were removed as burn-in.

Results

Phylogenetic analyses

The partial 28S rDNA sequence alignment consists of 1036 characters, with 596 phylogenetically informative positions. The maximum parsimony analysis resulted in the most parsimonious tree (Fig 1) with a length (TL) of 3,179 steps, consistency index (CI) of 0.4498, retention index (RI) of 0.6987, homoplasy index (HI) of 0.5502, and rescaled consistency index (RC) of 0.3143. The average standard deviation of split frequencies in Bayesian inference was 0.007557, below 0.01. In the strict consensus tree (Fig 1) generated by maximum parsimony, incorporating the posterior probabilities of Bayesian inference, *C. stilbeus* is placed in Clade III (BP = 78, PP = 1.0), closely to *C. lachnodes*, *C. thromboides*, *C. sinensis* and *C. stromoideus* with a relatively lower bootstrap support of 57%.

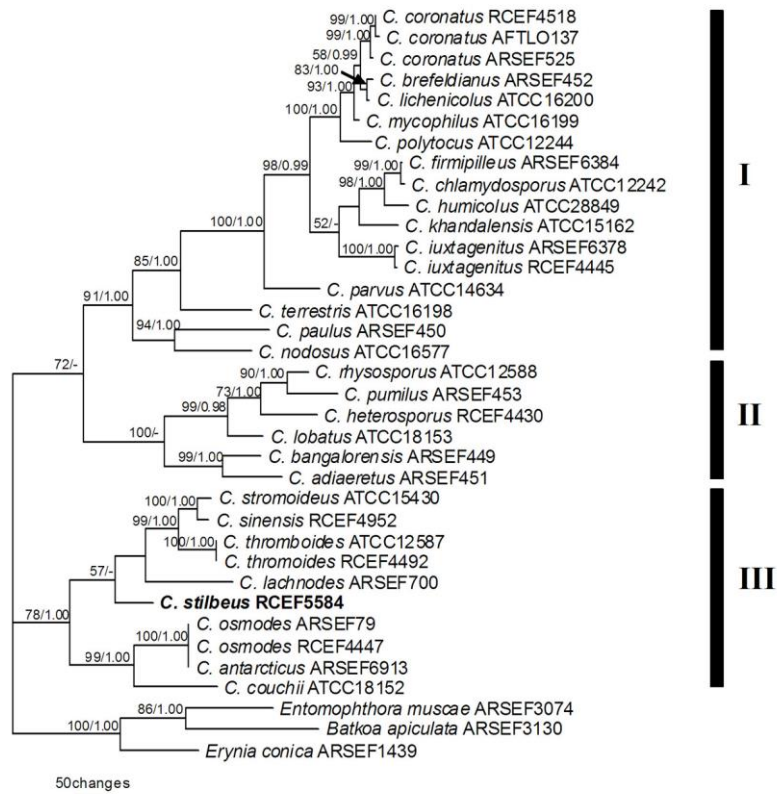


Figure 1 – Phylogram derived from maximum parsimony analysis of the partial 28S rDNA sequences of 33 *Conidiobolus* strains with *Entomophthora muscae*, *Batkoa apiculata* and *Erynia conica* as outgroups. Bootstrap support values (BP) > 50% from 1,000 replicates and Bayesian posterior probabilities (PP) > 0.98 (98%) are successively shown above respective branches.

***Conidiobolus stilbeus* Y. Nie & B. Huang, sp. nov.**

Figs 2–12

Mycobank 818142

FoF 02720 (<http://www.facesoffungi.org/>, Jayasiri SC et al. 2015)

