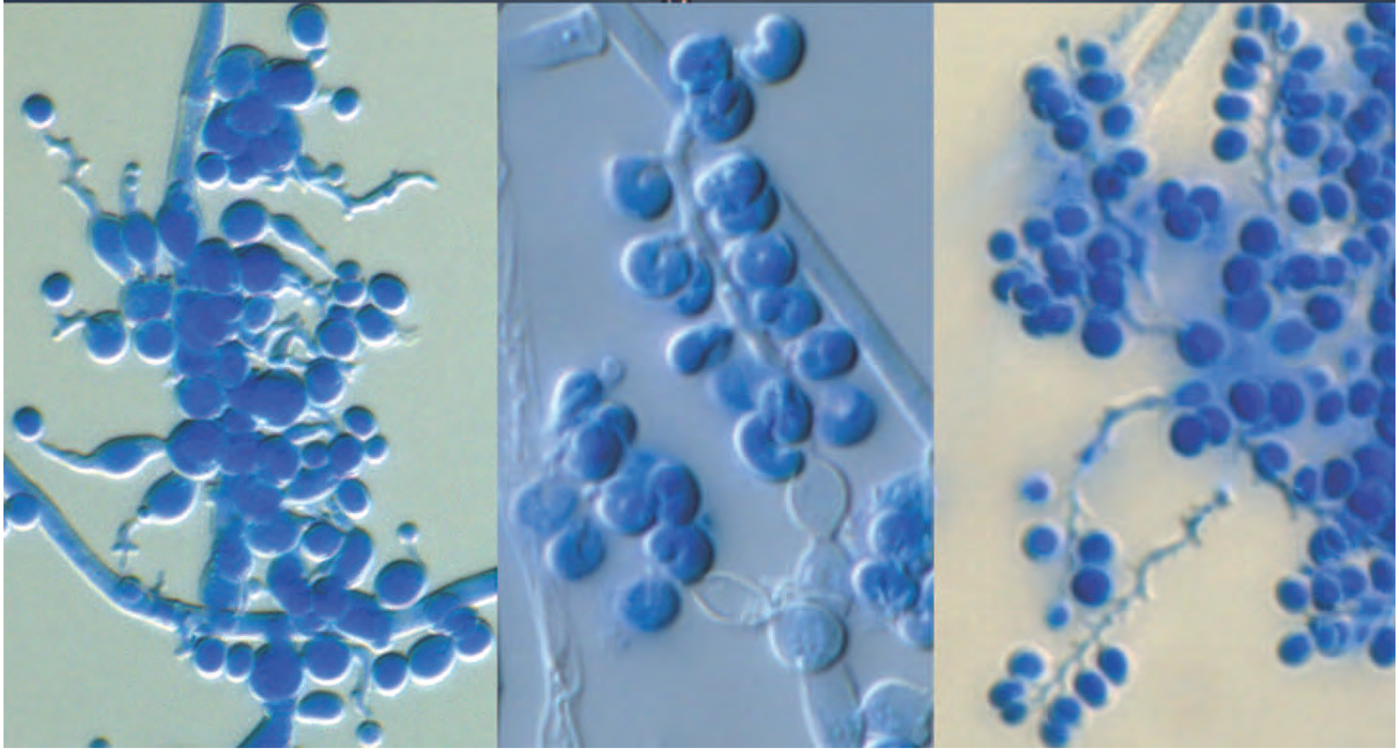
MYCOLOGIA

Vol. 103 No. 5

September
October 2011



OFFICIAL BIMONTHLY PUBLICATION *of the* MYCOLOGICAL SOCIETY OF AMERICA

Mycologia, 103(5), 2011, pp. 1055–1073. DOI: 10.3852/10-302

Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*

Stephen A. Rehner[1]
Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland 20705

Andrew M. Minnis
Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland 20705

Gi-Ho Sung
Mushroom Research Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon, 404-707, Republic of Korea

J. Jennifer Luangsa-ard
Phylogenetics Laboratory, National Center for Genetic Engineering and Biotechnology, Science Park, Pathum Thani, Thailand

Luis Devotto
Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Chillán, Chile

Richard A. Humber
Biological Integrated Pest Management, Research, Robert Holley Center for Agriculture and Health, USDA-ARS, Tower Road, Ithaca, New York 14853

Abstract: *Beauveria* is a cosmopolitan anamorphic genus of arthropod pathogens that includes the agronomically important species, *B. bassiana* and *B. brongniartii,* which are used as mycoinsecticides for the biological control of pest insects. Recent phylogenetic evidence demonstrates that *Beauveria* is monophyletic within the Cordycipitaceae (Hypocreales), and both *B. bassiana* and *B. brongniartii* have been linked developmentally and phylogenetically to *Cordyceps* species. Despite recent interest in the genetic diversity and molecular ecology of *Beauveria*, particularly as it relates to their role as pathogens of insects in natural and agricultural environments, the genus has not received critical taxonomic review for several decades. A multilocus phylogeny of *Beauveria* based on partial sequences of *RPB1*, *RPB2*, *TEF* and the nuclear intergenic region, Bloc, is presented and used to assess diversity within the genus and to evaluate species concepts and their taxonomic status. *B. bassiana* and *B. brongniartii*, both which represent species complexes and which heretofore have lacked type specimens, are redescribed and types are proposed. In addition six new species are described including *B. varroae* and *B. kipukae*, which form a biphyletic, morphologically cryptic sister lineage to *B. bassiana*, *B. pseudobassiana*, which also is morphologically similar to but phylogenetically distant from *B. bassiana*, *B. asiatica* and *B. australis*, which are sister lineages to *B. brongniartii*, and *B. sungii*, an Asian species that is linked to an undetermined species of *Cordyceps*. The combination *B. amorpha* is validly published and an epitype is designated.

