


Article

Blackwellomyces kaihuaensis and Metarhizium putuoense (Hypocreales), Two New Entomogenous Fungi from Subtropical Forests in Zhejiang Province, Eastern China

Yi Li ^{1,*},[†] , Xin-Chang Zhao ^{1,†}, Li-Xia Wu ², Yu Wang ¹, Ao Xu ¹ and Wen-Fei Lin ^{3,*}

¹ School of Food Science and Engineering, Yangzhou University, Yangzhou 225127, China; xinchang_1998@163.com (X.-C.Z.); 18252721892@163.com (Y.W.); xxxxuao@163.com (A.X.)

² Kaihua Branch of Quzhou City Bureau of Ecology and Environment, Quzhou 324300, China; shuzi0603@163.com

³ Institute of Edible and Medicinal Fungi, College of Life Science, Zhejiang University, Hangzhou 310058, China

* Correspondence: liyi062@yzu.edu.cn (Y.L.); 0920158@zju.edu.cn (W.-F.L.)

[†] These authors contributed equally to this work.

Abstract: Entomogenous fungi are one of the most diverse fungal groups among ascomycetes. During investigations of macro-fungi biodiversity in Kaihua and Putuo in Zhejiang Province, China, in 2021, several entomopathogenic fungal specimens were collected from subtropical forests. After a careful examination of morphology and multi-gene phylogenetic analyses applying *nrSSU*, *nrLSU*, *tef1*, *rpb1* and *rpb2*, two novel species were discovered, described herein as *Blackwellomyces kaihuaensis* and *Metarhizium putuoense*, and further compared with other similar species. Detailed descriptions, color photographs of fresh specimen and figures illustrating microscopic features of the two new species are provided.

Keywords: biodiversity; *Cordyceps* s. l.; Cordycipitaceae; Clavicipitaceae



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1. Introduction

Entomogenous fungi are those microorganisms which can infect and kill arthropods. They are commonly found in soil and are thought to play important ecological roles, not only as a regulator of arthropod populations, but also because they form complex relationships with plants, either as an endophyte or epiphyte [1]. A few species such as *Beauveria* Vuill., *Metarhizium* Sorokin, *Isaria* Pers. and *Lecanicillium* W. Gams & Zare have been used as biocontrol agents against a wide range of insect pests in forest, field and greenhouse environments [2]. All species in the hypocrealean families *Cordycipitaceae* and *Ophiocordycipitaceae* and some species in the family *Clavicipitaceae* are considered as entomopathogenic.

Blackwellomyces Spatafora & Luangsa-ard and *Metarhizium* are two genera that belong to the families *Cordycipitaceae* and *Clavicipitaceae*, respectively. The genus *Blackwellomyces* was segregated from *Cordyceps* Fr. and erected by the unique characters of the ascospore, which has irregularly spaced septa and does not disarticulate into part-spores at maturity [3]. Seven species of this genus have been described in Thailand, the United States and Japan [4–7]. Only one distribution record has been found from Sichuan Province within China for the genus, but without presenting any supporting specimen or sequence [8]. The genus *Metarhizium* was first described by Metchnikoff [9] and is one of the most ubiquitous genera of entomogenous fungi with diverse asexual morphologies and life cycles [10–12]. Due to the killing ability of various insect pests, certain species in this genus such as *Metarhizium anisopliae* (Metschn.) Sorokin have been widely used as eco-friendly biocontrol agents and have attracted global research interest [13,14]. Up until now, 77 species have been included in the Index Fungorum in *Metarhizium* (<http://www.indexfungorum.org/Names/Names.asp>, accessed on 20 October 2023). In China, a total of 24 species

have been described (<https://nmdc.cn/fungarium/fungi/chinadirectories>, accessed on 20 October 2023).

Zhejiang Province is located in the eastern coastal area of China; it has a humid subtropical climate and is characterized by a high forest coverage rate, making it particularly suitable for the growth of entomogenous fungi. About forty hypocrealean entomopathogenic species have been recorded in this province according to the Checklist of Fungi in China Database (<https://nmdc.cn/fungarium/fungi/chinadirectories>, accessed on 20 October 2023), mostly belonging to the genera *Cordyceps* and *Ophiocordyceps* Petch. The two genera of *Blackwellomyces* and *Metarhizium* have not been documented yet in the province. During field trips researching macro-fungi in Zhejiang Province in 2021, a good number of entomopathogenic fungal specimens were collected and identified. After a detailed morphological comparison and molecular studies, two novel species in the genera *Blackwellomyces* and *Metarhizium* were recognized and described in this study.

2. Materials and Methods

2.1. Field Collection

Specimens were collected from subtropical forests during field expeditions to Zhejiang Province, China, in March and June 2021. Strains were isolated from fresh specimens using potato dextrose agar (PDA) medium at room temperature and were then brought to the laboratory for further purification. After isolation, the fresh specimens were dried with silica gel. Dried specimens were deposited in the Fungarium (HMAS), Institute of Microbiology, Chinese Academy of Sciences.

2.2. Morphological Observation

Notes of stomata color and size were taken on fresh specimens. Color codes followed Kornerup and Wanscher (1981) [15]. Sections of ascomata were sliced by hand and mounted in a 5% KOH solution. Microscopic characters were observed using an Axio Imager A2 microscope (Carl Zeiss, Jena, Germany) with an Olympus XC50 digital camera (Olympus Corporation, Tokyo, Japan) and measured with Microsuite special edition software 3.1 (Soft Imaging Solutions GmbH, Münster, Germany).

2.3. DNA Isolation, PCR Amplification and Sequencing

DNA was extracted from either specimen or cultural mycelium. A method using Chelex-100 (Bio-Rad, Hercules, CA, USA) was applied for DNA isolation if dried specimen was used [16]. A modified CTAB method was otherwise applied if the fresh mycelium from cultured PDA plates was used for DNA isolation.

Six DNA fragments including internal transcribed spacers (ITS), the nuclear ribosomal small and large subunit subunit DNA (*nrSSU* and *nrLSU*), translation elongation factor 1- α (*tef1*), RNA polymerase II largest subunit (*rpb1*) and RNA polymerase II second largest subunit (*rpb2*) were amplified and sequenced for species identification and phylogenetic analysis. Primer pairs of ITS5/ITS4 [17], NS1/NS4 [17], LROR/LR5 [18], 983F/2218R [19], CRPB1/RPB1Cr [20] and fRPB2-5F/fRPB2-7cR [21] were used for PCR amplification and sequencing of ITS, *nrSSU*, *nrLSU*, *tef1*, *rpb1* and *rpb2*, respectively. PCR condition followed the above cited literatures except for *rpb2*, which was carried out as: 5 min of denaturation at 95 °C; 35 cycles at 95 °C for 1 min, 53 °C for 1 min, 72 °C for 2 min; and a final extension at 72 °C for 10 min. PCR amplification was performed in a thermocycler (GeneAmp R_PCR System 9700, Applied Biosystems, Foster City, CA, USA) and a total volume of 50 μ L reaction system was used containing: 25 μ L 2 \times Taq PCR Master Mix (Tiangen Biotech Co., Ltd., Beijing, China), 0.5 μ L of each primer (10 pM), 1 μ L template DNA and 23 μ L sterile deionized water. The PCR products were first visualized on 1.2% agarose gels and positive PCR amplicons were sent for sequencing in both directions. Sequences obtained in this study have been deposited in GenBank under accession numbers OQ980401–OQ980407 (*tef1*), OQ980408 (*rpb2*), OQ980409–OQ980414 (*rpb1*), OQ981961–OQ981967 (ITS), OQ981968–OQ981974 (*nrLSU*) and OQ981975–OQ981981 (*nrSSU*) (Table 1).