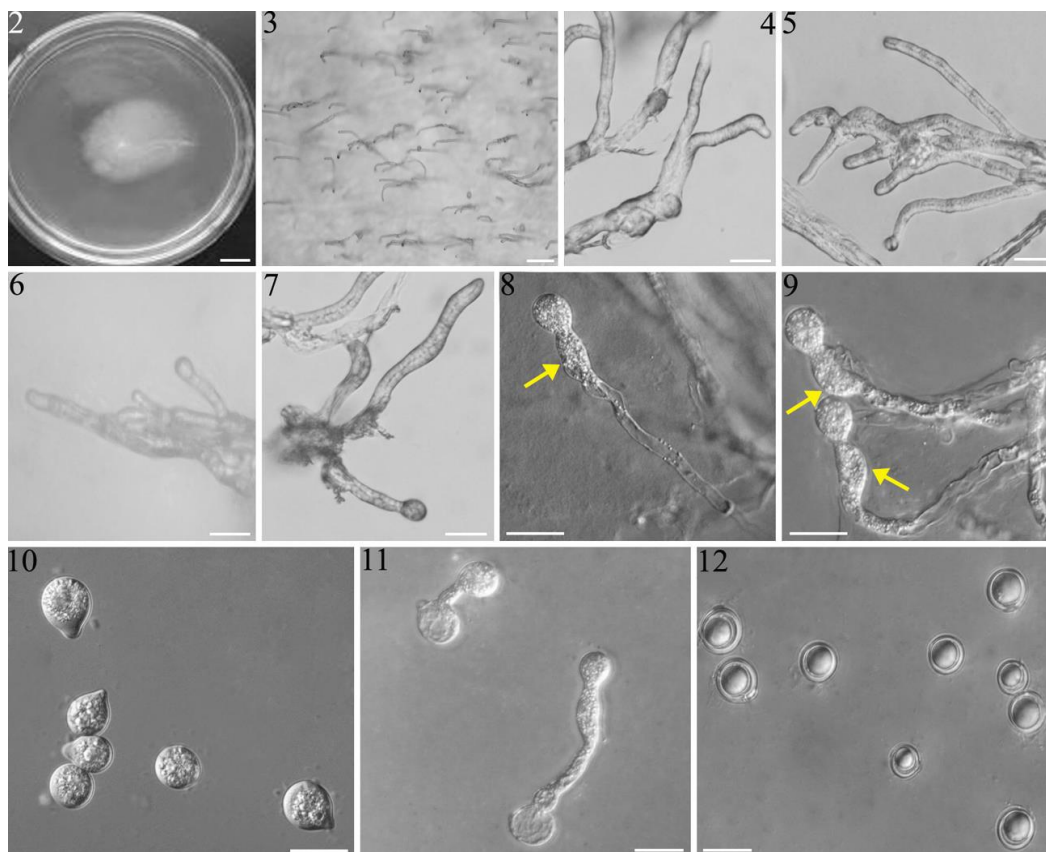
Etymology – *stilbeus* (Lat.) = fasciculation, referring to aerial hyphae forming mycelial strand.

This species differs from other *Conidiobolus* species by forming mycelial strand with 2–6 aerial phototropic hyphae, and by its two types of primary conidiophores: one is shorter and differentiated from aerial hyphae; the other is often longer and inflated, and arises from substrate mycelia.

Known distribution – widespread in soil and plant detritus

Material examined – China, Shandong Province, Mengshan National Forest Park, 34°22′–36°13′N, 117°24′–119°11′E, isolated from soil, 14 June 2011, Y. Nie, RCEF5584 (Holotype) – ex-type culture in RCEF.

Notes – **Colonies** grown on PDA for 3 days at 21°C, reaching *ca* 22–25 mm in diameter. **Mycelia** colorless, moderately branched, 5–10 µm wide, 2–6 aerial hyphae often oriented toward the main source of light and forming mycelial strand. **Primary conidiophores**, positively phototropic, colorless, unbranched and producing a single globose conidium, extending a length of 68–133 µm (commonly 80–100 µm) into the air, widening upward, 10–12 µm wide, some primary conidiophores without inflated upward are differentiated from aerial hyphae, 15–60 (commonly 25–40 µm). **Primary conidia** forcibly discharged, colorless, globose, measuring 17–21 µm in greatest width and 21–25 µm in total length including a basal papilla 2–6 µm high and 5–10 µm wide. After discharging onto 2% water-agar, similar and smaller **secondary conidia** arising from primary conidia. **Zygosporangia** formed after 5 days, mature zygosporangia smooth, colorless, subglobose, 15–19 µm in diameter with wall thickness of 1–2.5 µm.