Key words: Ascomycota, *Beauveria bassiana* complex, *B. brongniartii* complex, cryptic species, insect fungi, phylogeny

INTRODUCTION

Beauveria is a cosmopolitan anamorphic genus of soilborne facultative necrotrophic arthropod-pathogenic fungi (Roberts and Hajek 1992, Goettel et al. 2005) that occur also as saprotrophs and plant endophytes (Vega et al. 2008). Worldwide numerous registered mycoinsecticide formulations based on *B. bassiana* and *B. brongniartii* are used for control of insect pests (Faria and Wraight 2007). In addition *Beauveria* produces a diverse array of biologically active secondary metabolites that include non-peptide pigments and polyketides, nonribosomally synthesized peptide antibiotics and other secreted metabolites implicated in insect pathogenesis and virulence that have potential industrial, pharmaceutical and agricultural uses (Vey et al. 2001).

Genus *Beauveria* is characterized morphologically by globose to flask-shaped conidiogenous cells from which one-celled, terminal holoblastic conidia are produced in sympodial succession on an indeterminate, denticulate rachis. *Beauveria* species are distinguished principally according to characteristics of their conidia, which are typically smooth-walled, hyaline, 1.5–5.5 µm and globose to cylindrical or vermiform.

In the most recent taxonomic treatment of the genus, de Hoog (1972) recognized three species including *B. bassiana*, *B brongniartii* and *B. alba* (Limber) Saccas (now *Engyodontium album* (Limber) de Hoog (de Hoog 1978). Four additional *Beauveria* species have been recognized since including *B. vermiconia* de Hoog & Rao (1975), *B. amorpha* Samson & Evans (1982), *B. caledonica* Bisset &

Submitted 30 Sep 2010; accepted for publication 14 Feb 2011.

[1] Corresponding author. E-mail: stephen.rehner@ars.usda.gov

Widden (1986) and *B. malawiensis* Rehner et al. (2006b). Although previously classified with other anamorphic taxa in the now defunct Deuteromycota, cultural studies have linked *B. bassiana* and *B. brongniartii* developmentally to *Cordyceps* Fr. teleomorphs (Shimazu et al. 1988, Li et al. 2001, Huang et al. 2002) and molecular phylogenetic analyses confirm the placement of *Beauveria* within Cordycipitaceae (Hypocreales) (Sung et al. 2007). A combined ITS and *TEF* phylogeny of the genus (Rehner and Buckley 2005) corroborated its monophyly and delineated seven well supported terminal lineages corresponding to *B. amorpha*, *B. bassiana*, *B. brongniartii*, *B. caledonica* and *B. vermiconia* and two novel lineages, clade C, which is phylogenetically distinct from but morphologically similar to *B. bassiana*, and clade E, a taxon associated with a *Cordyceps* teleomorph. In addition this phylogenetic analysis also revealed that *B. bassiana* and *B. brongniartii* each embody cryptic phylogenetic species complexes. In particular *B. bassiana* includes an as yet undetermined number of cryptic lineages, many with intercontinental distributions (Rehner et al. 2006a, Ghikas et al. 2010, Rehner unpubl), and which occur as multispecies assemblages in both natural and agricultural habitats (Rehner et al. 2006a, Meyling et al. 2009).

As background to the present study, 1192 *Beauveria* strains mostly from the ARSEF culture collection (TABLE I) and representing all continents except Antarctica were analyzed for species diversity with Bloc, a nuclear intergenic region developed as a phylogenetic marker for *Beauveria* (Rehner et al. 2006a). Here a 68-strain ingroup dataset encompassing the range of phylogenetic diversity detected in the Bloc screen was subjected to multilocus phylogenetic analysis with an expanded nuclear genome sampling that included the Bloc nuclear intergenic region and three nuclear protein-encoding genes, translation elongation factor-1α (*TEF*), RNA polymerase II largest subunit (*RPB1*) and RNA polymerase II second largest subunit (*RPB2*). The principal objectives of this study were to (i) determine the phylogenetic structure of *Beauveria*, (ii) evaluate existing species concepts, (iii) revise the circumscription of *B. bassiana* and *B. brongniartii* and (iv) describe new species corresponding to novel lineages resolved in the phylogenetic analyses herein.

MATERIALS AND METHODS

Taxon sampling.—Exemplar strains of *Beauveria* used in the phylogenetic analysis included available ex-type cultures of currently recognized species, selected strains representing novel phylogenetic terminals discovered in a preliminary diversity screen with Bloc (data not shown) and outgroup taxa (TABLE I). Additional strains included for morphological examinations are listed under species treated in TAXONOMY. Strains were stored as small blocks of agar cultures and conidia in 10% glycerol at −80 C.

Colony growth and morphology.—Colony descriptions and measurements were determined from cultures grown on full strength Sabouraud's dextrose agar and potato dextrose agar (Difco™) at 23 C in darkness at 10 d from inoculation. Terms and notations used to describe colony coloration are those of Kornerup and Wanscher (1961). Microscopic measurements of conidiogenous cells and conidia were taken from agar cultures at 10–15 d and images acquired with a Nikon DSFi1 digital camera on a Nikon Eclipse E600 microscope with NIS Elements Basic Research 2.30, SP4 (Nikon) software. Length to width ratios are given as Q. Mean values for length, width and Q are indicated respectively by L^m, W^m and Q^m. Terminology for conidial shape follows Vellinga (1988). The images of conidia and conidiogenous cells were photographed in multiple focal planes with a Jenoptik CFscan digital camera (Jena, Germany) on an Olympus BS-51 microscope, and converted to photomontage to enhance depth of focus with Helicon Focus Pro 4.2.1.5 Pro software (Helicon Soft; www.heliconfocus.com).