2.4. Phylogenetic Analyses

Phylogenetic analyses applied five genes of *nrSSU*, *nrLSU*, *tef1*, *rpb1* and *rpb2*, which were widely used in the phylogenetic classification of clavicipitaceous fungi [22] and in the genus *Metarhizium* [23]. Available sequences of the genera *Blackwellomyces* and *Metarhizium* were retrieved from GenBank, especially those used in Mongkolsamrit et al. (2020a,b) [7,23], and then aligned with sequences generated in this study using the software ClustalW version 1.81 [24]. The alignment was manually edited using BioEdit ver. 7.2.5 [25]. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) with the combined five-gene dataset comprising *nrSSU*, *nrLSU*, *tef1*, *rpb1* and *rpb2*. The ML analysis was conducted with RAxML version 7.2.6 [26] using the GTRGAMMA model to obtain the best tree as well as the bootstrap supports (BS), which were assessed with 1000 replicates. Bayesian analyses were implemented with MrBayes v3.2.6. Two independent analyses of two parallel runs and four chains were carried out for 2,500,000 generations. Trees were sampled every 1000 generations; the first 20% were discarded as burn-in. Phylogenetic analyses of the two genera of *Blackwellomyces* and *Metarhizium* were constructed separately using the same approach. *Gamszarea wallacei* (H.C. Evans) Z.F. Zhang and L. Cai & *Cordyceps cylindrical* Petch were chosen as outgroups in phylogenetic reconstructions of *Blackwellomyces* and *Metarhizium*, respectively. Node supports greater than 70% in ML and above 0.95 in BI were shown on branches.

Table 1. Voucher information for materials used in phylogenetic analyses in this study. Bold: sequences obtained in this study; ^T: type specimen.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>Ascopolyporus polychrous</i>	P.C. 546	Bolivia	Coccoidea	–	DQ118737	DQ118745	DQ127236	–	[27]
<i>A. villosus</i>	ARSEF 6355	Panama	Coccoidea	–	AY886544	DQ118750	DQ127241	–	[27]
<i>Blackwellomyces aurantiacus</i>	BCC 85060 ^T	Thailand	Lepidoptera (larva)	–	MT003028	MK411598	MK411600	MT017819	[7]
<i>B. aurantiacus</i>	BCC 85061	Thailand	Lepidoptera (larva)	–	MT003029	MK411599	MK411601	MT017820	[7]
<i>B. calendulinus</i>	BCC 68500	Thailand	Coleoptera (larva)	–	MT003030	MT017842	MT017802	MT017821	[7]
<i>B. calendulinus</i>	BCC 68502 ^T	Thailand	Coleoptera (larva)	–	MT003031	MT017843	MT017803	MT017822	[7]
<i>B. cardinalis</i>	OSC 93610	USA	Lepidoptera (larva)	AY184974	AY184963	EF469059	EF469088	EF469106	[4]
<i>B. cardinalis</i>	CBS 113411 ^T	USA	Lepidoptera (larva)	NG_013131	MH874496	–	–	–	[28]
<i>B. cardinalis</i>	OSC 93609	USA	Lepidoptera (larva)	AY184973	AY184962	DQ522325	DQ522370	DQ522422	[4]
<i>B. cardinalis</i>	OSC 93619	Japan	Lepidoptera (larva)	AY184975	AY184964	–	–	–	[4]
<i>B. cardinalis</i>	OSC 93620	Japan	Lepidoptera (larva)	AY184976	AY184965	–	–	–	[4]
<i>B. kaihuaensis</i>	HMAS 285455 ^T	China	Lepidoptera (larva)	OQ981975	OQ981968	OQ980401	OQ980409	OQ980408	This study
<i>B. kaihuaensis</i>	HMAS 285456	China	Lepidoptera (larva)	OQ981976	OQ981969	OQ980402	OQ980410	–	This study
<i>B. lateris</i>	MFLU 18-0663 ^T	Thailand	Lepidoptera (larva)	NG_067678	NG_067857	MK069471	MK084615	MK079354	[6]
<i>B. minutus</i>	BCC 88269 ^T	Thailand	Coleoptera (larva)	–	MT003032	MT017844	MT017804	MT017823	[7]
<i>B. pseudomilitaris</i>	TBRC 3662	Thailand	Lepidoptera (larva)	–	MT003036	MT017848	MT017808	–	[7]
<i>B. pseudomilitaris</i>	BCC 73634	Thailand	Lepidoptera (larva)	–	–	MT017849	MT017809	MT017827	[7]
<i>B. pseudomilitaris</i>	BCC 1919 ^T	–	Lepidoptera (larva)	MF416588	MF416534	MF416478	–	MF416440	[3]
<i>B. pseudomilitaris</i>	BCC 2091	–	Lepidoptera (larva)	MF416589	MF416535	MF416479	–	MF416441	[3]
<i>B. pseudomilitaris</i>	NBRC 101409	Thailand	Lepidoptera (larva)	JN941748	JN941393	–	JN992482	–	[29]
<i>B. pseudomilitaris</i>	NBRC 101410	Thailand	Lepidoptera (larva)	JN941747	JN941394	–	JN992481	–	[29]
<i>B. pseudomilitaris</i>	NBRC 101411	Thailand	Lepidoptera (larva)	JN941746	JN941395	–	JN992480	–	[29]
<i>B. pseudomilitaris</i>	NBRC 101413	Thailand	Lepidoptera (larva)	JN941745	JN941396	–	JN992479	–	[29]
<i>B. roseostromatus</i>	BCC 91358 ^T	Thailand	Lepidoptera (larva)	–	MT003033	MT017845	MT017805	MT017824	[7]
<i>B. roseostromatus</i>	BCC 91359	Thailand	Lepidoptera (larva)	–	MT003034	MT017846	MT017806	MT017825	[7]
<i>B. roseostromatus</i>	BCC 91360	Thailand	Lepidoptera (larva)	–	MT003035	MT017847	MT017807	MT017826	[7]
<i>Lecanicillium psalliotae</i>	CBS 101270	UK	Soil	EF469128	EF469081	EF469066	EF469095	EF469113	[30]
<i>Gamszarea wallacei</i>	CBS 101237	Indonesia	Lepidoptera (pupa)	NG_062646	NG_042398	EF469073	EF469102	EF469119	[30]
<i>Aschersonia badia</i>	BCC 8105	Thailand	Hemiptera: scale insect	DQ522537	DQ518752	DQ522317	DQ522363	DQ522411	[31]
<i>A. placenta</i>	BCC 7869	Thailand	Hemiptera: scale insect	EF469121	EF469074	EF469056	EF469085	EF469104	[30]
<i>Balansia henningsiana</i>	A.E.G. 96-27a	USA	Poaceae: <i>Panicum</i> sp.	AY545723	AY545727	AY489610	AY489643	DQ522413	[20]
<i>B. pilulaeformis</i>	A.E.G. 94-2	–	Poaceae	AF543764	AF543788	DQ522319	DQ522365	DQ522414	[30]
<i>Claviceps fusiformis</i>	ATCC 26019	–	Poaceae	DQ522539	U17402	DQ522320	DQ522366	–	[30]
<i>C. paspali</i>	ATCC 13892	–	Poaceae	U32401	U47826	DQ522321	DQ522367	DQ522416	[30]

