MycoKeys 51: 55–64 (2019)
doi: 10.3897/mycokeys.51.30798
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Blastosporium persicolor gen. et sp. nov., a new helotialean fungus

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Academic editor: *T. Lumbsch* | Received 24 October 2018 | Accepted 9 April 2019 | Published 22 April 2019

Citation: Zheng H, Zhang ZN, Wen Z, Castañeda-Ruiz RF, Yu ZF (2019) *Blastosporium persicolor* gen. et sp. nov., a new helotialean fungus. MycoKeys 51: 55–64. https://doi.org/10.3897/mycokeys.51.30798

Abstract

A new genus and species, *Blastosporium persicolor*, is described and illustrated from leaves of mildewed tobacco. It is characterised by branched, septate hyphae from which arise macronematous, unbranched or spaced branched conidiophores and mono- or polyblastic conidiogenous cells that produced solitary and blastocatenate, obovoid, oblong, ellipsoidal, allantoid, broad fusiform to irregular, unicellular, hyaline conidia. The phylogenetic analyses, based on the combined sequence data from the small and large nuclear subunit ribosomal DNA (SSU and LSU), placed *B. persicolor* in the Leotiomycetes class, Helotiales order.

Keywords

Ascomycota, Pezizomycotina, phylogeny, *Nicotiana tabacum*

Introduction

The Kingdom Fungi contains a huge number of species, which continues to rise with more collections. With the advance in the studies of DNA sequence data, the fungal classification system has been updated over the years. Many described species obtained

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new taxonomic status after the molecular data and have been processed. Leotiomycetes is a large class in Ascomycota and has potential taxonomic value relating to the ecology and biology. The traditional classification of Leotiomycetes at high levels has experienced considerable challenges with the inclusion of the molecular techniques in systematics studies. For example, early research accepted five orders, 21 families and about 510 genera in the Leotiomycetes on the basis of both traditional classification and molecular phylogenetic studies (Eriksson 2005, Kirk et al. 2001), but a recent study reported a new classification of Leotiomycetes, including 11 orders, 44 families and about 590 genera (Wijayawardene et al. 2018) and this classification also lacks sufficient DNA sequence data. In Leotiomycetes, the order Helotiales, one of the largest non-lichen-forming ascomycetous groups, is composed of fungi of diverse morphology and ecology. Of these, members of the Helotiales thrive in various ecosystems and cover a broad range of niches and helotialean fungi have been found as plant pathogens, endophytes, nematode-trapping fungi, mycorrhizae, ectomycorrhizal parasites, fungal parasites, terrestrial saprobes, aquatic saprobes, root symbionts and wood rot fungi (Wang et al. 2006).

During a survey of fungi growing on mildewed tobacco leaves, an unknown fungus was found. Based on its morphological characters and DNA sequence data, it is proposed as a new asexual genus and species, *Blastosporium persicolor*.

Materials and methods

Isolation and morphological study of strain

Samples of the mildewed tobacco leaves were collected from Xiamen Logistics Warehousing Center. Samples were preserved in zip-locked plastic bags, labelled and transported to the laboratory. The procedure was as follows: samples (5g) were placed in PDA liquid medium (200 g potato, 20 g glucose, 1000 ml distilled water), shaken at 140 rpm/min for 1 h and the filtrate was collected. The filtrate was coated on a CMA plate (20 g cornmeal, 10 g agar, 1000 ml distilled water) at 28 °C, supplemented with two antibiotics (penicillin G, 0.5 g/l; and streptomycin, 0.5 g/l; Gams et al. 1998). After 3–5 days, single colonies were isolated into pure culture, grown on potato dextrose agar plates (PDA). The characteristics of the colonies were from PDA, CMA and SNA (synthetic low nutrient agar). Microscopic characteristics were made from cultures growing on CMA after incubation at room temperature for one week.

The pure cultures and dried cultures were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF, formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Pure cultures were grown on PDA for 5 days at 25 °C. Actively growing mycelium was scraped off the surface of a culture and transferred to 2 ml Eppendorf micro-centrifuge tubes. Total genomic DNA was extracted according to the procedures in Turner et al. (1997). Primers used for PCR amplification and sequencing of nucSSU rDNA, nucLSU rDNA and ITS rDNA were NS1-NS4, LROR-LR7 and ITS1-ITS4, respectively (White et al. 1990, Vilgalys and Hester 1990). Detailed protocols and PCR conditions for the amplification were fully described by Su et al. (2015). PCR products were then purified using a commercial Kit (Bioteke Biotechnology Co, Ltd, China) and forward and reverse sequences with a LI-COR 4000L automatic sequencer, using a Thermo Sequenase-kit, as described by Kindermann et al. (1998). The sequences were deposited in the National Center for Biotechnology Information (NCBI) and the accession numbers are listed in Table 1.

