Mycosphere 8(7): 809–816 (2017) www.mycosphere.org ISSN 2077 7019
Article
Doi 10.5943/mycosphere/8/7/1

A new species of *Conidiobolus* with chlamydospores from Dabie Mountains, Eastern China

Nie Y[1,2], Tang XX[1], Liu XY[3*] and Huang B[1*]

[1]*Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University, Hefei 230036, China*
[2]*School of Civil Engineering and Architecture, Anhui University of Technology, Ma·anshan 243002, China*
[3]*State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*

Nie Y, Tang XX, Liu XY, Huang B 2017 – A new species of *Conidiobolus* with chlamydospores from Dabie Mountains, Eastern China. Mycosphere 8(7), 809–816, Doi 10.5943/mycosphere/8/7/1

Abstract

Conidiobolus is a widely distributed genus throughout the world, and its resting spores include zygospores, chlamydospores and villose conidia. *Conidiobolus dabieshanensis* is confirmed as a new species with chlamydospores within the genus *Conidiobolus* based on the morphological evidence and the molecular data of LSU rDNA region. It is morphologically allied with *C. adiaeretus* and *C. firmipilleus* and it is phylogenetically closely related to *C. humicolus*, *C. chlamydosporus* and *C. firmipilleus*. The new species differs from *C. adiaeretus* by the absence of capilliconidia, from *C. firmipilleus* and *C. chlamydosporus* by its size of primary conidia and slender and curving germ hyphae arising from a single conidium, and from *C. humicolus* by its larger primary conidia and the absence of zygospores.

Key words – *Entomophthorales* – Morphology – Molecular phylogeny

Introduction

The genus *Conidiobolus* Bref. (*Ancylistaceae*, *Entomophthorales*) typified by *C. utriculosus* Bref. was originally isolated from basidiomycete fruit bodies (Brefeld 1884). It is widely distributed in soils and decaying leaves and sometimes isolated from keratin, living ferns and rotten vegetables (Kwon-Chung 2012, Nie et al. 2012). In addition, *C. coronatus*, *C. incongruous*, and *C. lamprauges* have been reported as pathogens of humans and animals (Vilela et al. 2010, Mendoza et al. 2014).

A total of 34 species are currently recognized within *Conidiobolus* (Huang et al. 2007; King 1976a, b, 1977; Nie et al. 2012, 2016). Three subgenera *Delacroixia*, *Capillidium* and *Conidiobolus* were introduced based on the types of secondary conidia (Ben-Ze'ev & Kenneth 1982), and followed by Humber (1989, 1997). Recent multigene phylogenetic analyses revealed at least 3 lineages in the genus *Conidiobolus*, but the subgeneric boundaries of Ben-Ze'ev & Kenneth are not supported (Gryganskyi et al. 2013).

Three types of resting spores are presented in *Conidiobolus* species. They are zygospores, chlamydospores and villose conidia. The zygospores are the most common, which are double walled and formed inside the larger one of the two conjugating cells (Humber 1981, 1989). Within *Conidiobolus*, 22 species produce zygospores. Among the remaining species, only *C. coronatus* produces villose conidia, and *C. adiaeretus*, *C. eurymitus*, *C. firmipilleus*, *C. humicolus*, *C. lachnodes*, *C. pumilus*, and *C. rhysosporus* form chlamydospores with a single thickened wall layer

Submitted 5 March 2017, Accepted 25 May 2017, Published 29 May 2017
Corresponding Author: Huang B – e-mail – bhuang@ahau.edu.cn, Liu XY – e-mail – Liuxiaoyong@im.ac.cn

(King 1977). The present study introduces a new species of *Conidiobolus* with chlamydospores based on morphological features and phylogenetic data.

Materials & Methods

Sample collection, strain isolation and morphological identification

Soil samples were collected from Dabie Mountains, Huoshan County, Anhui Province, Eastern China. The canopy plating approach in this study followed Drechsler (1952) and King (1976a). In the laboratory, a Petri dish containing potato dextrose agar (PDA, Difco) was inverted over the soil, incubated at 21ºC and examined daily. When fungi were detected on the PDA canopy, discharged conidia transferred to a new PDA plate for morphological study. The measurements of different fungal structures were taken as described by King (1976a). The specimen has been deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University (RCEF) and duplicated in the China General Microbiological Culture Collection Center (CGMCC). The dried cultures were deposited in RCEF and the Herbarium Mycologicum Academiae Sinicae (HMAS).