Table 1. Cont.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>C. purpurea</i>	S.A. cp11	–	Poaceae	EF469122	EF469075	EF469058	EF469087	EF469105	[30]
<i>Cordyceps cylindrica</i>	CBS 744.73	Japan	Arachnida: spider	EF468987	EF468841	EF468786	EF468892	–	[30]
<i>Hypocrella schizostachyi</i>	BCC 14123	Thailand	Hemiptera	DQ522557	DQ518771	DQ522346	DQ522392	DQ522447	[30]
<i>H. nectrioides</i>	GJS 89-104	–	Hemiptera: scale insect	–	DQ518772	DQ522347	DQ522393	DQ522448	[31]
<i>Keithomyces acicularis</i>	JCM 33284	Japan	Soil	LC435738	LC435741	LC462188	–	–	[32]
<i>K. acicularis</i>	JCM 33285	Japan	Soil	LC435739	LC435742	LC462189	–	–	[32]
<i>K. carneus</i>	CBS 239.32	France	Sand dune	EF468988	EF468843	EF468789	EF468894	EF468938	[31]
<i>K. carneus</i>	CBS 399.59	USA	Soil	EF468989	EF468842	EF468788	EF468895	EF468939	[31]
<i>K. neogunnii</i>	BUM 415	China	Lepidoptera (larva)	MH143845	MH143828	MH143861	MH143876	MH143891	[33]
<i>K. neogunnii</i>	GZUH SB13050305	China	Lepidoptera (larva)	KU729724	–	KU729729	KU729734	–	[34]
<i>Keithomyces</i> sp.	CBS 126563	Tanzania	Soil	MT078871	MT078856	MT078848	MT078864	MT078921	[23]
<i>Keithomyces</i> sp.	CBS 127407	USA	Soil	MT078873	MT078858	MT078850	MT078866	MT078923	[23]
<i>Marquandomyces marquandii</i>	CBS 127132	USA	Soil	MT078872	MT078857	MT078849	MT078865	MT078922	[23]
<i>M. marquandii</i>	CBS 129413	USA	Soil	MT078874	MT078859	MT078851	MT078867	–	[23]
<i>M. marquandii</i>	CBS 182.27	USA	Soil	EF468990	EF468845	EF468793	EF468899	EF468942	[30]
<i>Metacordyceps chlamydosporia</i>	CBS 101244	–	Diplopoda: egg of slug	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424	[31]
<i>M. chlamydosporia</i>	CBS 504.66	Canada	Soil	AF339593	AF339544	EF469069	EF469098	EF469120	[30]
<i>Metapochonia bulbillosa</i>	CBS 145.70	Denmark	<i>Picea abies</i>	AF339591	AF339542	EF468796	EF468902	EF468943	[30]
<i>M. goniodes</i>	CBS 891.72	Germany	Fungi	AF339599	AF339550	DQ522354	DQ522401	DQ522458	[30]
<i>M. rubescens</i>	CBS 464.88	Scotland	Nematode eggs	AF339615	AF339566	EF468797	EF468903	EF468944	[30]
<i>Metarhizium acridum</i>	ARSEF 7486	Niger	Orthoptera	–	–	EU248845	EU248897	EU248925	[10]
<i>M. album</i>	ARSEF 2082	Indonesia	Hemiptera	DQ522560	DQ518775	DQ522352	DQ522398	DQ522452	[30]
<i>M. alvesii</i>	CG1123	Brazil	Soil	–	–	KY007614	KY007612	KY007613	[35]
<i>M. anisopliae</i>	ARSEF 7487	Ethiopia	Orthoptera	–	–	DQ463996	DQ468355	DQ468370	[10]
<i>M. anisopliae</i>	CBS 130.71	Ukraine	Avena sativa	MT078868	MT078853	MT078845	MT078861	MT078918	[23]
<i>M. argentiniense</i>	CEP424	Argentina	Blaberidae: Epilamprinae	–	–	MF966624	MF966625	MF966626	[36]
<i>M. atrovirens</i>	TNM F10184	Japan	Coleoptera	JF415950	JF415966	–	JN049884	–	[11]
<i>M. baoshanense</i>	BUM 63.4	China	Soil	KY264178	KY264175	KY264170	KY264181	KY264184	[37]
<i>M. baoshanense</i>	CCTCCM 2016589	China	Soil	KY264177	KY264174	KY264169	KY264180	KY264183	[37]
<i>M. bionidarum</i>	CBS 648.67	France	Coleoptera	–	–	LC126075	LC125907	LC125923	[38]
<i>M. bionidarum</i>	NBRC 112661	Japan	Diptera	–	–	LC126076	LC125908	LC125924	[38]
<i>M. biotecense</i>	BCC 51812 ^T	Thailand	Hemiptera: Delphacidae	MN781937	MN781838	MN781693	MN781745	MN781792	[23]
<i>M. biotecense</i>	BCC 51813	Thailand	Hemiptera: Delphacidae	MN781938	MN781839	MN781694	MN781746	MN781793	[23]

Table 1. Cont.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>M. blattodeae</i>	MY00896	Thailand	Blattodea	HQ165657	HQ165719	HQ165678	HQ165739	HQ165638	[12]
<i>M. brachyspermum</i>	CM1	Japan	Coleoptera	–	LC469749	LC469751	–	–	[39]
<i>M. brasiliense</i>	ARSEF 2948	Brazil	Hemiptera	–	–	KJ398809	KJ398620	–	[40]
<i>M. brittlebankisoides</i>	Hn1	China	Coleoptera	–	–	AB778556	AB778555	AB778554	[41]
<i>M. brunneum</i>	ARSEF 2107	USA	Coleoptera	–	–	EU248855	EU248907	EU248935	[10]
<i>M. candelabrum</i>	BCC 29224 ^T	Thailand	Hemiptera: leafhopper	MN781952	MN781853	MN781708	MN781755	MN781804	[23]
<i>M. cercopidarum</i>	BCC 31660 ^T	Thailand	Hemiptera: leafhopper	MN781953	MN781854	MN781709	MN781756	MN781805	[23]
<i>M. chaiyaphumense</i>	BCC 78198	Thailand	Hemiptera: Cicadidae	KX369596	KX369593	KX369592	KX369594	KX369595	[12]
<i>M. chaiyaphumense</i>	BCC 28241	Thailand	Hemiptera: Cicadidae	MN781932	MN781831	MN781684	MN781740	MN781784	[23]
<i>M. cicadae</i>	BCC 48696	Thailand	Hemiptera: Cicadidae	MN781948	MN781848	MN781703	–	MN781800	[23]
<i>M. cicadae</i>	BCC 48881 ^T	Thailand	Hemiptera: Cicadidae	MN781949	MN781849	MN781704	MN781752	–	[23]
<i>M. clavatum</i>	BCC 84543 ^T	Thailand	Coleoptera (larva)	–	MN781834	MN781689	MN781741	MN781789	[23]
<i>M. clavatum</i>	BCC 84558	Thailand	Coleoptera (larva)	–	MN781835	MN781690	MN781742	–	[23]
<i>M. culicidarum</i>	BCC 7625	Thailand	Diptera: Culicidae	–	MN781850	MN781705	–	MN781801	[23]
<i>M. culicidarum</i>	BCC 2673	Thailand	Diptera: Culicidae	MN781950	MN781851	MN781706	MN781753	MN781802	[23]
<i>M. culicidarum</i>	BCC 7600 ^T	Thailand	Diptera: Culicidae	MN781951	MN781852	MN781707	MN781754	MN781803	[23]
<i>M. cylindrosporum</i>	RCEF 3632	China	Hemiptera: Cicadidae	JF415964	JF415987	JF416022	–	–	[11]
<i>M. cylindrosporum</i>	TNS 16371	Japan	Hemiptera: Cicadidae	JF415963	JF415986	JF416027	JN049902	–	[11]
<i>M. dendrolimatilis</i>	GZAC-IFR1006	China	Lepidoptera	–	–	KT166031	KT961694	KT166032	[42]
<i>M. eburneum</i>	BCC 79267	Thailand	Lepidoptera (pupa)	–	MN781826	–	MN781735	–	[23]
<i>M. eburneum</i>	BCC 79252 ^T	Thailand	Lepidoptera (pupa)	–	MN781829	MN781682	MN781736	–	[23]
<i>M. ellipsoideum</i>	BCC 49285 ^T	Thailand	Hemiptera (adult)	MN781957	MN781858	MN781713	MN781759	MN781808	[23]
<i>M. ellipsoideum</i>	BCC 12847	Thailand	Hemiptera (adult)	MN781959	MN781860	MN781715	MN781761	MN781810	[23]
<i>M. ellipsoideum</i>	BCC 53509	Thailand	Hemiptera (adult)	MN781958	MN781859	MN781714	MN781760	MN781809	[23]
<i>M. flavoviride</i>	CBS 218.56	Czech Republic	Coleoptera	–	–	KJ398787	KJ398598	–	[40]
<i>M. flavoviride</i>	CBS 125.65	USA	Soil	MT078869	MT078854	MT078846	MT078862	MT078919	[23]
<i>M. flavum</i>	BCC 90870 ^T	Thailand	Coleoptera (larva)	MN781965	MN781874	MN781731	MN781776	MN781822	[23]
<i>M. flavum</i>	BCC 90874	Thailand	Coleoptera (larva)	MN781966	MN781875	MN781732	MN781777	MN781823	[23]
<i>M. frigidum</i>	ARSEF 4124	Australia	Coleoptera	–	–	DQ464002	DQ468361	DQ468376	[43]
<i>M. fusoidium</i>	BCC 41242	Thailand	Psocoptera	MN781942	MN781825	MN781679	–	MN781780	[23]
<i>M. fusoidium</i>	BCC 53130	Thailand	Psocoptera	MN781943	MN781843	MN781698	–	MN781795	[23]
<i>M. fusoidium</i>	BCC 28246 ^T	Thailand	Lepidoptera	MN781944	MN781844	MN781699	MN781749	MN781796	[23]
<i>M. gaoligongense</i>	BUM 3.5	China	Soil	KY087810	KY087814	KY087818	KY087822	–	[44]
<i>M. gaoligongense</i>	CCTCCM 2016588	China	Soil	KY087812	KY087816	KY087820	KY087824	KY087826	[44]
<i>M. globosum</i>	ARSEF 2596	India	Lepidoptera	–	–	EU248846	EU248898	EU248926	[11]
<i>M. granulomatis</i>	UAMH 11028 ^T	Denmark	<i>Chamaeleo calypttratus</i>	HM635076	HM195304	KJ398781	–	–	[45]