Sequence alignment and phylogenetic analysis

Other fungal sequences were obtained from the GenBank nucleotide database. DNA sequence data were aligned using ClustalX 1.83 (Higgins 1994) with default parameters and the consensus sequences were manually adjusted and linked in BioEdit v.7.0 (Hall 1999). Manual gap adjustments were made to improve the alignment and ambiguously aligned regions were also excluded. Portions of the 5'- and 3'-ends of the nuclear small and large subunits ribosomal DNA (nucSSU and nucLSU) were excluded from all analyses and coded by a question mark (?). MrBayes (Ronquist and Huelsenbeck 2003) was used to calculate the SSU rRNA and LSU rRNA sequence-based Bayesian inference of the phylogeny tree, with the following parameters: ngen=1,000,000; samplefr=1,000; printfr=1,000. The GenBank accession numbers of sequences used in the phylogenetic analysis are shown in Table 1 including the classes of Leotiomycetes, Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Orbiliomycetes, Pezizomycetes and Sordariomycetes. *Candida albicans* (C.P. Robin) Berkhout (Saccharomycetes) was used as outgroup.

Results

Sequence analyses

In BLAST searches, the ITS sequence *B. persicolor*, MH992518, had the highest similarity of 88% with *Tetracladium* and 87% with *Chalara* (Corda) Rabenh., both belonging to Leotiomycetes. Therefore, most sequences are mainly from Leotiomycetes in the dataset. The dataset comprised 57 taxa representing 7 classes, 11 orders, 22

Table 1. Strains and the GenBank accession numbers of sequences used in the molecular phylogenetic analyses in this study.