DNA extraction, PCR amplification and sequencing.—Genomic DNA was prepared either from ground lyophilized mycelium and purified according to Rehner and Buckley (2005) or extracted with PrepMan Ultra Sample Preparation Reagent® (Applied Biosystems, Foster City, California) according to the manufacturer's instructions.

We used partial sequences of four nuclear loci in the molecular phylogenetic analysis including RNA polymerase II largest subunit (*RPB1*), RNA polymerase II second largest subunit (*RPB2*), translation elongation factor-1α (*TEF*) and the nuclear intergenic region Bloc. PCR amplification and sequencing primers for these loci are provided (TABLE II). The loci *RPB1*, *RPB2* and *TEF* were selected for analysis because of their wide usage in phylogenetic reconstructions of fungi (AFTOL, http://aftol.org/data.php) including their demonstrated utility in phylogenetic studies of *Beauveria* and related clavicipitaceous fungi (Rehner and Buckley 2005, Sung et al. 2007). Bloc is a nuclear intergenic region initially developed for cryptic speciation investigations within *B. bassiana* (Rehner et al. 2006a). Sequences for the nuclear ribosomal internal transcribed spacer region (ITS) also were determined for available *Beauveria* ex-type cultures with ITS5 and ITS4 primers (White et al. 1990) to serve as authenticated reference sequences for these species. However the ITS was not included in the present phylogenetic data matrix due to its limited information within the ingroup and because of difficulties in resolving alignment ambiguities, particularly between outgroup and ingroup taxa.

PCR amplification, sequencing template preparation and sequencing methods are described in Rehner and Buckley (2005), Rehner et al. (2006a) and Rojas et al. (2010).

Data editing and phylogenetic analysis.—Raw sequence chromatograms were assembled and edited with Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan). Multiple sequence alignments were created with MAFFT

TABLE I. Strains used in *Beauveria* phylogenetic analysis, their origin, substrate and GenBank numbers

Strain	Species	Locality	Host/Substrate	GenBank number				
				ITS	Bloc	*tef*	*rpb1*	*rpb2*
ARSEF [a] 1969	*B. amorpha*	Peru	Coleoptera: Curculionidae	AY31998	HQ880738	AY531907	HQ880879	HQ880950
ARSEF 2641	″	Brazil	Hymenoptera: Formicidae	AY532008	HQ880739	AY531917	HQ880880	HQ880952
ARSEF 4149	″	Australia	Coleoptera: Scarabaeidae	HQ880804	HQ880735	HQ881006	HQ880876	HQ880948
ARSEF 7542	″	USA, Colorado	Hymenoptera: Formicidae	HQ880805	HQ880736	HQ881007	HQ880877	HQ880949
B518a[b]	″	Chile	Soil	HQ880806	HQ880737	HQ881008	HQ880878	HQ880950
ARSEF 4384	*B. asiatica*	China	Coleoptera: Scarabaeidae	AY532026	HQ880716	AY531935	HQ880857	HQ880929
ARSEF 4474	″	China	Coleoptera: Scarabaeidae	AY531936	HQ880717	AY531936	HQ880858	HQ880930
ARSEF 4850	″	Republic of Korea	Coleoptera: Cerambycidae	AY531936	HQ880718	AY531937	HQ880859	HQ880931
ARSEF 4580	*B. australis*	Australia	Orthoptera: Acridiidae	HQ880788	HQ880719	HQ880994	HQ880860	HQ880932
ARSEF 4598	″	Australia	Soil	HQ880789	HQ880720	HQ880995	HQ880861	HQ880933
ARSEF 4622	″	Australia	Orthoptera: Acridiidae	HQ880790	HQ880721	HQ880996	HQ880862	HQ880934
ARSEF 300	*B. bassiana*	Australia	Hemiptera: Lygaeidae	AY532015	HQ880690	AY531924	HQ880831	HQ880903
ARSEF 751	″	Vietnam	Coleoptera: Chrysomelidae	AY532045	HQ880694	AY531954	HQ880835	HQ880907
ARSEF 1040	″	Japan	Lepidoptera: Bombycidae	AY531972	HQ880689	AY531881	HQ880830	HQ880902
ARSEF 1478	″	Brazil	Hemiptera: Pentatomidae	AY531981	HQ880695	AY531890	HQ880836	HQ880908
ARSEF 1564	″	Italy	Lepidoptera: Arctiidae	HQ880761	HQ880692	HQ880974	HQ880833	HQ880905
ARSEF 1811	″	Morocco	Coleoptera: Curculionidae	AY531992	HQ880696	AY531901	HQ880837	HQ880909
ARSEF 1848	″	Belgium	Coleoptera: Rhizophagidae	AY531995	HQ880696	AY531904	HQ880832	HQ880904
ARSEF 7518	″	Japan	Hymenoptera: Pamphiliidae	HQ880762	HQ880693	HQ880975	HQ880834	HQ880906
ARSEF 617	*B. brongniartii*	France	Coleoptera: Scarabaeidae	HQ880782	HQ880713	HQ880991	HQ880854	HQ880926
ARSEF 979	″	France	Coleoptera: Scarabaeidae	HQ880783	HQ880714	HQ880992	HQ880855	HQ880927
ARSEF 985	″	Japan	Coleoptera: Scarabaeidae	HQ880768	HQ880699	HQ880978	HQ880840	HQ880912
ARSEF 2271	″	USA, Kentucky	Coleoptera: Curculionidae	HQ880779	HQ880710	HQ880988	HQ880851	HQ880923
ARSEF 2831	″	USA, Maryland	Lepidoptera	HQ880778	HQ880709	HQ880987	HQ880850	HQ880922
ARSEF 4362	″	Japan	Soil	AY532025	HQ880708	AY531934	HQ880849	HQ880921
ARSEF 4363	″	Japan	Soil	HQ880776	HQ880707	HQ880986	HQ880848	HQ880920
ARSEF 6213	″	USA, New York	Coleoptera: Curculionidae	HQ880775	HQ880706	HQ880985	HQ880847	HQ880919
ARSEF 6214	″	USA, New York	Coleoptera: Curculionidae	HQ880774	HQ880705	HQ880984	HQ880846	HQ880918
ARSEF 6215	″	USA, New York	Coleoptera: Curculionidae	HQ880781	HQ880712	HQ880990	HQ880853	HQ880925
ARSEF 7058	″	USA, Maine	Hymenoptera: Formicidae	HQ880773	HQ880704	HQ880983	HQ880845	HQ880917
ARSEF 7268	″	Republic of Korea	Coleoptera: Carabidae	HQ880772	HQ880703	HQ880982	HQ880844	HQ880916
ARSEF 7376	″	USA, Maryland	Homoptera: Cicadidae	HQ880770	HQ880701	HQ880980	HQ880842	HQ880914
ARSEF 7516	″	Japan	Coleoptera: Scarabidae	HQ880766	HQ880697	HQ880976	HQ880838	HQ880910
ARSEF 7517	″	Japan	Coleoptera: Scarabidae	HQ880767	HQ880698	HQ880977	HQ880839	HQ880911
ARSEF 10277	″	USA, Oregon	Soil: Rhizosphere	HQ880780	HQ880711	HQ880989	HQ880852	HQ880924
ARSEF 10278	″	USA, Oregon	Soil: Rhizosphere	HQ880769	HQ880700	HQ880979	HQ880841	HQ880913
ARSEF 10280	″	USA, Oregon	Soil: Rhizosphere	HQ880771	HQ880702	HQ880981	HQ880843	HQ880915