Table 1. Cont.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>M. granulomatis</i>	UAMH 11176	Denmark	<i>Chamaeleo calyptratus</i>	–	HM635078	KJ398782	KJ398593	–	[45]
<i>M. gryllidicola</i>	BCC 37918	Thailand	Orthoptera: Gryllidae	MN781935	MN781836	MN781691	MN781743	MN781790	[46]
<i>M. gryllidicola</i>	BCC 82988	Thailand	Orthoptera: Gryllidae	MK632117	MK632091	MK632062	MK632166	MK632143	[46]
<i>M. guizhouense</i>	ARSEF 6238	China	Lepidoptera	–	–	EU248857	EU248909	EU248937	[10]
<i>M. guizhouense</i>	CBS 258.90	China	Lepidoptera	–	–	EU248862	EU248914	EU248942	[10]
<i>M. huainamdangense</i>	BCC 32190	Thailand	Hemiptera: leafhopper	MN781954	MN781855	MN781710	MN781757	–	[23]
<i>M. huainamdangense</i>	BCC 44270 ^T	Thailand	Hemiptera: leafhopper	MN781956	MN781857	MN781712	–	MN781807	[23]
<i>M. huainamdangense</i>	BCC 7672	Thailand	Hemiptera: leafhopper	MN781955	MN781856	MN781711	MN781758	MN781806	[23]
<i>M. humberi</i>	IP46	Brazil	Soil	–	–	MH837574	MH837556	MH837565	[47]
<i>M. indigoticum</i>	TNS F18553	Japan	Lepidoptera	JF415953	JF415968	JF416010	JN049886	JF415992	[11]
<i>M. indigoticum</i>	TNS F18554	Japan	Lepidoptera	JF415952	JF415969	JF416011	JN049887	JF415993	[11]
<i>M. kalasinense</i>	BCC 53582	Thailand	Coleoptera (larva)	KC011175	KC011183	KC011189	–	–	[12]
<i>M. koreanum</i>	ARSEF 2039	Republic of Korea	Hemiptera: Delphacidae	–	–	KJ398806	KJ398616	–	[40]
<i>M. koreanum</i>	BCC 27998	Thailand	Hemiptera: Fulgoromorpha	MN781945	MN781845	MN781700	–	MN781797	[23]
<i>M. koreanum</i>	BCC 30455	Thailand	Hemiptera: Fulgoromorpha	MN781946	MN781846	MN781701	MN781750	MN781798	[23]
<i>M. lepidiotae</i>	ARSEF 7488	Australia	Coleoptera	–	–	EU248865	EU248917	EU248945	[10]
<i>M. majus</i>	ARSEF 1015	Japan	Lepidoptera	–	–	EU248866	EU248918	EU248946	[10]
<i>M. majus</i>	ARSEF 1914	Philippines	Coleoptera	–	–	EU248868	EU248920	EU248948	[10]
<i>M. megapomponiae</i>	BCC 25100 ^T	Thailand	Hemiptera: Megopomponia	MN781947	MN781847	MN781702	MN781751	MN781799	[23]
<i>M. minus</i>	ARSEF 2037	Philippines	Hemiptera	AF339580	AF339531	DQ522353	DQ522400	DQ522454	[30]
<i>M. minus</i>	ARSEF 1099	Philippines	Hemiptera	–	–	KJ398799	KJ398608	KJ398706	[40]
<i>M. niveum</i>	BCC 52400 ^T	Thailand	Hemiptera: Cicadidae	MN781933	MN781832	MN781685	–	MN781785	[23]
<i>M. nornnoi</i>	BCC 19364	Thailand	Lepidoptera (larva)	MN781940	MN781841	MN781696	MN781747	–	[23]
<i>M. nornnoi</i>	BCC 25948 ^T	Thailand	Coleoptera (adult beetle)	MN781941	MN781842	MN781697	MN781748	–	[23]
<i>M. novozealandicum</i>	ARSEF 4661	Australia	Soil	–	–	KJ398811	KJ398622	–	[40]
<i>M. novozealandicum</i>	ARSEF 4674	Australia	Soil	–	–	KJ398812	KJ398623	–	[40]
<i>M. ovoidosporum</i>	BCC 29223	Thailand	Hemiptera: Cercopidae	MN781960	MN781861	MN781716	MN781762	–	[23]
<i>M. ovoidosporum</i>	BCC 32600 ^T	Thailand	Hemiptera: Eurybrachidae	MN781961	MN781862	MN781717	MN781763	–	[23]
<i>M. ovoidosporum</i>	BCC 7634	Thailand	Hemiptera (adult)	MN781962	MN781863	MN781718	MN781764	MN781811	[23]
<i>M. owariensis</i>	NBRC 33258	Japan	Hemiptera	HQ165669	HQ165730	HQ165689	HQ165747	–	[12]
<i>M. pemphigi</i>	ARSEF 7491	UK	Hemiptera	–	–	KJ398819	KJ398629	DQ468379	[40]
<i>M. pemphigi</i>	ARSEF 6569	UK	Hemiptera	–	–	KJ398813	KJ398624	DQ468378	[40]
<i>M. phasmatodeae</i>	BCC 49272	Thailand	Orthoptera: Phasmatodea	MK632119	MK632093	MK632064	–	MK632145	[46]
<i>M. phasmatodeae</i>	BCC 2841	Thailand	Orthoptera: Phasmatodea	MN781931	MN781828	MN781681	MN781738	MN781782	[46]
<i>M. phuwiangense</i>	BCC 78206	Thailand	Coleoptera (adult)	–	–	MN781719	MN781765	MN781812	[23]
<i>M. phuwiangense</i>	BCC 85068	Thailand	Coleoptera (adult)	–	MN781864	MN781720	MN781766	MN781813	[23]

Table 1. Cont.