Name	Strain	GenBank accession number	
		LSU	SSU
Arthonia caesia (Flot) Körb.	AFTOL-ID 775	FJ469668	–
Arthrobotrys elegans (Subram & Chandrash) Seifert & W.B. Kendr.	AFTOL-ID 1252	FJ176864	FJ176810
Arthrocladiella mougeotii (Lév) Vassilkov	–	AB022379	AB033477
Blastosporium persicolor Z. F. Yu & H. Zheng	**YMF1.05546**	**MH992517**	**MH992516**
Blumeria graminis (DC.) Speer	–	AB022362	AB033476
Brasiliomyces trinus (Harkn) R.Y. Zheng	–	AB022350	–
Bryoglossum gracile (P. Karst.) Redhead	MBH52481	AY789420	AY789419
Bulgaria inquinans (Pers.) Fr.	ZW-Geo52-Clark	AY789344	AY789343
Candida albicans (C.P. Robin) Berkhout	WO1	L28817	X53497
Capnodium coffeae Pat.	CBS 147.52	DQ247800	DQ247808
Chlamydotubeufia huaikangplaensis Boonmee & K.D. Hyde	MFLUCC10-0926	JN865198	–
Ciboria batschiana (Zopf) N. F. Buchw.	WZ-JXD-22	AY789322	–
Cudonia circinans (Pers.) Fr.	OSC56399	AF279379	AF107343
Cyttaria darwinii Berk.	14	EU107208	EU107181
Dermea acerina (Peck) Rehm	CBS 161.38	DQ247801	DQ247809
Disciotis venosa (Pers.) Arnould	AFTOL-ID 179	AY544667	AY544711
Dothidea sambuci (Pers.) Fr.	AFTOL-ID 274	AY544681	AY544722
Erysiphe australiana (McAlpine) U. Braun & S. Takam.	–	AB022407	–
Erysiphe cornicola Meeboon & S. Takam.	–	AB022389	–
Erysiphe glycines F. L. Tai	MUMH52	AB022397	AB120748
Erysiphe gracilis R. Y. Zheng & G. Q. Chen	–	AB022357	–
Erysiphe mori (I. Miyake) U. Braun & S. Takam.	–	AB022418	AB033484
Erysiphe simulans (E. S. Salmon) U. Braun & S. Takam.	–	AB022395	–
Eupenicillium limosum S. Ueda	AFTOL-ID 2014	EF411064	EF411061
Fabrella tsugae (Farl) Kirschst.	–	AF356694	–
Geoglossum glabrum Pers.	OSC60610	AY789317	AY789316
Geoglossum umbratile Sacc.	Mycorec1840	AY789303	AY789302
Helicoma chlamydosporum Shearer	CBS 160.69	AY856875	AY856923
Helicoma vaccinii Carris	CBS 216.90	AY856879	AY856926
Helicomyces roseus Link	CBS 283.51	AY856881	AY856928
Helicosporium guianense Linder	CBS 269.52	AY856893	AY856938
Holwaya mucida (Schulzer) Korf & Abawi	B 70 0009352	DQ257356	DQ257355
Lachnum bicolor (Bull.) P. Karst.	AFTOL-ID 177	AY544674	AY544690
Lachnum virgineum (Batsch) P. Karst.	AFTOL-ID 49	AY544646	AY544688
Leotia lubrica (Scop.) Pers.	ZW-Geo59-Clark	AY789359	AY789358
Monascus purpureus Went	AFTOL-ID 426	DQ782908	DQ782881
Morchella esculenta (L.) Pers.	AFTOL-ID 60	AY544664	AY544708
Mycosphaerella punctiformis (Pers.) Starbäck	AFTOL-ID 942	DQ470968	DQ471017
Neoerysiphe galeopsidis (DC.) U. Braun	–	AB022369	–
Neofabraea malicorticis (Cordley) H.S. Jacks.	AFTOL-ID 149	AY544662	AY544706
Orbilia vinosa (Alb. & Schwein.) P. Karst.	AFTOL-ID 905	DQ470952	DQ471000
Penicillium freii Frisvad & Samson	DAMO 216705	AY640958	AY640998
Phyllactinia moricola (Henn.) Homma	–	AB022401	AB033481
Piceomphale bulgarioides (P. Karst.) Svrček	1589.P	Z81415	–
Pleochaeta shiraiana (Henn.) Kimbr. & Korf	MUMH36	AB022403	AB120750
Podosphaera tridactyla (Wallr.) de Bary	–	AB022393	–
Roccellographa cretacea J. Steiner	AFTOL-ID 93	DQ883696	DQ883705
Rutstroemia bolaris (Batsch) Rehm	1526.P	Z81419.1	–
Sawadaea polyfida (C.T. Wei) R.Y. Zheng & G.Q. Chen	–	AB022364	–
Schismatomma decolorans (Erichsen) Clauzade & Vězda	DUKE 0047570	NG_027622	NG_013155
Scleromitrula shiraiana (Henn.) S. Imai	Hirayama062001	AY789407	AY789406
Sclerotinia sclerotiorum (Lib.) de Bary	WZ0067	AY789347	AY789346
Spathularia flavida Pers.	wz138	AF433142	AY789356
Thaxteriella inthanonensis Boonmee & K.D. Hyde	MFLUCC11-0003	JN865199	–
Trichoglossum hirsutum (Pers.) Boud.	AFTOL-ID 64	AY789313	AY789312
Vibrissea flavovirens (Pers.) Korf & J.R. Dixon	MBH39316	AY789426	AY789425
Vibrissea truncorum (Alb. & Schwein) Fr.	CUP-62562	AY789402	AY789401