DNA extraction, PCR and sequencing

After incubation on PDA at 21ºC for seven days, total genomic DNA was extracted from the mycelium scraped from the cellophane with modified CTAB method (Watanabe et al. 2010), and stored at -20ºC. The LSU rDNA region was amplified with the primers LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') (Vilgalys et al. 1990), and the PCR reaction used in this study has been described by Liu (Liu et al. 2005). PCR products were sequenced by Shanghai Genecore Biotechnologies Company (Shanghai, China) with the same primers. Accession numbers for sequences used for phylogenetic analyses are provided (Table 1).

Phylogenetic analysis

Referring to King (1976b), more LSU rDNA sequences were downloaded from GenBank. *Batkoa apiculata*, *Entomophthora muscae*, and *Erynia conica* were retrieved as outgroups. Multiple sequences were aligned with BioEdit (Hall 1999) and Clustal X (Thompson et al. 1997), and the alignment was deposited to TreeBASE (Submission ID: 20453; Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S20453). Maximum Parsimony (MP) and Bayesian Inference (BI) were applied to the LSU rDNA dataset. All characters were weighted, and gaps were treated as missing data. PAUP* 4.0b10 (Swofford 2002) was used to perform the MP analysis. Branch swapping algorithm was tree bisection-reconnection (TBR) and MulTrees were used. Branch support was estimated with bootstrapping by using 1,000 replicates (Felsenstein 1985). Modeltest 3.7 (Posada & Crandall 1998) was used to determine the best-fit evolution mode for BI. The BI was implemented by Markov Chain Monte Carlo (MCMC) sampling within the software MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The MCMC chains ran until a critical value for the topological convergence diagnostic was less than 0.005.

Results

Phylogenetic analyses

The final partial LSU sequence alignment includes 39 strains, and consists of 1025 characters, of which 604 characters are parsimony-informative and 112 characters are parsimony-uninformative. The heuristic search using maximum parsimony (MP) generated three parsimonious trees (TL = 3378, CI = 0.4307, HI = 0.5693, RI = 0.7035, RC = 0.3030), one of which is selected and shown in Figure 1. The GTR+I+G nucleotide substitution model is used based on the Akaike Information Criterion (AIC). One tree is saved to a file within every 100 generations for a total of 1,000,000 MCMC generations, and the average standard deviation of split frequencies in Bayesian Inference is 0.004833, below 0.005. Three clades present on the phylogenetic tree, and *C. dabieshanensis* grouped in Clade I, being most closely related to *C. humicolus*, *C. firmipilleus* and *C. chlamydosporus.*

Table 1 The species in this study and their GenBank accession numbers of LSU rDNA*.

Species	Strains	GeneBank Number
Conidiobolus adiaeretus	ARSEF 451 (T)	KC461182
C. antarcticus	ARSEF 6913 (T)	DQ364207
C. bangalorensis	ARSEF 449 (T)	DQ364204
C. brefeldianus	ARSEF 452 (T)	EF392382
C. chlamydosporus	ATCC 12242 (T)	JF816212
C. coronatus	NRRL 28638	AY546691
C. coronatus	ARSEF 525	DQ364205
C. coronatus	RCEF 4518	JN131537
C. couchii	ATCC 18152 (T)	JN131538
C. dabieshanensis	CGMCC 3.15763 (T)	KY398125
C. firmipilleus	ARSEF 6384	JX242592
C. gonimodes	ATCC 14445 (T)	JF816221
C. heterosporus	RCEF 4430	JF816225
C. humicolus	ATCC 28849 (T)	JF816220
C. iuxtagenitus	ARSEF 6378 (T)	KC788410
C. iuxtagenitus	RCEF 4445	JX946695
C. khandalensis	ATCC 15162 (T)	KX686994
C. lachnodes	ARSEF 700	KC788408
C. lichenicolus	ATCC 16200 (T)	JF816216
C. lobatus	ATCC 18153 (T)	JF816218
C. marcosporus	ATCC 16578 (T)	KY398124
C. mycophilus	ATCC 16199 (T)	KX686995
C. nodosus	ATCC 16577 (T)	JF816217
C. osmodes	ARSEF 79	EF392371
C. osmodes	RCEF 4447	JN131539
C. parvus	ATCC 14634 (T)	KX752051
C. paulus	ARSEF 450 (T)	KC788409
C. polytocus	ATCC 12244 (T)	JF816213
C. pumilus	ARSEF 453 (T)	EF392383
C. rhysosporus	ATCC 12588 (T)	JN131540
C. sinensis	RCEF 4952 (T)	JF816224
C. stilbeus	RCEF 5584 (T)	KP218522
C. stromoideus	ATCC 15430 (T)	JF816219
C. terrestris	ATCC 16198 (T)	KX752050
C. thromboides	ATCC 12587 (T)	JF816214
C. thromboides	RCEF 4492	JF816223
Batkoa apiculata	ARSEF 3130	EF392404
Entomophthora muscae	ARSEF 3074	DQ273772
Erynia conica	ARSEF 1439	EF392396