TABLE I. Continued

Strain	Species	Locality	Host/Substrate	GenBank number				
				ITS	Bloc	*tef*	*rpb1*	*rpb2*
JE276[c]	*B. brongniartii*	Switzerland	Coleoptera: Scarabaeidae	HQ880784	HQ880715	HQ880993	HQ880856	HQ880928
ARSEF 1567	*B. caledonica*	Switzerland	Coleoptera: Scolytidae	AY531986	HQ880747	AY531894	HQ880888	HQ880960
ARSEF 2251	″	Brazil	Coleoptera	AY532003	HQ880750	AY531912	HQ880891	HQ880963
ARSEF 2567	″	Scotland	Soil	AY532006	HQ880748	AY531915	HQ880889	HQ880961
ARSEF 4302	″	Australia	Soil	HQ880821	HQ880752	HQ881014	HQ880893	HQ880965
ARSEF 7117	″	USA, Georgia	Orthoptera: Gryllacrididae	HQ880820	HQ880751	HQ881013	HQ880892	HQ880964
ARSEF 8024	″	Denmark	Coleoptera: Scarabaeidae	HQ880818	HQ880749	HQ881012	HQ880890	HQ880962
ARSEF 7032	*B. kipukae*	USA, Hawaii	Homoptera: Delphacidae	HQ880803	HQ880734	HQ881005	HQ880875	HQ880947
ARSEF 4755	*B. malawiensis*	Australia	Soil	HQ880825	HQ880754	HQ881015	HQ880895	HQ880967
ARSEF 7760	″	Malawi	Coleoptera: Cerambycidae	DQ376247	HQ880756	DQ376246	HQ880897	HQ880969
BCC17613[d]	″	Australia	NA	HQ880824	HQ880755	HQ881016	HQ880896	HQ880968
ARSEF 1855	*B. pseudobassiana*	Canada	Coleoptera: Scolytidae	HQ880796	HQ880727	HQ880999	HQ880868	HQ880940
ARSEF 2997	″	Canada	Hymenoptera: Vesidae	HQ880797	HQ880728	HQ881000	HQ880869	HQ880941
ARSEF 3216	″	USA, Wisconsin	Thysanoptera: Thripidae	AY532019	HQ880725	AY531927	HQ880866	HQ880938
ARSEF 3220	″	Portugal	Lepidoptera: Tortricidae	AY532020	HQ880722	AY532928	HQ880863	HQ880935
ARSEF 3405	″	USA, Kentucky	Lepidoptera: Tortricidae	AY532022	HQ880723	AY531931	HQ880864	HQ880936
ARSEF 3529	″	USA, Maryland	Lepidoptera: Lymantriidae	HQ880795	HQ880726	HQ880998	HQ880867	HQ880939
ARSEF 4933	″	France	Coleoptera: Curculionidae	AY532029	HQ880729	AY531938	HQ880870	HQ880942
ARSEF 6229	″	China	Coleoptera: Scolytidae	HQ880799	HQ880730	HQ881001	HQ880871	HQ880943
ARSEF 7242	″	Republic of Korea	Hymenoptera	HQ880793	HQ880724	HQ880997	HQ880865	HQ880937
ARSEF 1685	*B. sungii*	Japan	Coleoptera: Scarabaeidae	AY531990	HQ880740	AY531899	HQ880881	HQ880953
ARSEF 5689	″	Republic of Korea	Coleoptera: Scarabaeidae	AY532030	HQ880741	AY531939	HQ880882	HQ880954
ARSEF 7043	″	Republic of Korea	Coleoptera: Scarabaeidae	AY532039	HQ880742	AY531948	HQ880883	HQ880955
ARSEF 7044	″	Republic of Korea	Coleoptera: Scarabaeidae	AY532040	HQ880743	AY531949	HQ880884	HQ880956
ARSEF 7279	″	Republic of Korea	Coleoptera: Scarabaeidae	HQ880813	HQ880744	HQ881009	HQ880885	HQ880957
ARSEF 7280	″	Republic of Korea	Coleoptera: Scarabaeidae	HQ880814	HQ880745	HQ881010	HQ880886	HQ880958
ARSEF 7281	″	Republic of Korea	Coleoptera: Scarabaeidae	HQ880815	HQ880746	HQ881011	HQ880887	HQ880959
ARSEF 2694	*B. varroae*	Switzerland	Coleoptera: Curculionidae	HQ880802	HQ880733	HQ881004	HQ880874	HQ880946
ARSEF 8257	″	France	Acari: Varroidae	HQ880800	HQ880731	HQ881002	HQ880872	HQ880944
ARSEF 8259	″	France	Acari: Varroidae	HQ880801	HQ880732	HQ881003	HQ880873	HQ880945
ARSEF 2922	*B. vermiconia*	Chile	Soil	AY532012	HQ880753	AY531920	HQ880894	HQ880966