Species	Voucher No.	Location	Host	nrSSU	nrLSU	tef1	rpb1	rpb2	References
<i>M. phuwiangense</i>	BCC 85069 ^T	Thailand	Coleoptera (adult)	–	MN781865	MN781721	MN781767	MN781814	[23]
<i>M. pingshaense</i>	CBS 257.90	China	Coleoptera	–	–	EU248850	EU248902	EU248930	[10]
<i>M. prachinense</i>	BCC 47950	Thailand	Lepidoptera	KC011172	KC011180	KC011186	KC011184	–	[12]
<i>M. prachinense</i>	BCC 47979	Thailand	Lepidoptera	KC011173	KC011181	KC011187	KC011185	–	[12]
<i>M. pseudoatrovirens</i>	TNS F16380	Japan	Coleoptera	–	JF415977	–	JN049893	JF415997	[11]
<i>M. purpureogenum</i>	MAFF 243305	Japan	Soil	–	AB700552	LC126078	LC125913	LC125920	[38]
<i>M. purpureogenum</i>	MAFF 244762	Japan	Soil	–	–	LC126079	LC125911	LC125922	[38]
<i>M. purpureonigrum</i>	BCC 89247 ^T	Thailand	Coleoptera (larva)	–	–	MN781725	MN781771	MN781817	[23]
<i>M. purpureonigrum</i>	BCC 89249	Thailand	Coleoptera (larva)	MN781963	MN781869	MN781726	MN781772	MN781818	[23]
<i>M. purpureonigrum</i>	BCC 89248	Thailand	Coleoptera (larva)	MN781964	MN781870	MN781727	–	MN781819	[23]
<i>M. purpureum</i>	BCC 82173	Thailand	Coleoptera (larva)	–	MN781866	MN781722	MN781768	MN781815	[23]
<i>M. purpureum</i>	BCC 82642 ^T	Thailand	Coleoptera (larva)	–	MN781867	MN781723	MN781769	MN781816	[23]
<i>M. purpureum</i>	BCC 83548	Thailand	Coleoptera (larva)	–	MN781868	MN781724	MN781770	–	[23]
<i>M. putuoense</i>	HMAS 285457 ^T	China	Coleoptera (larva)	OQ981977	OQ981970	OQ980403	OQ980411	–	This study
<i>M. putuoense</i>	HMAS 285458	China	Coleoptera (larva)	OQ981978	OQ981971	OQ980404	OQ980412	–	This study
<i>M. putuoense</i>	HMAS 285459	China	Coleoptera (larva)	OQ981979	OQ981972	OQ980405	OQ980413	–	This study
<i>M. putuoense</i>	HMAS 285460	China	Coleoptera (larva)	OQ981980	OQ981973	OQ980406	OQ980414	–	This study
<i>M. putuoense</i>	LY 280	China	Coleoptera (larva)	OQ981981	OQ981974	OQ980407	–	–	This study
<i>M. reniforme</i>	IndGH96	Philippines	–	HQ165670	HQ165732	–	–	HQ165649	[12]
<i>M. reniforme</i>	ARSEF 429	Philippines	Orthoptera: Tettigoniidae	HQ165671	HQ165733	HQ165690	–	HQ165650	[12]
<i>M. rileyi</i>	CBS 806.71	USA	Trichoplusia ni	AY526491	–	EF468787	EF468893	EF468937	[30]
<i>M. rileyi</i>	NBRC 8560	Japan	Lepidoptera	HQ165667	HQ165729	HQ165688	–	–	[12]
<i>M. robertsii</i>	ARSEF 4739	Australia	Soil	–	–	EU248848	EU248900	EU248928	[10]
<i>M. samlanense</i>	BCC 17091	Thailand	Hemiptera (adult)	HQ165665	HQ165727	HQ165686	–	HQ165646	[12]
<i>M. samlanense</i>	BCC 39752	Thailand	Hemiptera (adult)	MN781939	MN781840	MN781695	–	MN781794	[12]
<i>M. sulphureum</i>	BCC 36585	Thailand	Lepidoptera (larva)	–	–	MN781686	–	MN781786	[23]
<i>M. sulphureum</i>	BCC 39045	Thailand	Lepidoptera (larva)	MK632120	MK632095	MK632066	–	MK632147	[23]
<i>M. sulphureum</i>	BCC 36592 ^T	Thailand	Lepidoptera (larva)	–	–	MN781687	–	MN781787	[23]
<i>M. takense</i>	BCC 30939	Thailand	Hemiptera	HQ165659	HQ165721	–	HQ165741	HQ165640	[12]
<i>M. takense</i>	BCC 30934	Thailand	Hemiptera	HQ165658	HQ165720	HQ165679	HQ165740	HQ165639	[12]
<i>M. viride</i>	CBS 659.71	–	<i>Chameleo lateralis</i>	HQ165673	HQ165735	HQ165692	–	HQ165652	[12]
<i>M. viridulum</i>	BCC 36261	Thailand	Hemiptera: Cicadidae	MN781930	MN781827	MN781680	MN781737	MN781781	[23]
<i>M. viridulum</i>	ARSEF 6927	Taiwan	Hemiptera	–	–	KJ398815	–	–	[40]
<i>Myriogenospora atramentosa</i>	A.E.G. 96-32	–	Plant	AY489701	AY489733	AY489628	AY489665	DQ522455	[30]
<i>Nigelia aurantiaca</i>	BCC 19950	Thailand	Lepidoptera	GU979934	GU979943	GU979952	GU979961	GU979967	[12]
<i>N. aurantiaca</i>	BCC 37621	Thailand	Lepidoptera	GU979937	GU979946	GU979955	GU979964	GU979970	[12]

Table 1. Cont.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>N. martialis</i>	EFCC 6863	Republic of Korea	Lepidoptera	–	JF415975	JF416016	–	JF415995	[12]
<i>N. martialis</i>	HMAS 197472	China	Coleoptera: Cerambycidae	JF415955	JF415973	JF416015	JN049892	JF415994	[12]
<i>Papiliomyces liangshanensis</i>	EFCC 1452	Republic of Korea	Lepidoptera	EF468962	EF468815	EF468756	–	–	[30]
<i>P. liangshanensis</i>	EFCC 1523	Republic of Korea	Lepidoptera	EF468961	EF468814	EF468755	–	EF468918	[30]
<i>P. shibinensis</i>	GZUH SB13050311	China	Lepidoptera	KR153588	–	KR153589	KR153590	–	[48]
<i>Petchia siamensis</i>	BCC 68420	Thailand	Ootheca of Mantidae	MK632113	MK632087	–	MK632163	MK632140	[46]
<i>P. siamensis</i>	BCC 73636	Thailand	Ootheca of Mantidae	MK632115	MK632089	MK632060	–	MK632138	[46]
<i>Purpureocillium lavendulum</i>	FMR 10376	Venezuela	Soil	–	FR775489	FR775516	FR775512	–	[49]
<i>P. lilacinus</i>	CBS 284.36	USA	Soil	AY526475	FR775484	EF468792	EF468898	EF468941	[30]
<i>P. takamizusanense</i>	NHJ 3582	Thailand	Hemiptera: Cicadidae	EU369097	EU369034	EU369015	–	–	[50]
<i>Purpureomyces khaoyaiense</i>	BCC 1376	Thailand	Lepidoptera (larva)	KX983468	KX983462	KX983457	–	KX983465	[12]
<i>P. khaoyaiensis</i>	BCC 14290	Thailand	Lepidoptera (larva)	–	JF415970	KJ398797	JN049888	–	[11]
<i>P. maesotensis</i>	BCC 88441	Thailand	Lepidoptera (larva)	–	MN781877	MN781734	MN781779	MN781824	[23]
<i>P. maesotensis</i>	BCC 89300 ^T	Thailand	Lepidoptera (larva)	–	MN781876	MN781733	MN781778	–	[23]
<i>P. pyriformis</i>	BCC 85074 ^T	Thailand	Lepidoptera (larva)	–	MN781873	MN781730	MN781775	MN781821	[23]
<i>P. pyriformis</i>	BCC 85348	Thailand	Lepidoptera (larva)	–	MN781871	MN781728	MN781773	MN781820	[23]
<i>P. pyriformis</i>	BCC 85349	Thailand	Lepidoptera (larva)	–	MN781872	MN781729	MN781774	–	[23]
<i>Regiocrella camerunensis</i>	ARSEF 7682	–	Hemiptera	–	DQ118735	DQ118743	DQ127234	–	[51]
<i>Rotiferophthora angustispora</i>	CBS 101437	–	Bdelloid rotifers	AF339584	AF339535	AF543776	DQ522402	DQ522460	[30]
<i>Shimizuomyces paradoxus</i>	EFCC 6279	Republic of Korea	<i>Smilax sieboldii</i>	EF469131	EF469084	EF469071	EF469100	EF469117	[30]
<i>S. paradoxus</i>	EFCC 6564	Republic of Korea	<i>Smilax sieboldii</i>	EF469130	EF469083	EF469072	EF469101	EF469118	[30]
<i>Sungia yongmunensis</i>	EFCC 2131	Republic of Korea	Lepidoptera	EF468977	EF468833	EF468770	EF468876	–	[30]
<i>S. yongmunensis</i>	EFCC 2135	Republic of Korea	Lepidoptera	EF468979	EF468834	EF468769	EF468877	–	[30]
<i>Torrubiella luteorostrata</i>	NHJ 11343	Thailand	Hemiptera: scale insect	EF468995	EF468850	EF468801	EF468906	–	[30]

Table 1. Cont.