Figures 2–12 – *Conidiobolus stilbeus*. **2**. Colony on PDA after 3 days at 21°C. **3,4,5**. Aerial phototropic hyphae forming mycelial strand. **6,7**. Primary conidiophores differentiated from aerial hyphae. **8,9**. Primary conidiophores with inflation arising from substrate mycelia. **10**. Primary conidia. **11**. Producing secondary conidia. **12**. Zygospores. – Bars: 2 = 10 mm, 3 = 100 µm, 4–12 = 20 µm).

Key to *Fungus* species

1. Microconidia and capilliconidia produced *Conidiobolus adiaeretus*
1. Microconidia produced, capilliconidia not produced 2
2. Villose resting spores produced *C. coronatus*
2. Villose resting spores not produced 3
3. Zygospores produced 4
3. Zygospores not produced 8
4. Globose chlamydospores produced *C. humicolus*
4. Globose chlamydospores not produced 5
5. Primary conidiophores longer (up to 100 µm or more) 6
5. Primary conidiophores short (50 µm or less) 7
6. Primary conidia larger, maximum not under 45×54 µm *C. macrosporus*
6. Primary conidia small, maximum not over 37×42 µm *C. incongruus*
7. Primary conidia small, less than 26×30 µm *C. mycophilus*
7. Primary conidia small, up to 31×36 µm *C. brefeldianus*
8. Primary conidiophores not branched, produced a single primary conidia *C. firmipilleus*
8. Primary conidiophores branched, produced more than 2 primary conidium 9
9. Primary conidia larger, up to 42×44 µm *C. megalotocus*
9. Primary conidia larger, less than 25×29 µm *C. polytocus*
10. Microconidia not produced, capilliconidia produced 11

10. Microconidia and capilliconidia not produced	15
11. bi- or trifurcate with each branch bearing a single capilliconidia.....	<i>C. heterosporus</i>
11. A single capilliconidia arised from primary conidia	12
12. Zygosporeres produced.....	13
12. Zygosporeres not produced.....	14
13. Zygosporeres smooth.....	<i>C. bangalorensis</i>
13. Zygosporeres mostly rough	<i>C. rhyosporus</i>
14. Primary conidia small, less than 14×18 µm, capilliconidia small, less than 7.5×12 µm.....	<i>C. pumilus</i>
14. Primary conidia larger, maximum not under 24×26 µm, capilliconidia small, maximum not over 10×25 µm.....	<i>C. lobatus</i>
15. Resting spores not produced.....	<i>C. multivagus</i>
15. Resting spores produced	16
16. Only chlamydo-sporeres produced	<i>C. lachnodes</i>
16. Zygosporeres produced.....	20
17. Elongate secondary conidia produced	18
17. Elongate secondary conidia not produced	19
18. Chlamydo-sporeres produced.....	<i>C. eurymitus</i>
18. Chlamydo-sporeres not produced	19
19. Each zygosporere in a position separated by a short, but relatively constant, distance from a lateral conjugation outgrowth or beak	<i>C. iuxtagenitus</i>
19. Each zygosporere in a position not separated by a short, but relatively constant, distance from a lateral conjugation outgrowth or beak	<i>C. couchii</i>
20. Primary conidiophores produced from cushion mycelium.....	21
20. Primary conidiophores not produced from cushion mycelium.....	22
21. Usually branched at edge mycelia, much shorter conidiophores (12 – 40µm) produced	<i>C. stromioideus</i>
21. Rarely branched at edge mycelia, much longer conidiophores (32.5 – 110µm) produced	<i>C. sinensis</i>
22. Primary conidiophores bifurcated and bearing 2 conidium.....	<i>C. margaritatus</i>
22. Primary conidiophores not bifurcated and bearing a single conidia.....	23
23. Forming mycelial strand with 2-6 aerial phototropic hyphae and two types of primary conidiophores.....	<i>C. stilbeus</i>
23. Not forming mycelial strand and forming one type of primary conidiophores	24
24. The optimal temperature for the culture growth appears to be between 40 and 45°C	<i>C. thermophilus</i>
24. The optimal temperature for the culture growth appears to be between 20 and 30°C	25
25. Zygosporeres yellowish	26
25. Zygosporeres colorless	27
26. Zygosporeres usually rough (a few smooth ones may be present)	<i>C. osmodes</i>
26. Zygosporeres smooth.....	<i>C. paulus</i>
27. Zygosporeres larger, more than 60 µm	<i>C. utriculosis</i>
27. Zygosporeres small, less than 40 µm.....	28
28. Primary conidiophores longer, maximum not under 100 µm.....	29
28. Primary conidiophores short, maximum not over 30 µm	30
29. Mycelium lustrous, zygosporeres small, 12 – 18 µm	<i>C. lamprauges</i>
29. Mycelium not lustrous, zygosporeres larger, 17.5 – 27 µm.....	<i>C. thromboides</i>
30. Primary conidia (15-18×17-21 µm) and zygosporeres (17-22×21-30 µm) small.....	<i>C. khandalensis</i>
30. Primary conidia (20-25×25-31 µm) and zygosporeres (25-40 µm) larger.....	<i>C. antarcticus</i>