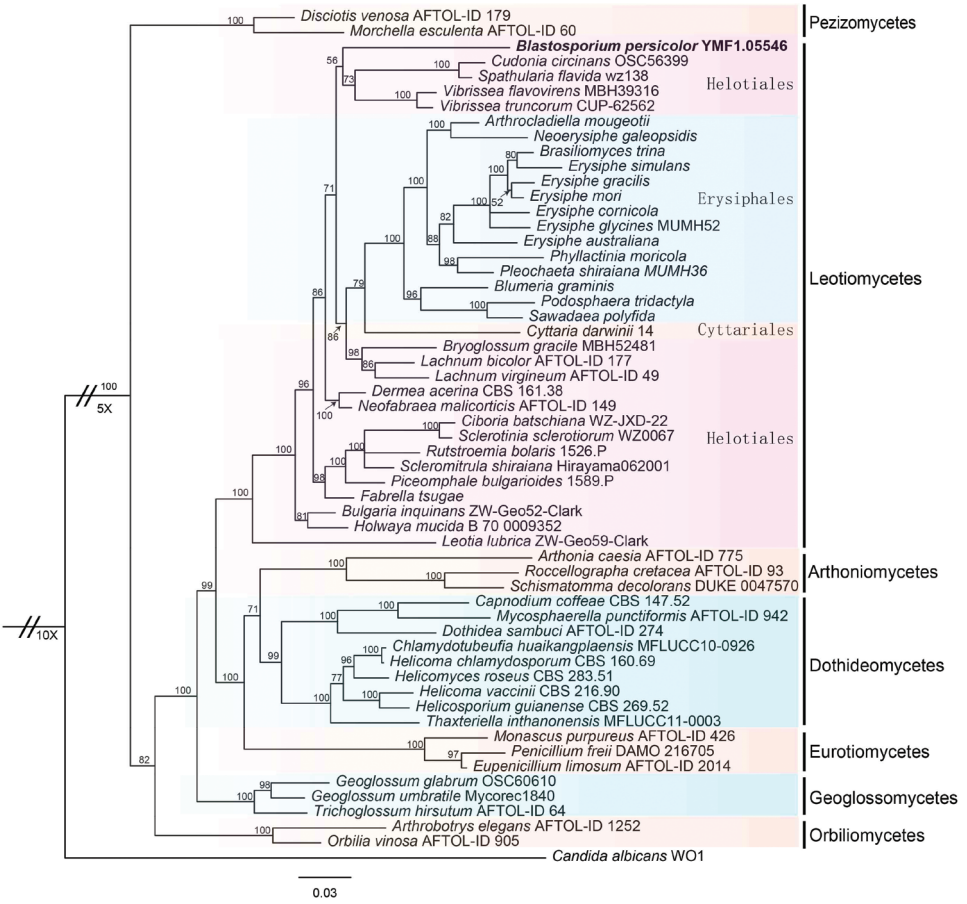

Figure 1. Phylogenetic tree based on Bayesian analysis of the combined LSU and SSU sequences. *Candida albicans* is used as outgroup. Bayesian bootstraps were indicated by the nodes and the scale bar shows the expected changes per site. The new genus proposed is in boldface.

families and 57 species with *Candida albicans* as outgroup. Other DNA sequences were obtained from the GenBank. The final alignment comprised a total of 1635 base pairs (TreeBASE accession number: 23451), which combined the SSU rRNA and LSU rRNA sequences and the dataset was analysed by the Bayesian Inference method. The topologies of the tree are shown with the Bayesian posterior probabilities values for clades of analyses (Figure 1). In this tree, the new genus is phylogenetically placed in the Leotiomycetes. This monophyletic group formed a close relationship with several genera, which are grouped in this class, e.g. *Vibrissea flavovirens* and *Vibrissea truncorum* (Vibrisseaceae), *Cudonia circinans* and *Spathularia flavida* (Cudoniaceae) that are grouped with the new genus in the same clade. Therefore, analysis of partial LSU and SSU nuc rDNA sequences placed the new genus in the Leotiomycetes. Additionally, the tree also supports the fact that the Helotiales is not monophyletic.

Taxonomy

Blastosporium Z. F. Yu & H. Zheng, gen. nov.

MycoBank MB828280

Etymology. Latin, *Blasto-*, referring to the blastic conidial ontogeny, + Latin, *sporium*, referring to the conidia.

Type species. *Blastosporium persicolor* Z. F. Yu & H. Zheng

Diagnosis. Characterised by mono- and polyblastic, integrated or discrete conidiogenous cells, solitary or blastocatenate, unicellular, obovoid, oblong, ellipsoidal, allantoid conidia (5–8 × 2.3–4.1 μm). Differs from the genus *Tetracladium* De Wild. by macronematous or semi-macronematous conidiophores and mono- and polyblastic conidiogenous cells.

Description. Mycelium partly superficial and partly immersed, composed of branched, septate, smooth, hyaline hyphae. *Conidiophores* macronematous or semi-macronematous, erect or prostrate, smooth, hyaline, sometimes reduced to conidiogenous cells. *Conidiogenous cells* mono- and polyblastic, terminal, integrated or discrete, determinate, sometimes with sympodial elongations, smooth, hyaline. *Conidia* solitary or blastocatenate, acrogenous, unicellular, obovoid, oblong, ellipsoidal, allantoid, broad fusiform to irregular, smooth, hyaline.

Distribution. China.