Species	Voucher No.	Location	Host	<i>nrSSU</i>	<i>nrLSU</i>	<i>tef1</i>	<i>rpb1</i>	<i>rpb2</i>	References
<i>T. luteostrata</i>	NHJ 12516	Thailand	Hemiptera: scale insect	EF468994	EF468849	EF468800	EF468905	EF468946	[30]
<i>T. petchii</i>	NHJ 6240	Thailand	Hemiptera: scale insect	EU369103	EU369038	EU369022	EU369060	EU369082	[50]
<i>T. petchii</i>	NHJ 6209	Thailand	Hemiptera: scale insect	EU369104	EU369039	EU369023	EU369061	EU369081	[50]
<i>T. tenuis</i>	NHJ 6293	Thailand	Hemiptera: scale insect	EU369112	EU369044	EU369029	EU369068	EU369087	[50]
<i>T. tenuis</i>	NHJ 345.01	Thailand	Hemiptera: scale insect	EU369111	EU369045	EU369030	–	EU369088	[50]
<i>Tyrannicordyceps fratricida</i>	TNS 19011	–	Fungi	JQ257022	JQ257023	JQ257028	JQ257016	JQ257021	[52]
<i>Yosiokobayasia kusanaginensis</i>	TNS F18494	Japan	Coleoptera	JF415954	JF415972	JF416014	JN049890	–	[11]

3. Results

3.1. Phylogeny

The combined five-gene dataset for phylogenetic reconstruction of the genus *Blackwellomyces* comprises 28 taxa and includes 4832 nucleotide positions, among which 768 are variable and 434 informative. The topology of *Blackwellomyces* revealed by ML and BI was almost identical, despite slight differences in statistical support for certain branches (Figure 1). The monophyly of the genus *Blackwellomyces* is strongly supported (BS = 100%, PP = 1.00). In the phylogeny (Figure 1), the new collections from China (HMAS 285455 and HMAS 285456) are grouped together with *B. aurantiacus* Mongkols., Noisrip., Himaman & Luangsa-ard and *B. lateris* Y.P. Xiao, T.C. Wen & K.D. Hyde, two species that hailed from Thailand.

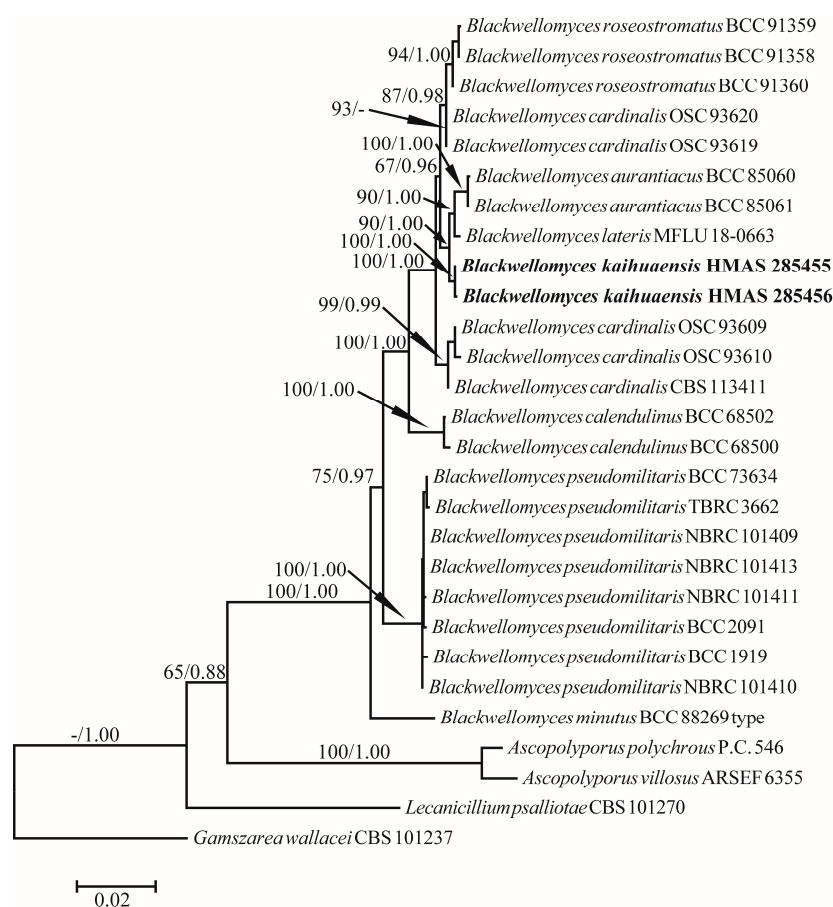


Figure 1. Maximum likelihood (ML) phylogenetic tree of *Blackwellomyces* inferred from five-gene (nrSSU, nrLSU, *tef1*, *rpb1* and *rpb2*) dataset. Values above branches are ML bootstrap proportions (BP, left) and Bayesian posterior probabilities (PP, right). BP values < 50% and PP values < 0.70 were not shown.

The five-gene dataset used for phylogenetic analysis for the genus *Metarhizium* includes 178 taxa and 4832 characters (base pairs) among which 1866 were variable and 1474 were parsimony informative. Both ML and BI analyses provided high supports for the terminal clades at the species/genus level except for *Metarhizium*, which formed a monophyletic group in ML but was poorly supported (Figure 2). The monophyly of *Metarhizium* was not supported in BI analyses as *Nigelia* Luangsa-ard, Tasan. & Thanak. merged into the clade. Sequences of the five collections (HMAS 285457, HMAS 285458, HMAS 285459, HMAS 285460 and LY 280) included in this study were identical. They formed a highly supported clade (BS = 100%, PP = 1.00) and showed a close sister relationship to *Metarhizium flavum* Luangsa-ard, Mongkols., Thanakitp. & Samson (Figure 2).

3.2. Taxonomy

Blackwellomyces kaihuaensis Yi Li, X.C. Zhao, A. Xu & W.F. Lin, sp. nov. (Figure 3).

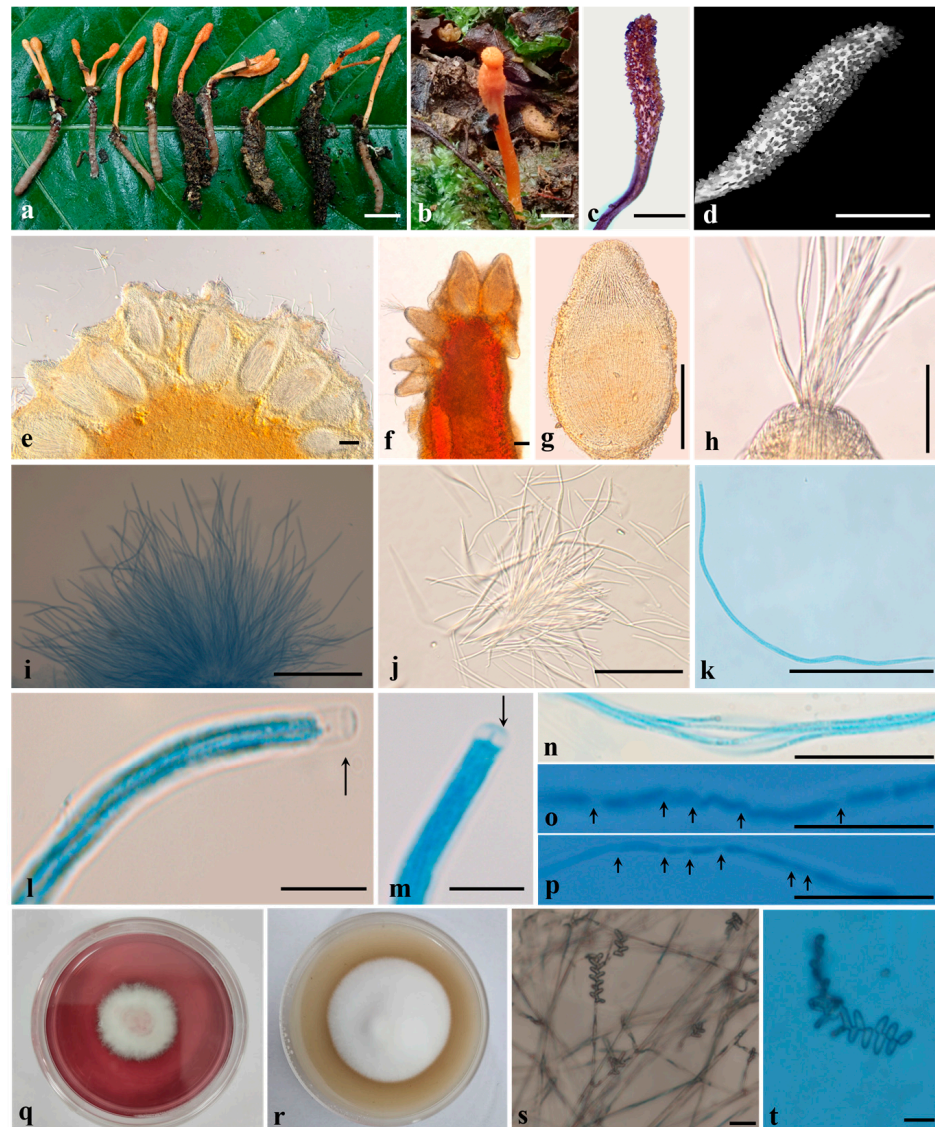


Figure 3. Macro- and microscopic features of *Blackwellomyces kaihuaensis* (HMAS 285455, holotype): (a,b) stromata; (c,d) fertile part; (e–g) perithecia; (h–k) asci; (l,m) ascus tip, arrows indicate apical cap; (n–p) ascospores with septations (arrows); (q) colony grows for 10 days on PDA; (r) colony grows for 10 days on OA; (s,t) arrangement of conidia. Scale bars: (a) = 5 mm; (b–d) = 2 mm; (e–k), (n–p) = 100 μ m; (l,m), (s,t) = 10 μ m.