Discussion

In comparing the morphological features with earlier described *Conidiobolus* species, the present isolate differs from other species by forming mycelial strand with 2–6 aerial phototropic hyphae, and by its two types of primary conidiophores: one is shorter and differentiated from aerial hyphae, the other is often longer and inflated, and arises from substrate mycelium. To some extent, the length of primary conidia of *C. stilbeus* resembles six *Conidiobolus* species: it is distinguished from *C. couchii* mainly by its lack of elongated secondary conidia and longer primary conidiophores (Srinivasan & Thirumalachar 1968), from *C. lamprauges* (25 – 50 µm), *C. lachnodes* (15 – 40 µm), *C. thermophilus* (37.5 – 50 µm), *C. multivagus* (20 – 75 µm) and *C. khandalensis* (10 – 30 µm) by its longer primary conidiophores (80 – 100 µm) (Drechsler 1953, 1955, 1960, Waingankar et al. 2008, Srinivasan & Thirumalachar 1962).

Gryganskyi et al. (2013) conducted a preliminary molecular work for *Conidiobolus* and suggested that more ex-type should be needed to reveal the phylogenetic lineages in *Conidiobolus*. The phylogenetic analysis of partial 28S rDNA in this study shows that the genus *Conidiobolus* splits into three clades (Fig1). *Conidiobolus stilbeus* is located in Clade III composing of 10 isolates which lacks of microconidia and capilliconidia. Within this Clade, it is most closely related to *C. lachnodes* and relatively distant to *C. couchii*, which are both morphologically allied to *C. stilbeus*. Another morphological relative *C. khandalensis* is phylogenetically distantly related, placing in the Clade I. The remaining three morphologically similar species *C. lamprauges*, *C. multivagus* and *C. thermophilus* need to be investigated in the future upon the availability of their living cultures.

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References

- Bałaży S, Wiśniewski J, Kaczmarek S. 1987 – Some note worthy fungi occurring on mites. Bulletin of the Polish Academy of Sciences, Biological Sciences 35, 199–224.
- Bałaży S. 1993 – *Entomophthorales*: Flora of Poland (Flora Polska), Fungi (Mycota), v. 24. Kraków, Poland, Polish Academy of Science, W. Szafer Inst. Botany.
- Ben-Ze'ev IS, Kenneth RG. 1982 – Features-criteria of taxonomic value in the *Entomophthorales*: I. A revision of the Batkoan classification. Mycotaxon 14, 393–455.
- Drechsler C. 1952 – Widespread distribution of *Delacroixia coronata* and other saprophytic *Entomophthoraceae* in plant detritus. Science 115, 575–576.
- Drechsler C. 1953 – Three new species of *Conidiobolus* isolated from leaf mold. Journal of the Washington Academy of Science 43(2), 29–34.
- Drechsler C. 1955 – Three new species of *Conidiobolus* isolated from decaying plant detritus. American Journal of Botany 42, 437–443.
- Drechsler C. 1960 – Two new species of *Conidiobolus* found in plant detritus. American Journal of Botany 47, 368–377.
- Felsenstein J. 1985 – Confidence limits on the bootstrap: an approach using the bootstrap. Evolution 38, 783–791.
- Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R. 2012 – Molecular phylogeny of the *Entomophthoromycota*. Molecular Phylogenetics and Evolution 65, 682–694.
- Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2013 – Phylogenetic lineages in *Entomophthoromycota*. Persoonia 30, 94–105.
- Hall TA. 1999 – Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