Notes. *Blastosporium* is superficially similar to the genera, *Acaromyces* Boekhout et al. and *Meira* Boekhout et al. Their conidiophores are reduced to conidiogenous cells, which produce solitary or sometimes blastocatenate, unicellular, hyaline conidia by blastic conidial ontogeny. These genera are yeast-like hyphomycetes that have been connected phylogenetically with Exobasidiomycetidae (Ustilaginomycetes, Basidiomycota) (Boekhout et al. 2003, Seifert et al. 2011).

Hyphozyma de Hoog & M.T.Sm. also superficially resembles *Blastosporium*, but *Hyphozyma* is a typical yeast-like hyphomycete, characterised by undifferentiated conidiophores and conidia are unicellular, hyaline, solitary or produced in basipetal chains (de Hoog and Smith 1981, Seifert et al. 2011).

Blastosporium persicolor Z. F. Yu & H. Zheng, sp. nov.

MycoBank MB828281

Figure 2

Etymology. Latin, *persicolor*, referring to the apricot colour of the colonies on PDA medium.

Description. Colonies on CMA with 1–2 concentric rings slightly curled, entire at the margin, light orange-yellowish-pinkish colour. Reverse yellowish-orange. Mycelium partly superficial and partly immersed, composed of branched, septate, smooth-walled, creeping, 2.0–3.3 μm wide hyphae. *Conidiophores* macronematous or semi-