*The taxonomy of *Conidiobolus* refers to the scheme of King (1976b). ARSEF = ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC = American Type Culture Collection (Manassas, U.S.A). CGMCC = China General Microbiological Culture Collection Center (Beijing, China). NRRL = ARS Culture Collection (Peoria, U.S.A). RCEF = Research Center for Entomogenous Fungi (Hefei, China). T = ex-type.

Conidiobolus dabieshanensis Y. Nie & B. Huang, *sp. nov.* Figs 2–13

Index Fungorum number: IF 552756; Facesoffungi number: FoF 02877 (Jayasiri et al. 2015)

Etymology – *dabieshanensis* (Lat.) = Referring to the region where the fungus was isolated.

This species is mainly characterized by the formation of chlamydospores and the slender and curving germ hyphae arising from a single conidium. It differs from *C. adiaeretus* by the absence

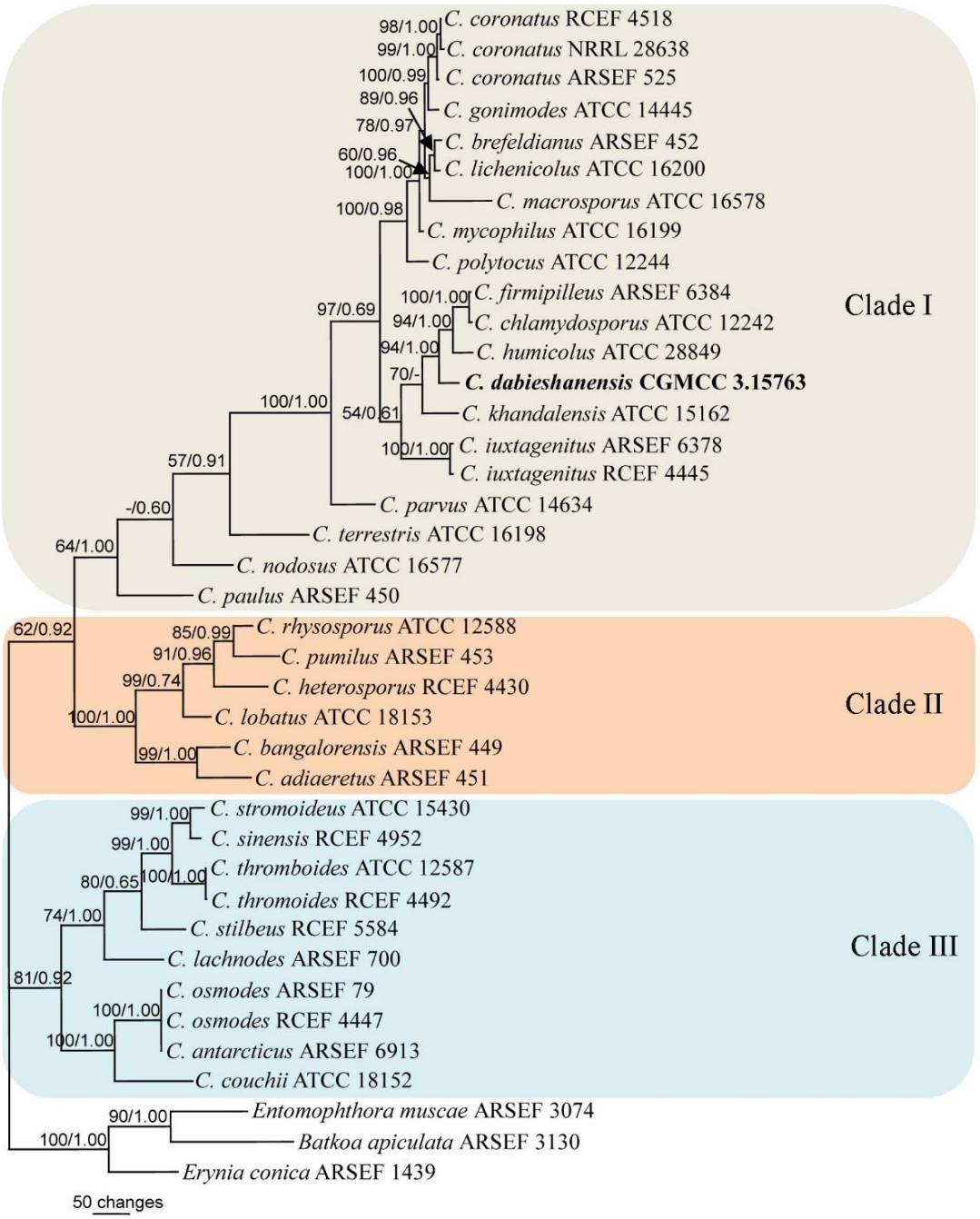
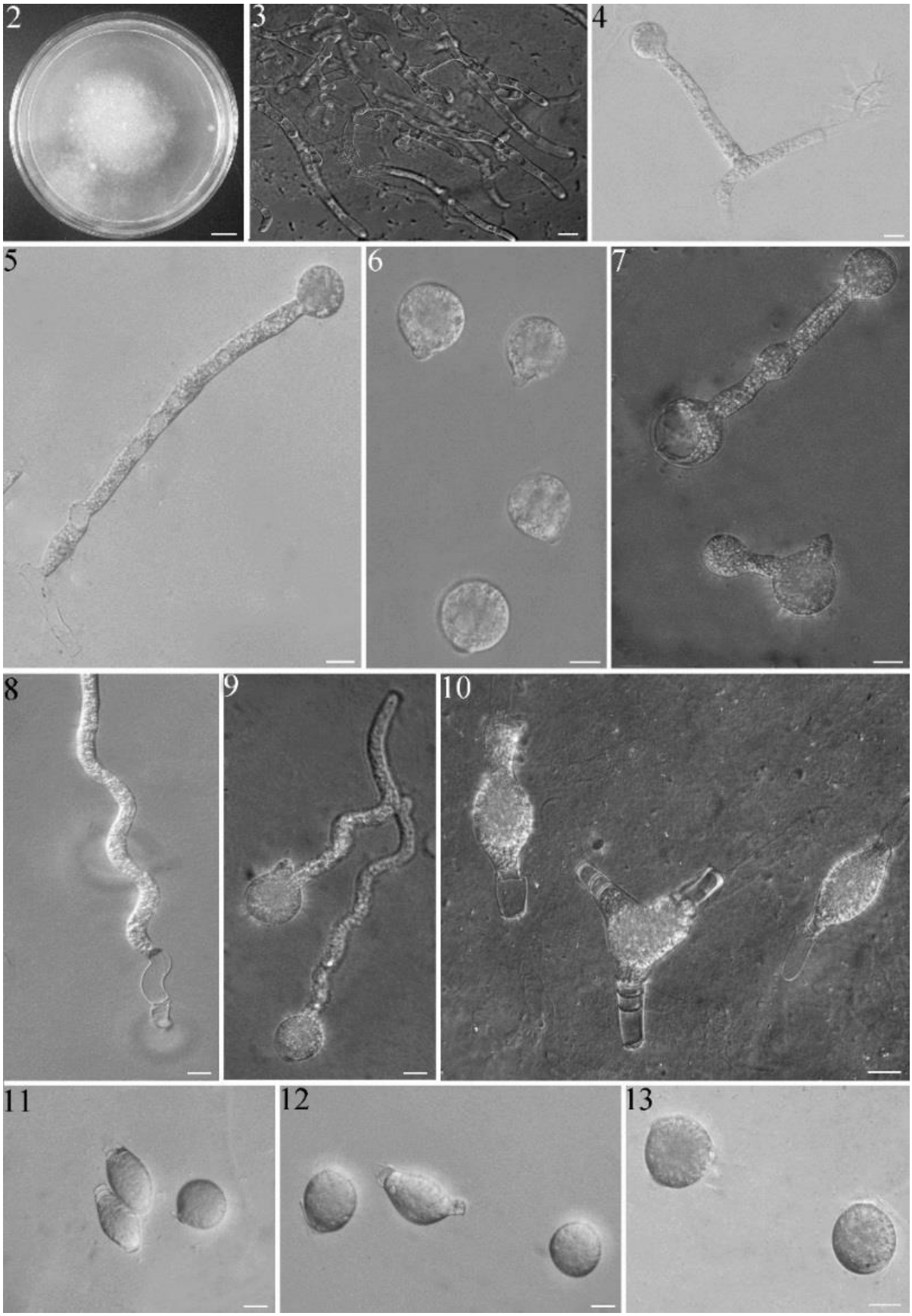