TABLE I. Continued

Strain	Species	Locality	Host/Substrate	GenBank number				
				ITS	Bloc	*tef*	*rpb1*	*rpb2*
ARSEF 7260	*Isaria cicadae*	Republic of Korea	Hymenoptera: Formicidae	HQ880826	HQ880757	HQ881017	HQ880898	HQ880970
ARSEF 4096	*Isaria tenuipes*	Brazil	Lepidoptera: Noctuidae	HQ880827	NA	HQ881018	HQ880899	HQ880971
ARSEF 4029	*Isaria farinosa*	Denmark	Coleoptera: Carabidae	HQ880828	NA	HQ881019	HQ880900	HQ880972
ARSEF 5050	*Cordyceps militaris*	USA, New York	Lepidoptera	HQ880829	NA	HQ881020	HQ880901	HQ880973

[a] ARS Entomopathogenic Fungus Collection, Ithaca, New York, USA

[b] Luis Devotto, Universidad Austral de Chile, Valdivia, Chile

[c] Jürg Enkerli, Agroscope Reckenholz-Tänikon ART, Zürich, Switzerland

[d] BCC, Thailand BIOTEC Culture Collection, Pathumthani, Thailand

(Katoh et al. 2005, Katoh and Toh 2008) using the FFT-NS-2 alignment option. Length variable regions in *RPB1* and *RPB2* introns and Bloc intergenic regions were excluded from phylogenetic analyses; thus no attempt was made to optimize alignments for these regions.

Maximum parsimony (MP) analyses were performed on individual and concatenated datasets. MP and nonparametric MP bootstrapping (MP BS) analyses were conducted with PAUP* 4b10 (Swofford 2003; Sinauer Associates, Sunderland, Massachusetts) with all nucleotide characters unordered, equally weighted and gaps excluded. MP searches for the shortest trees for all datasets implemented tree-bisection and reconnection branch-swapping (TBR) and 1000 random taxon-addition sequence replicates. Nonparametric MP BS analyses (Felsenstein 1985) were performed to assess clade support with 1000 BS replicates. We used a conditional combination criterion (Mason-Gamer and Kellogg 1996) to determine whether different data partitions could be concatenated and analyzed jointly. Data partitions were combined if monophyly statements were congruent or where either one or both conflicting monophyletic groups received less than 70% bootstrap support.

Selection of models of nucleotide substitution for the maximum likelihood (ML) and Bayesian (BI) analyses, implementing the Akaike information criterion (AIC), was determined with Modeltest 3.7 (Posada and Crandall 1998). The four-gene dataset was divided into 10 partitions, and these and their substitution models are a single partition for Bloc (HKY + G), *TEF* first and second codon positions (F81 + I + G), *TEF* 3rd codon positions (TrN + I + G), *RPB1* first codon positions (TrN + I + G), *RPB1* second codon positions (TVM + I), *RPB1* third codon positions (TVM + G), *RPB1* intron (K80 + G), *RPB2* first and second codon positions (TIM + I), *RPB2* third codon positions (TVM + G) and *RPB2* intron (TIM + G). ML (Felsenstein and Churchill 1996) searches and 800 ML bootstrap (ML BS) replicates were conducted with GARLI 0951 (Zwickl 2006).