Fungal Names: FN 571325.

Etymology—The epithet “kaihuaensis” refers to the type locality of Kaihua County, Quzhou City, Zhejiang Province, China, where the species was originally found.

Diagnosis—Differs from the closest species, *B. lateris*, by the shorter length of stromata and fertile head, stromata in the side face and the existence of septa. It also differs from another closely related species, *B. aurantiacus*, with shorter lengths of asci and ascospores.

Holotype—China, Zhejiang Province, Kaihua County, Linyuan Village, 29°10′21.89″ N, 118°36′20.80″ E, 343 m alt., on a lepidopteran larva buried in soil under mixed broadleaf-conifer forest, 29 May 2021, Yi Li and Ao Xu, HMAS 285455. GenBank accession numbers: ITS = OQ981961, nrSSU = OQ981975, nrLSU = OQ981968, tef1 = OQ980401, rpb1 = OQ980409, rpb2 = OQ980408.

Description—Sexual morph: *Stromata*: single or multiple, typically unbranched, 6–12 mm in length and 0.8–1.2 mm in width, orange-red to bright red, cylindrical to clavate. Rhizoids flexuous, emerging from the head area of lepidoptera larva, 5–25 mm below ground. Fertile part bright reddish-orange, clavate or palmated, 1.5–4.0 mm in length and 1.0–1.6 mm in width. Perithecia semi-immersed, narrowly ovoid, (280–)320–370(–410) × (100–)120–180(–200) μm. Asci eight-spored, cylindrical, (140–)170–240(–270) × 3.5–4.5 μm, with a 3–4 mm thick hemispheric top. Ascospores filiform, hyaline, irregularly multiseptated, (130–)160–220(–250) × 1.0–1.5 μm, do not separate into part-spores when they reach maturity. —**Asexual morph:** Colonies growing with white to purplish red on PDA, cottony with a high mycelium density and 20 mm in diameter after 10 days at 25 °C, purplish pigment spreading in PDA and no pigment in OA. Phialides *Evlachovaea*-like (conidia arranged in alternate orientations with a zipper-like arrangement), arising from aerial hyphae, solitary or in whorls of 2–5 on each branch, swollen at the base, 8–15 × 1.5–2.5 μm, tapering from the base to a thin neck, 5–10 × 1 μm. Conidia hyaline, ovoid to cylindrical, smooth, one-celled, 3–4 × 1.5–2.0 μm.

Additional specimen examined—China, Zhejiang Province, Kaihua County, Zaodi Village, 29°21′54.41″ N, 118°17′30.55″ E, 293 m alt, under mixed broadleaf–conifer forest, 24 May 2021, Jiao-Jiao Lu and Wen-Fei Lin, HMAS 285456. GenBank accession numbers: ITS = OQ981962, nrSSU = OQ981976, nrLSU = OQ981969, tef1 = OQ980402, rpb1 = OQ980410.

Host: larvae of Pyralidae (Lepidoptera).

Known distribution: Zhejiang Province, China.

Metarhizium putuoense Yi Li, X.C. Zhao, Yu Wang & W.F. Lin, sp. nov. (Figure 4).

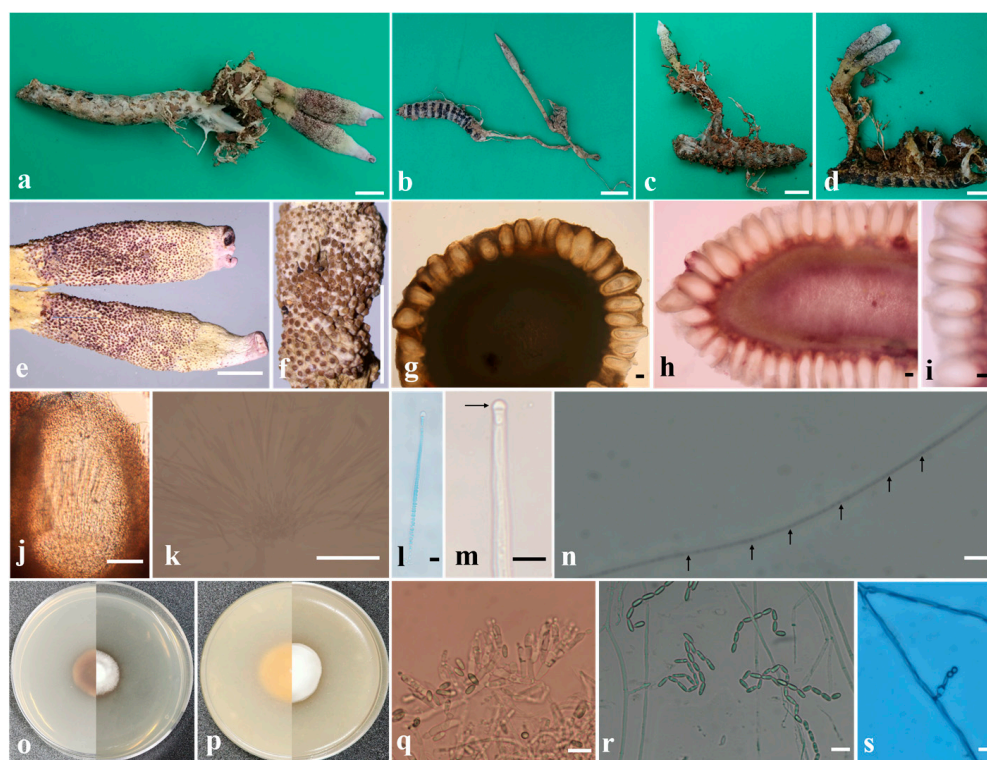


Figure 4. Macro- and microscopic features of *Metarhizium putuoense* (HMAS 285457, holotype): (a–d) stromata; (e,f) fertile part; (g–j) perithecia; (k,l) asci; (m) ascus tip, arrow indicates apical cap; (n) ascospores with septations (arrows); (o) colony grows for 14 days on PDA; (p) colony grows for 14 days on OA; (q) germination of conidia; (r) conidia; (s) chlamydospores. Scale bars: (a–f) = 5 mm; (g–k) = 100 μm; (l–n), (q–s) = 10 μm.

Fungal Names: FN 571581.

Etymology—The epithet “putuoense” refers to the type locality of Putuo District, Zhoushan City, Zhejiang Province, China, where the species was originally found.

Diagnosis—Differs from closely related species, i.e., *M. flavum*, *M. purpureonigrum* Luangsa-ard, Tasan., Thanakitp. & Samson and *M. purpureum* Luangsa-ard, Mongkols., Lamlerthton, Thanakitp. & Samson by the pale yellow to light brown stomata with an infertile upper part.

Holotype—China, Zhejiang Province, Zhoushan City, Putuo District, Taohua Island, 29°47′06.57″ N, 122°18′09.94″ E, 183 m alt., on a coleoptera larva buried in soil of bare road slopes on the edge of a broad-leaved forest, 16 June 2021, Yi Li, Xin-Chang Zhao, Yu Wang and Wen-Fei Lin, HMAS 285457. GenBank accession numbers: ITS = OQ981963, *nrSSU* = OQ981977, *nrLSU* = OQ981970, *tef1* = OQ980403, *rpb1* = OQ980411.