Figure 1 – Phylogenetic tree of *Conidiobolus* constructed with maximum parsimony analysis of LSU rDNA sequence data, with *Entomophthora muscae*, *Batkoa apiculata* and *Erynia conica* as outgroup taxa. Branches are labeled with maximum parsimony bootstrap value (BP), and Bayesian posterior probabilities (BPP) more than 50% and 0.60, respectively. The new species of *C. dabieshanensis* is in bold. Bar at the bottom left represents 50 changes in the parsimony analyses.

of capilliconidia, and from *C. firmipilleus* by slightly larger primary conidia and slender and curving germ hyphae. Phylogenetically, it is closely related to *C. humicolus*, *C. firmipilleus* and *C. chlamydosporus*, but differs from *C. humicolus* by its larger primary conidia and the absence of zygospores, and from *C. chlamydosporus* by its slightly smaller primary conidia and curving germ hyphae.

Known distribution – Anhui province, China

Material examined – China, Anhui Province, Huoshan County, Dabie Mountains, 31˚18′N, 116˚01′E, isolated from soil, 12 April 2016, XX Tang, (RCEF6329, Holotype; HMAS255215, Isotype) – ex-type culture CGMCC 3.15763.



Figures 2–13 – *Conidiobolus dabieshanensis*. 2. Colony on PDA after 3 days at 21˚C. 3. Moderately branched at the colony edge. 4, 5. Primary conidiophores arising from mycelia. 6. Primary conidia. 7. Producing secondary conidia. 8, 9. Slender and curving germ hyphae arising from a single conidium. 10. Forming chlamydospores. 11, 12, 13. Chlamydospores. – Bars: 2 = 10 mm, 3–13 = 20 μm.

Notes – Colonies on PDA at 21˚C after 3 days, white, reaching *ca* 52 mm in diameter. Mycelia colorless, moderately branched at the colony edge, 8–16 μm wide. Primary conidiophores, colorless, unbranched and producing a single globose conidium without widening upward, extending a length of (60–)80–155(–287) μm into the air, 9–17 μm wide. Primary conidia forcibly discharged, colorless, globose to subglobose, measuring 32.5–45 μm in greatest length and 29–38 μm in total width including a basal papilla 4–11 μm high and 9–16 μm wide. After discharging onto 2% water-agar, similar and smaller secondary conidia arising from primary conidia, slender and curving germ hyphae arising from a single conidium. Chlamydospores formed intercalarily within assimilative hyphae after 3 days, mostly subspherical to elongate ellipsoidal, rarely barrel-shaped, 33–50 × 22–35μm.

Discussion

The presence and nature of zygospores or azygospores is a useful ancillary characteristic in *Conidiobolus* (Humber 1989). Taking into account the types of resting spores in *Conidiobolus* presented by King (1976a,b, 1977), morphological features were compared among seven *Conidiobolus* species which form chlamydospores, and the length of primary conidia of the new species *C. dabieshanensis* are mostly related to *C. adiaeretus* (15–46 × 13–45 μm) and *C. firmipilleus* (8–40 × 6.5–33 μm) . However, *C. dabieshanensis* differs from *C. adiaeretus* by the absence of capilliconidia (Drechsler 1953a, Callaghan et al. 2000), and from *C. firmipilleus* due to the slightly larger primary conidia and curving germ hyphae (Drechsler 1953b, King 1977). As a note, microconidia in *C. dabieshanensis* are not observed.