- Huang B, Humber RA, Hodge KT. 2007 – A new species of *Conidiobolus* from Great Smoky Mountains National Park. *Mycotaxon* 100, 227–233.
- Humber RA. 2012 – *Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon* 120, 477–492.
- Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J, Buyck B, Cai L, Dai YC, Abd-Elsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bahkali AH, Karunarathna SC, Liu JK, Luangsa-Ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo JM, Ghobad-Nejhad M, Nilsson H, Pang KL, Pereira OL, Phillips AJL, Raspe O, Rollins AW, Romero AI, Etayo J, Selcuk F, Stephenson SL, Suetrong S, Taylor JE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen TC, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena RS, Li WJ, Perera RH, Phookamsak R, De Silva NI, Thambugala KM, Tian Q, Wijayawardene NN, Zhao RL, Zhao Q, Kang JC, Promputtha I. 2015 – The faces of fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity* 74, 3–18.
- Jensen AB, Gargas A, Eilenberg J, Rosendahl S. 1998 – Relationships of the insect-pathogenic order *Entomophthorales* (*Zygomycota*, *Fungi*) based on phylogenetic analyses of nucleus small subunit ribosomal DNA sequences (SSU rDNA). *Fungal Genetics and Biology* 24(3), 325–334.
- King DS. 1976a – Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy I. Taxonomic considerations. *Canadian Journal of Botany* 54, 45–65.
- King DS. 1976b – Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy II. Taxonomic considerations. *Canadian Journal of Botany* 54, 1285–1296.
- King DS. 1977 – Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy III. Descriptions of recognized species. *Canadian Journal of Botany* 55, 718–729.
- Liu M, Rombach MC, Humber RA, Hodge KT. 2005 – What's in a name? *Aschersoniainsperata*: a new pleoanamorphic fungus with characteristics of *Aschersonia* and *Hirsutella*. *Mycologia* 97, 249–256.
- Nie Y, Yu CZ, Liu XY, Huang B. 2012 – A new species of *Conidiobolus* (*Ancylistaceae*) from Anhui, China. *Mycotaxon* 120, 427–435.
- Ronquist F, Huelsenbeck JP. 2003 – MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Srinivasan MC, Thirumalachar MJ. 1962 – Studies on species of *Conidiobolus* from India–III. *Mycologia* 54(6), 685–693.
- Srinivasan MC, Thirumalachar MJ. 1968 – Two new species of *Conidiobolus* from India. *Journal of the Mitchell Society* 84, 211–212.
- Swofford DL. 2003 – PAUP*. Phylogenetic analysis using parsimony and other methods. Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Thompson JD, Gibson TJ, Plewniak F. 1997 – The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 63, 215–228.
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172, 4238–4246.
- Waingankar VM, Singh SK, Srinivasan MC. 2008 – A new thermophilic species of *Conidiobolus* from India. *Mycopathologia* 165, 173–177.
- Wang CF, Li KP, Huang B. 2010a – A new record to China – *Conidiobolus iuxtagenitus*. *Journal of Fungal Research* 1, 12–14.
- Wang CF, Li KP, Liu YJ, Li ZZ, Huang B. 2010b – Three new Chinese records of *Conidiobolus*. *Mycosystema* 4, 595–599.
- Watanabe M, Lee K, Goto K, Kumagai S, Sugita-Konishi Y, Hara-Kudo Y. 2010 – Rapid and effective DNA extraction method with bead grinding for a large amount of fungal DNA. *Journal of Food Protection* 73(6), 1077–1084.

Waters SD, Callaghan AA. 1989 – *Conidiobolus iuxtagenitus*, a new species with discharge delongate repetitional conidia and conjugation tubes. *Mycological Research* 93, 223–226.