Partitioned Bayesian analyses were carried out with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with nucmodel = 4by4 and samplefreq = 500 for 25 × 10^6 generations. All parameters were unlinked across partitions except branch lengths and topology. Branch-length rate multipliers were unlinked (prset ratepr = variable). All analyses were carried out with parallel processing (one chain per CPU) with eight chains per run and two runs per analysis (nruns = 2) and brlenspr = unconstrained:Exp (100). The props command was used to increase the proposal rate from 1000 to 10 000 and to decrease the Dirichlet alpha parameter from 500 to 250 for the rate multipliers (proposal mechanism 26 in MrBayes). Burn-in and convergence were assessed with Tracer 1.5 (Rambaut A, Drummond AJ [2007].Tracer 1.4, available at http://beast.bio.ed.ac.uk/Tracer) by examining PSRF values in the MrBayes.stat output files and by using Bayes Factor comparisons of marginal likelihoods of pairs of runs in Tracer.

RESULTS

Data matrix and phylogenetic analysis.—The combined *Beauveria* data matrix included four loci, 72

TABLE II. PCR amplification (×) and sequencing primers

Locus	Primer	PCR	Sequence (5′–3′)[a]	Reference
Bloc	B5.1F	×	CGACCCGGCCAACTACTTTGA	Rehner et al. 2006a
	B3.1R	×	GTCTTCCAGTACCACTACGCC	Rehner et al. 2006a
	B822Ldg		AGATYCGYAACGTCAACTTT	Rehner et al. 2006a
	B22Udg		GTCGBAGCCAGAGCAACT	this study
	B5.4F		CATTCGMGGCYTGTTCTTTGG	this study
	BRn2		CTCCACGCATTCCGCACCAG	this study
	Bfint		GTTCCTTGCCCTCGGTAATGAA	this study
	BFn2		TCTCGATGCCGTTACCTACA	this study
	Brint		AGCATATCGGGCATGACTGA	this study
	BFn6		TGGTGCGGAATGCGTGGAGC	this study
	B3.3R		TTCCAGTACCACTACGCCGGC	Rehner et al. 2006a
*rbp*1	RPB1A	×	GARTGYCCDGGDCAYTTYGG	Matheny et al. 2002
	RPB1A_VH6R	×	ATGACCCATCATRGAYTCCTTRTG	Stiller and Hall 1997
	RPB1B_VH6Fa	×	CAYAAGGARTCYATGATGGGTCAT	Hofstetter et al. 2007
	RPB1B_G2R	×	GTCATYTGDGTDGCDGGYTCDCC	Hoffstetter et al. 2007
	Bv-RPB1b_R2		TTGAGBARRCCRTACATRAGC	this study
	Bv-RPB1B_R4		GGGCTRACRAARGCYCKRTCCC	this study
	Bv-RPB1B_F1		CCHTACAAYGCYGATTTYGACG	this study
	Bv-RPB1B_F2		GCTYATGTAYGGYYTVCTCAA	this study
	Bv-RPB1B_F4		GCTYTDCCKGGYATGAAYGTYCG	this study
	Bv-RPB1B_R7		CMGCWGTATCAATGAGACCYTC	this study
rpb2	fRPB2_5F	×	GACGAYAGAGAYCAYTTYGG	Liu et al. 1999
	RPB2A_7cR	×	CCCATRGCTTGYTTRCCCAT	Liu et al. 1999
	fRPB2-7cF	×	ATGGGYAARCAAGCYATGGG	Liu et al. 1999
	RPB2-3053bR	×	TGRATYTTRTCRTCSACCAT	Reeb et al. 2004
	Bv-RPB2A_R1		CCCCTGTTGATCATRAAGTCA	this study
	Bv-RPB2A_F3		CCMGCCGARCCRCTYATTGA	this study
	Bv-RPB2A_F4		CGCCTGAAGACDAARACMAACC	this study
	Bv-RPB2B_R4		CRGCGTTRACAGRCACRATGA	this study
	Bv-RPB2B_F1		AAGCGTCTTGATTTRGCRGGYCC	this study
	Bv-RPB2B_R2		GCGTGAATYTTRTCRTCCAC	this study
tef	983F	×	GCYCCYGGHCAYCGTGAYTTYAT	Rehner and Buckley 2005
	2218R	×	ATGACACCRACRGCRACRGTYTG	Rehner and Buckley 2005
	1567RintB		ACHGTRCCRATACCACCRAT	Rehner and Buckley 2005
	1577F		CARGAYGTBTACAAGATYGGTGG	Rehner and Buckley 2005

[a] IUPCA degenerate nucleotides: D, AGT; H, ACT; N, ACGT; S, CG; R, AG; Y, CT.

isolates and 7628 characters (Bloc: 1574, *TEF*: 992, *RPB1*: 2928, *RPB2*: 2166,) and is available from TreeBASE as submission 11129. After removal of 56 ambiguously aligned and gapped characters the data matrix contained 7437 characters (Bloc: 1425; *TEF*: 986; *RPB1*: 2916; *RPB2*: 2166), including 1603 parsimony informative characters (Bloc: 454; *TEF*: 105; *RPB1*: 580; *RPB2*: 464). Character and MP tree statistics for the individual genes and the concatenated dataset are provided (TABLE III). The log-likelihood score of the best tree from the GARLI ML analysis was −28973.86. All Bayesian analyses converged on a stationary phase that yielded topologically identical 50% majority rule consensus trees (data not shown). The BS values from MP BS and ML BS analyses and the PPs from one of the Bayesian analyses are provided (FIG. 1).