The sequences of LSU rDNA region have been proved to be effective in distinguishing the *Conidiobolus* species (Nie et al. 2012, 2016), and the phylogenetic tree (Fig. 1) inferred from this locus demonstrated that 36 *Conidiobolus* strains (contain 26 ex-types) form three Clades which was almost consistent with the phylogenetic analysis of *Conidiobolus* based on the datas of nucLSU, nucSSU, RPB2 and mtSSU by Gryganskyi et al. (2013). In the Clade I, *C. dabieshanensis* forms a well-supported clade with *C. humicolus*, *C. firmipilleus* and *C. chlamydosporus* (BP = 94, PP = 1.00), but morphologically differs from *C. humicolus* (26–38 × 23–34 μm) by its larger primary conidia and the absence of zygospores, and from *C. chlamydosporus* (18–50 × 15–45 μm) by its slightly smaller primary conidia and curving germ hyphae (Drechsler 1955, Srinivasan & Thirumalachar 1961). The morphological relative *C. adiaeretus*, is phylogenetically distantly related and falls in the Clade II.

Both morphological and phylogenetic evidences support that the specimen isolated from Eastern China is a new species within the genus *Conidiobolus*.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (No. 30770008 and 31370068) and the Key Science Research Project of Anhui Province (No. TD200708).

References

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.

Brefeld O. 1884 – *Conidiobolus utriculosus* und *minor*. Untersuchungen aus der Gesammtgebiete der Mykologie 6(2), 35–78.

Callaghan AA, Waters SD, Manning RJ. 2000 – Alternative repetitional conidia in *Conidiobolus adiaeretus*: development and germination. Mycological Research 104, 1270–1275.

Drechsler C. 1952 – Widespread distribution of *Delacroixia coronata* and other saprophytic *Entomophthoraceae* in plant detritus. Science 115, 575–576.

Drechsler C. 1953a – Three new species of *Conidiobolus* isolated from leaf mold. Journal of the Washington Academy of Science 43(2), 29–34.

Drechsler C. 1953b – Two new species of *Conidiobolus* occurring in leaf mold. American Journal of Botany 40(3), 104–115.

Drechsler C. 1955 – Two new species of *Conidiobolus* that produce microconidia. American Journal of Botany 42(9), 793–802.
Felsenstein J. 1985 – Confidence limits on the bootstrap: an approach using the bootstrap. Evolution 38, 783–791.
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. 1981– An alternative view of certain taxonomic criteria used in the *Entomophthorales* (*Zygomycetes*). Mycotaxon 13, 191–240.
Humber RA. 1989 – Synopsis of a revised classification for the *Entomophthorales* (*Zygomycotina*). Mycotaxon 34, 441–460.
Humber RA. 1997 – Fungi: identification. In: LA Lacey (ed.). Manual of Techniques in Insect Pathology. London, Academic Press.
Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. 2015 – The faces of fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
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.
Kwon-Chung KJ. 2012 – Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: Molecular mycologic perspectives. Clinical Infectious Diseases 54, S8–S15.
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.
Mendoza L, Vilela R, Voelz K, Ibrahim AS, Voigt K, Lee SC. 2014 – Human fungal pathogens of *Mucorales* and *Entomophthorales*. Cold Spring Harbor Perspectives in Medicine 5(4), 1–33.
Nie Y, Yu CZ, Liu XY, Huang B. 2012 – A new species of *Conidiobolus* (*Ancylistaceae*) from Anhui, China. Mycotaxon 120, 427–435.
Nie Y, Tang XX, Liu XY, Huang B. 2016 – *Conidiobolus stilbeus*, a new species with mycelial strand and two types of primary conidiophores. Mycosphere 7(6), 801–809.
Posada D, Crandall KA. 1998 – MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Ronquist F, Huelsenbeck JP. 2003 – MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Srinivasan MC, Thirumalachar MJ. 1961 – Studies on species of *Conidiobolus* from India - I. Sydowia, Annales Mycologici15, 237–241.
Swofford DL. 2002 – PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland.
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.
Vilela R, Silva SMS, Correa FR, Dominguez E, Mendoza L. 2010 – Morphologic and phylogenetic characterization of *Conidiobolus lamprauges* recovered from infected sheep. Journal of Clinical Microbiology 48, 427–432.
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.

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.