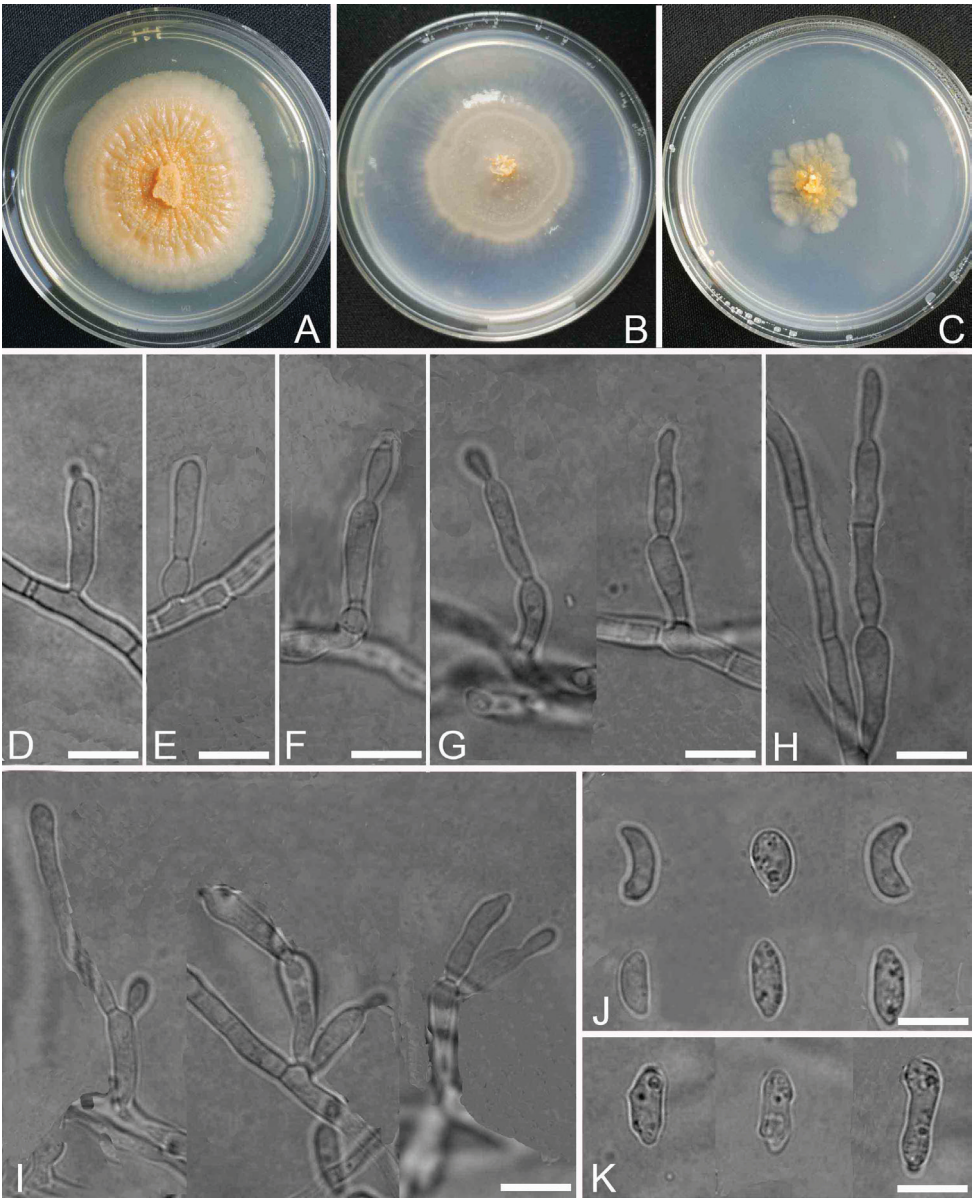

Figure 2. Cultures and anamorph of *Blastosporium persicolor* (YMF 1.05546). **A–C** Cultures (**A** on PDA **B** on CMA **C** on SNA) at 25 °C after 12 days **D–H** conidiophores and monoblastic conidiogenous cells **I** conidiophores and polyblastic conidiogenous cells **J, K** conidia (**J** one scar on conidia **K** multi-scars on conidia); Scale bar: 10 µm (**D–K**).

macronematous, mononematous, erect or prostrate, straight or flexuous, unbranched or slightly branched, hyaline, smooth-walled, 35–14.4 × 1.8–3.5 µm. *Conidiogenous cells* mostly monoblastic, sometime polyblastic after several sympodial elongations,

integrated or discrete, terminal or intercalary, 7.0–13.1× 2.6–3.3 μm, clavate or cylindrical, with a distinct or inconspicuous denticle at the conidiogenous loci. *Conidia* solitary or blastocatenate, acrogenous, obovoid, oblong, ellipsoidal, subcylindrical, allantoid, broad fusiform to irregular, slightly attenuated, truncate at the base or at the ends, unicellular, smooth, hyaline, 5–8 × 2.3–4.1 μm. Sexual form unknown.

Culture characteristics. (in darkness, at 25 °C after 10 d). Colonies attaining 1.5–1.7 cm diam. on PDA, 1.0–1.2 cm diam. on SNA, 1.5–1.7 cm on CMA. On PDA, colonies plicated, orange, reverse pale yellow, margin smooth and entire; sporulation abundant. On SNA, colonies flat, white to cream-coloured, flocculent, reverse white, growing slowly, sporulation abundant. The fungus does not grow at 35 °C on PDA, CMA and SNA.

Type. CHINA. Xiamen, Fujian Province, 24°33'9.6"N, 117°55'7.4"E, 23 m alt., from mildewed tobacco (*Nicotiana tabacum* L.) leaves, June 2018, Z.N. Zhang (dried slide YMFT 1.05546, holotype; ex-type YMF 1.05546).

Discussion

To determine the phylogenetic placement of this species, *Blastosporium persicolor* was analysed with species from 7 classes, Leotiomycetes, Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Orbiliomycetes, Pezizomycetes and Geoglossomycetes (Wang et al. 2006). By Bayesian analysis, the new genus was placed in the Helotiales, Leotiomycetes. In the tree, *B. persicolor* grouped with the *Cudonia-Spathularia* clade and *Vibrissea* clade, but the placement did not receive strong support. Therefore, we have temporarily designated this species as a new genus and family *incertae sedis*.

In the Helotiales, many genera, such as *Bulgaria* Fr. (Bulgariaceae), *Rutstroemia* P. Karst. (Rutstroemiaceae) and *Hegermila* Raitv. (Hyaloscyphaceae), were only observed as sexual morphs, but *Neofabraea* H.S. Jacks (Dermateaceae) and *Articulospora* Ingold (Helotiaceae) were observed as having asexual and sexual morphs (Chen et al. 2015, Wijayawardene et al. 2018, Wang et al. 2015a). In this study, we just observed the asexual morph of *B. persicolor*.

Based on ITS sequence data, *B. persicolor* is 88% similar to the genus *Tetracladium* De Wild. (*T. marchalianum* De Wild. as the type species), which was placed in the Helotiales and family incertae sedis. Moreover, *Blastosporium* shares some morphological features with *Tetracladium* as pale yellow and compact colonies and hyphae branched, septate and hyaline and both *Blastosporium* and *Tetracladium* sporulated abundantly on natural substrates (Sati et al. 2009, Wang et al. 2015b). However, *B. persicolor* is obviously distinct from the genus *Tetracladium* by the size and shape of conidia.