Phylogenetic diversity and relationships within Beauveria.—ML, MP and Bayesian phylogenetic analyses of *Beauveria* yielded compatible trees that each resolved 12 well supported terminal lineages or branches (FIG. 1). We summarized the MP BS support values for species clades (TABLE IV). Six terminal clades correspond to previously described species that include *B. amorpha*, *B. bassiana*, *B. brongniartii*, *B. caledonica*, *B. malawiensis* and *B. vermiconia*. *B. bassiana* represents a highly speciose complex of morphologically cryptic phylogenetic species (Rehner unpubl). Details of the composition and structure of the *B. bassiana* complex are beyond the scope of the taxon and gene sampling presented here and will be described elsewhere. Six additional clades or lineages represent novel species that we describe herein and include *B. asiatica*, *B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. sungii* and *B.*

TABLE III. Character, MP tree statistics for Bloc, *TEF*, *RPB1*, *RPB2* and combined gene dataset

Locus	No. characters	Number PIC[a] (PIC/bp)[b]	MPT[c] Number	Length	CI[d]	RC[e]
Bloc	1574	454 (28.8)	192	1326	0.5226	0.4454
TEF	986	105 (10.6)	20	229	0.5240	0.4458
RPB1	2928	580 (19.8)	14	1318	0.5622	0.4485
RPB2	2166	464 (21.4)	8	899	0.6585	0.5821
Combined	7437	1603 (21.6)	12	3844	0.5583	0.4797

[a] PIC, parsimony-informative base pairs.
[b] (PIC/bp), parsimony-informative characters/total base pairs.
[c] MPTs, most parsimonious trees.
[d] CI, consistency index.
[e] RI, retention index.

varroae. *B. varroae* and *B. kipukae* form a biphyletic clade that is the sister to the *B. bassiana* clade. *B. asiatica* and *B. australis* are sisters to *B. brongniartii*, and these three species comprise a lineage that is sister to the *B. bassiana/varroae/kipukae* clade. In all analyses *B. amorpha* was the sister to the *B. bassiana/brongniartii* group clade, although this relationship received significant bootstrap support only from *RPB1* and *RPB2*. *B. malawiensis* was placed as the basal-most taxon with significant support from Bloc, *RPB1* and *RPB2*. Sister group relationships and branching order among the remaining basal taxa, including *B. pseudobassiana*, *B. sungii* and the sister pair *B. vermiconia*/*B. caledonica*, were not unanimously supported by the three analytical methods used and thus remain equivocal.

Colony characteristics and micromorphology of Beauveria.—*Beauveria* presents few morphological characteristics that are taxonomically diagnostic at species rank. Colony growth characteristics and appearance are similar among the majority of *Beauveria* species with colonies appearing white to light yellow and having a lanose or velutinous texture that frequently becomes farinaceous as conidia accumulate on the surface of aging cultures. Exceptions are *B. malawiensis* with colonies that are light to intensely pink and *B. sungii*, which becomes strongly yellow. Conidiogenous cells, although diagnostic for the genus, are largely uninformative for distinguishing species. The only species uniquely characterized on the basis of conidial features is *B. vermiconia*, which produces short comma- or sickle-shaped conidia. Otherwise conidial shapes in other *Beauveria* species are more or less either globose/subglobose/broadly ellipsoid or ellipsoid/oblong/cylindrical. Species within each conidial shape category overlap in size variation and are difficult to differentiate. *B. bassiana* group species are characterized by globose/subglobose/broadly ellipsoid conidia, as are the related *B. varroae* and *B. kipukae*. In addition *B. australis* and *B. pseudobassiana*, which are not immediately related to *B. bassiana* or to one another, also produce similar-sized globose, subglobose to broadly ellipsoid conidia that cannot be readily distinguished from those of *B. bassiana* or each other. The remaining species, *B. amorpha*, *B. asiatica*, *B. brongniartii*, *B. caledonica*, *B. malawiensis* and *B. sungii*, are characterized by ellipsoid/oblong/cylindrical conidia but are hard to separate diagnostically due to overlapping size and shape variation.

TAXONOMY

B. bassiana and *B. brongniartii* are redescribed, and typifications are proposed for these species. Genealogical criteria were used to delimit six novel clades or phylogenetic terminals that are described here as new species including *B. asiatica*, *B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. sungii and B. varroae*. *B. amorpha* is validly published and an epitype specimen is proposed.

Beauveria Vuill., Bull Soc Bot France 59:40. 1912.

Typus genericus. *Beauveria bassiana* (Bals.-Criv.) Vuill.

Colonies on full-strength Sabouraud's dextrose and potato dextrose agar at 10 d at 23 C 15–42 mm diam, cottony, powdery, velutinous, or woolly, white or yellow or rarely pinkish, margin white, reverse uncolored or yellow and occasionally with pinkish to red soluble pigment that discolors the medium, at times producing synnemata or irregular synnema-like structures. Aerial hyphae branching, hyaline, septate, walls thin and smooth. Conidiogenous cells scattered or gregarious in dense clusters or whirls, sessile on aerial hyphae or formed one or more at a time on subtending cells borne on aerial hyphae, hyaline, base typically subspherical or ampulliform, with walls thin and smooth, apex proliferating sympodially as a geniculate rachis with prominent denticles resulting from conidial secession. Cells subtending the conid-