Description—**Sexual morph:** *Stromata* single or multiple, unbranched or dichotomous, 25–60 mm long and 2–4 mm broad, pale yellow to light brown, usually clavate. *Rhizoids* flexuous, arising from the head or abdominal region of coleoptera larvae buried 2–4 cm deep underground. Fertile part light brown to pale purple, clavate or irregularly shaped, 10–15 mm long, 2–4 mm broad, the top is purple and infertile, *Perithecia* immersed, narrowly ovoid, (420–)450–500(–530) × (220–)250–300(–330) μm. *Asci* eight-spored, cylindrical, (190)210–260(280) × 5–7 μm, with a 6–8 × 5–7 μm hemispheric top. *Ascospores* filiform, hyaline, irregularly multiseptated, (170–)190–240(–260) × 1.0–1.5 μm, do not separate into part-spores when they reach maturity. —**Asexual morph:** *Colonies* on OA or PDA grow somewhat slowly and reach a diameter of 15–20 mm in 21 days at 25 °C, with low mycelium density, velvety, flocculent margin, white at the beginning of growth, the center of the colony produces green conidia after 14 days, reverse pale brown on PDA and yellow on OA. *Phialides* *Metarhizium*-like, arising from aerial hyphae, with 2–3 phialides per branch, wine bottle shape, 7–11 × 1.5–3.0 μm, swollen at the base and tapering suddenly into a thin neck 0.5–0.8 μm wide. *Chlamydospores* present, singly or multiple in short chains, subglobose, up to 5 μm in diameter. *Conidia* arranged in chains, smooth, oval, olive green, one-celled, 6–7 × 3–4 μm.

Additional specimen examined: China, Zhejiang Province, Zhoushan City, Putuo District, Taohua Island, 29°49′39.26″ N, 122°17′40.60″ E, 130 m alt., on a coleoptera larva buried in soil of bare road slopes on the edge of a broad-leaved forest, 17 June 2021, Yi Li, Xin-Chang Zhao, Yu Wang and Wen-Fei Lin, HMAS 285458 (GenBank accession numbers: ITS = OQ981964, *nrSSU* = OQ981978, *nrLSU* = OQ981971, *tef1* = OQ980404, *rpb1* = OQ980412), HMAS 285459 (GenBank accession numbers: ITS = OQ981965, *nrSSU* = OQ981979, *nrLSU* = OQ981972, *tef1* = OQ980405, *rpb1* = OQ980413), HMAS 285460 (GenBank accession numbers: ITS = OQ981966, *nrSSU* = OQ981980, *nrLSU* = OQ981973, *tef1* = OQ980406, *rpb1* = OQ980414).

Host: larvae of *Campsosternus* sp. (Coleoptera).

Known distribution: Zhejiang Province, China.

4. Discussion

Nearly 75% of Zhejiang province is covered in mountains and 61.24% is covered by forest, with a typically humid subtropical climate, making it suitable for the growth of entomogenous fungi. However, the biodiversity of this particular fungal group has not been studied in-depth in this region. During the investigation in the past two years, over 70 specimens have been collected with more than 15 species recognized (unpublished data). The present study reported two novel species, *B. kaihuaensis* and *M. putuoense*, based on morphological studies and five-gene phylogenies. The species biodiversity of this fungal group was considered far more rich than is currently known in this province.

Since the genus *Blackwellomyces* was segregated from *Cordyceps*, seven species have been described [3], including five species recorded from Thailand, i.e., *B. pseudomilitaris* (Hywel-Jones & Sivichai) Spatafora & Luangsa-ard, *B. lateri*, *B. aurantiacus*, *B. calendulinus* Mongkols., Noisrip., Khons. & Luangsa-ard, *B. minutus* Mongkols., Noisrip., Himaman & Luangsa-ard and *B. roseostromatus* Mongkols., Noisrip., Khonsanit & Luangsa-ard [5–7] and *B. cardinalis* (G.H. Sung & Spatafora) Spatafora & Luangsa-ard, which has been recorded in the eastern United States, southeastern Japan, Republic of Korea and Sichuan Province in

China [4,8,52]. The new species *B. kaihuaensis* described in this study is supported as most closely related to *B. lateris* and *B. aurantiacus* by the five-gene phylogeny (Figure 1). The species differs from *B. lateris* by the shorter length of stromata, the fertile head with stromata in the side face and the existence of septa. *Blackwellomyces lateris* was reported to possess aseptate ascospores [6], which is different from all the known species and contradicts the concept of the genus; the genus is diagnosed by the irregularly multiseptated ascospores which do not disarticulate into part-spores at maturity [3]. The new species also differs from *B. aurantiacus* in lengths of asci and ascospores; the later species possesses longer asci and ascospores of $350 \times 4\text{--}5 \mu\text{m}$ and $(200\text{--})235\text{--}295\text{--}(330) \times 1.0\text{--}1.5 \mu\text{m}$, respectively, compared with $(140\text{--})170\text{--}240\text{--}(270) \times 3.5\text{--}4.5 \mu\text{m}$ and $(130)160\text{--}220(250) \times 1\text{--}1.5 \mu\text{m}$ for *B. kaihuaensis*. Notably, the five specimens of the species *B. cardinalis* did not form a monophyletic clade. Three collections from the United States clustered together and showed an earlier origination, whereas the other two collections from Japan showed a more recent speciation and a close relationship with *B. roseostromatus* (Figure 1). It is apparently necessary to check the materials that identified as *B. cardinalis*.

Metarhizium is a group of well-known insect pathogens with various sexual and asexual morphological traits [10,12]. In this study, 178 taxa and 66 species were selected for phylogenetic analysis, and it was found that *M. putuoense* formed a distinct lineage and was most closely related to *M. flavum*, *M. purpureonigrum* and *M. purpureum*, all of which were found in Thailand [23]. *Metarhizium putuoense* has pale yellow to light brown stomata with a purple, infertile upper part while *M. flavum* has pale yellow to olive yellow stomata with a fertile upper part. Moreover, the species differ from each other in the length of their asci and ascospores. *Metarhizium putuoense* has longer asci and ascospores than *M. flavum*. Another difference between the two species is that *M. putuoense* produced chlamydospores when cultured on PDA plates, while *M. flavum* produced chlamydospores only on SDAY/4 but not on PDA. *Metarhizium putuoense* also differs from *M. purpureonigrum* and *M. purpureum* in the color of the stromata. The latter two species have purple to dark stromata with a fertile upper part, while *M. putuoense* has pale yellow to light brown stromata with an infertile upper part.

Finally, it is noteworthy that the new species *B. kaihuaensis* is found in troops, i.e., a large number of (>100) individuals were found in a relatively small (approximately 50 m²) region. In other words, the host insects can occur in high density and will probably cause outbreaks; in the meantime, a good number of Lepidoptera larva have been killed by the fungus. The fungus showed a good killing ability and can be considered as a potential biocontrol agent of forest pests, even though the host species has not been identified yet at the species level.

Entomopathogenic fungi, with special emphasis on the species in the order Hypocreales, widely exist in almost all terrestrial ecosystems, especially in tropical [53] and subtropical forests [22]. It is thought that entomogenous fungi may help to maintain stability in forest ecosystems as they are not only involved in the regulation of arthropod populations [53], but also act as endophytes assisting the plant host with growth, pathogen resistance and tolerance to environmental challenges such as drought and fire [54]. On the other hand, knowledge of the interactions between entomopathogenic fungi and its host insects, and plants in certain cases, is deficient. In addition, it is frequently observed that multiple strains belonging to different species have been isolated simultaneously in a single fungal-insect association. The phenomenon of hyperparasitism has often been reported particularly in the genus *Pleurocordyceps* Y.J. Yao, Y.H. Wang, S. Ban, W.J. Wang, Yi Li, Ke Wang & P.M. Kirk (also known as *Polycephalomyces Kobayasi*) [55]. A clear understanding of this interaction between insects and their fungal parasites requires more solvable technologies such as pyrosequencing, genome sequencing and pan-genome analysis, rather than just using a regular strategy of strain isolation, species identification and description only.

5. Conclusions

In the present study, two novel species were introduced based on a detailed morphologic study and multi-gene phylogenetic analyses from subtropical forests in Zhejiang Province, China, as *Blackwellomyces kaihuaensis* and *Metarhizium putuoense*, respectively.

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