By molecular phylogeny analysis, *Blastosporium* belongs to the order Helotiales that currently contains 27 families (Wijayawardene et al. 2018). Moreover, members of the Helotiales cover a broad range of niches, such as plant pathogens, endophytes and aquatic hyphomycetes. *Blastosporium persicolor* was discovered from mildewed tobacco; therefore, it may be a plant pathogen.

Acknowledgements

This work was financed by the National Natural Science Foundation Program of PR China (31570023, 31770026). We are grateful to two reviewers for critically reviewing the manuscript and for providing helpful suggestions to improve this paper.

References

Boekhout T, Theelen B, Houbraken J, Robert V, Scorzetti G, Gafni A, Gerson U, Sztejnberg A (2003) Novel anamorphic mite-associated fungi belonging to the Ustilaginomycetes: *Meira geulakonigii* gen. nov., sp. nov., *Meira argovae* sp. nov. and *Acaromyces ingoldii* gen. nov., sp. nov. International Journal of Systematic and Evolutionary Microbiology 53(5): 1655–1664. https://doi.org/10.1099/ijs.0.02434-0

Chen C, Verkley GJ, Sun G, Groenewald JZ, Crous PW (2015) Redefining common endophytes and plant pathogens in *Neofabraea*, *Pezicula*, and related genera. Fungal Biology 120: 1291–1322. https://doi.org/10.1016/j.funbio.2015.09.013

de Hoog GS, Smith MT (1981) *Hyphozyma*, a new genus of yeast-like hyphomycetes. Antonie van Leeuwenhoek 47: 339–352. https://doi.org/10.1007/BF02350784

Eriksson OE (2005) Outline of Ascomycota-2005. Myconet 11: 1–113.

Gams W, Hoekstra ES, Aptroot A (1998) CBS course of mycology, 4th edn. Centraalbureau voor Schimmelcultures, Baarn.

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. https://doi.org/10.1007/978-1-4757-0905-6_31

Higgins DG (1994) CLUSTAL V: multiple alignment of DNA and protein sequences. Methods in Molecular Biology 25: 307–318. https://doi.org/10.1385/0-89603-276-0:307

Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP (1998) Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genetics and Biology 24(3): 298–309. https://doi.org/10.1006/fgbi.1998.1049

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth and Bisby's dictionary of the fungi (9th edn). CAB International, Wallingford.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sati SC, Arya P, Belwal M (2009) *Tetracladium nainitalense* sp. nov. a root endophyte from Kumaun Himalaya, India. Mycologia 101(5): 692–695. https://doi.org/10.3852/08-192

Seifert KA, Morgan-Jones G, Gams W, Kendrick B (2011) The genera of hyphomycetes. CBS Biodiversity Series 9.

Su HY, Udayanga D, Luo ZL, Manamgoda DS, Zhao YC, Yang J, Liu XY, Mckenzie EH, Zhou DQ, Hyde KD (2015) Hyphomycetes from aquatic habitats in Southern China: species of *Curvularia* (Pleosporaceae) and *Phragmocephala* (Melannomataceae). Phytotaxa 226(3): 201–216. https://doi.org/10.11646/phytotaxa.226.3.1

Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B, Arisan-Atac I, Strauss J, Samuels GJ, Börner T, Kubicek CP (1997) Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycological Research 101(4): 449–459. https://doi.org/10.1017/S0953756296002845

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. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang L, Sun X, Wei JG, Lou JF, Guo LD (2015a) A new endophytic fungus *Neofabraea illicii*, isolated from *Illicium verum*. Mycoscience 56(3): 332–339. https://doi.org/10.1016/j.myc.2014.10.002

Wang M, Jiang X, Wu W, Hao Y, Su Y, Cai L, Xiang M, Liu X (2015b) Psychrophilic fungi from the world's roof. Persoonia 34: 100–112. https://doi.org/10.3767/003158515X685878

Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS (2006) Toward a phylogenetic classification of the Leotiomycetes based on rdna data. Mycologia, 98(6): 1065–1075. https://doi.org/10.3852/mycologia.98.6.1065

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: a guide to methods and applications 18(1): 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK, Maharachchikumbura SS, Ekanayaka AH, Tian Q, Phookamsak R (2018) Outline of ascomycota: 2017. Fungal Diversity 88(1): 167–263. https://doi.org/10.1007/s13225-018-0394-8