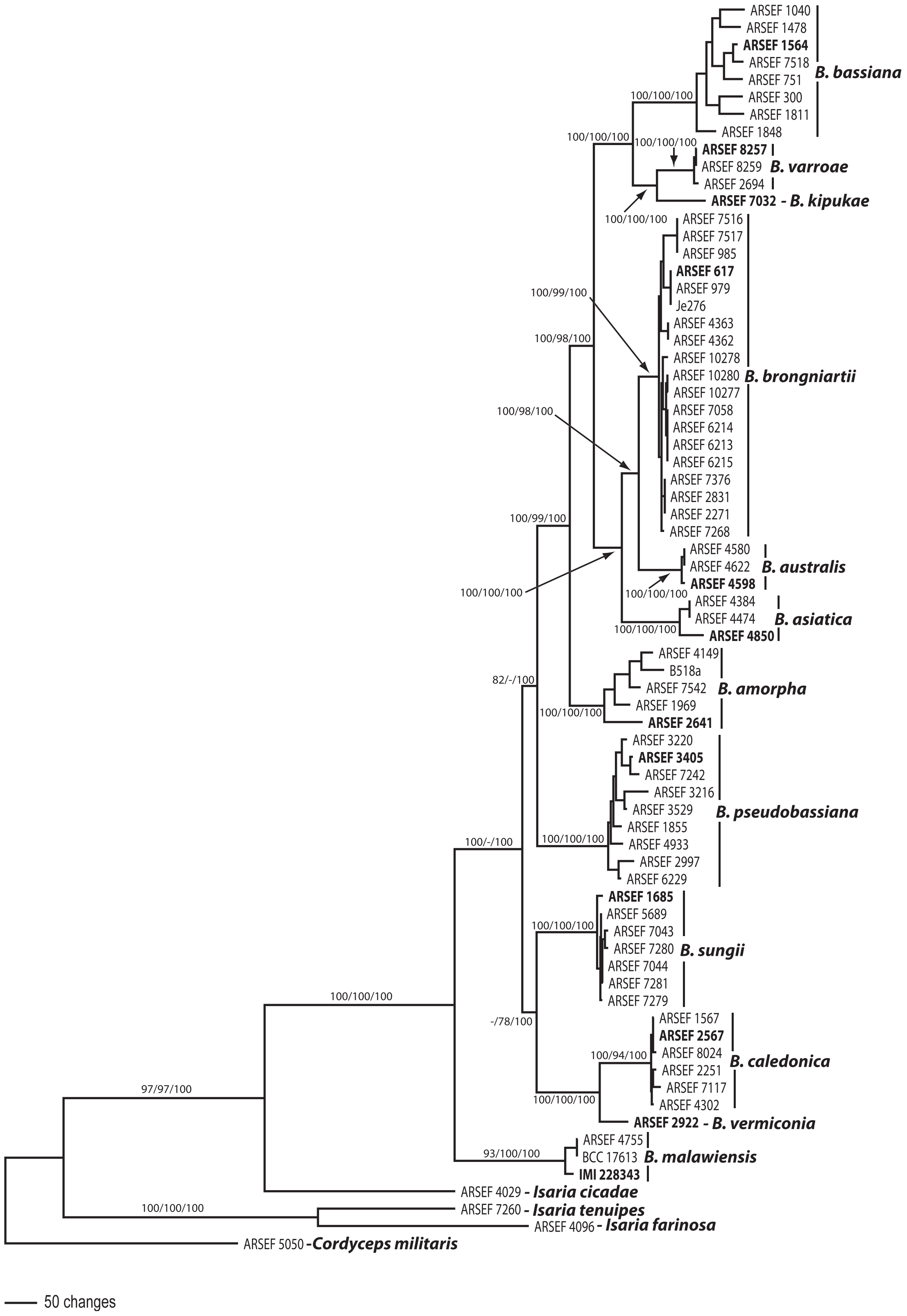


FIG. 1. Phylogeny of *Beauveria* illustrating species relationships inferred from joint ML analysis of Bloc, *TEF*, *RPB1* and *RPB2*. The series of three values above internal branches correspond respectively to MP BS proportions (≥ 70), ML BS proportions (≥ 70) and PPs (≥ PP 95) from Bayesian analyses, respectively. Species clades are indicated by vertical bars. Strains associated with type material are indicated in boldface.

TABLE IV. MP bootstrap support (> 50%) for species by individual gene partitions and combined (All) dataset for 72-isolate *Beauveria* analysis. Bootstrap support for *B. kipukae* and *B. vermiconia* were not evaluated because only single isolates were available for these species

Species	Bloc	*TEF*	*RPB1*	*RPB2*	*All*
B. amorpha	*98*	*71*	*99*	*99*	*99*
B. asiatica	*100*	*91*	*100*	*100*	*100*
B. australis	*100*	*97*	*100*	*100*	*100*
B. bassiana	*100*	*75*	*100*	*100*	*100*
B. brongniartii	*79*	*NA*	*100*	*99*	*99*
B. caledonica	*100*	*97*	*100*	*100*	*100*
B. malawiensis	*100*	*100*	*100*	*100*	*100*
B. pseudobassiana	*99*	*100*	*99*	*100*	*100*
B. sungii	*100*	*100*	*100*	*100*	*100*
B. varroae	*100*	*99*	*100*	*100*	*100*

iogenous cells subspherical, fusiform, cylindrical or shield-shaped, forming short chains that sometimes branch, hyaline, walls thin and smooth. Conidia holoblastic, acrogenous, produced singly, globose, subglobose, ellipsoid, oblong, cylindrical, reniform or sickle-shaped, hyaline, aseptate, walls thin and smooth, at times with inconspicuous hilum at base. Chlamydospores absent. Some species known to be associated with *Cordyceps* teleomorphs.

Beauveria bassiana (Bals.-Criv.) Vuill., Bull Soc Bot France 59 40. 1912. FIGS. 2–4

≡ *Botrytis bassiana* Bals.-Criv., Linnaea 10:611. 1835 (basionym).

See de Hoog (1972) for a nomenclatural discussion and additional synonyms.

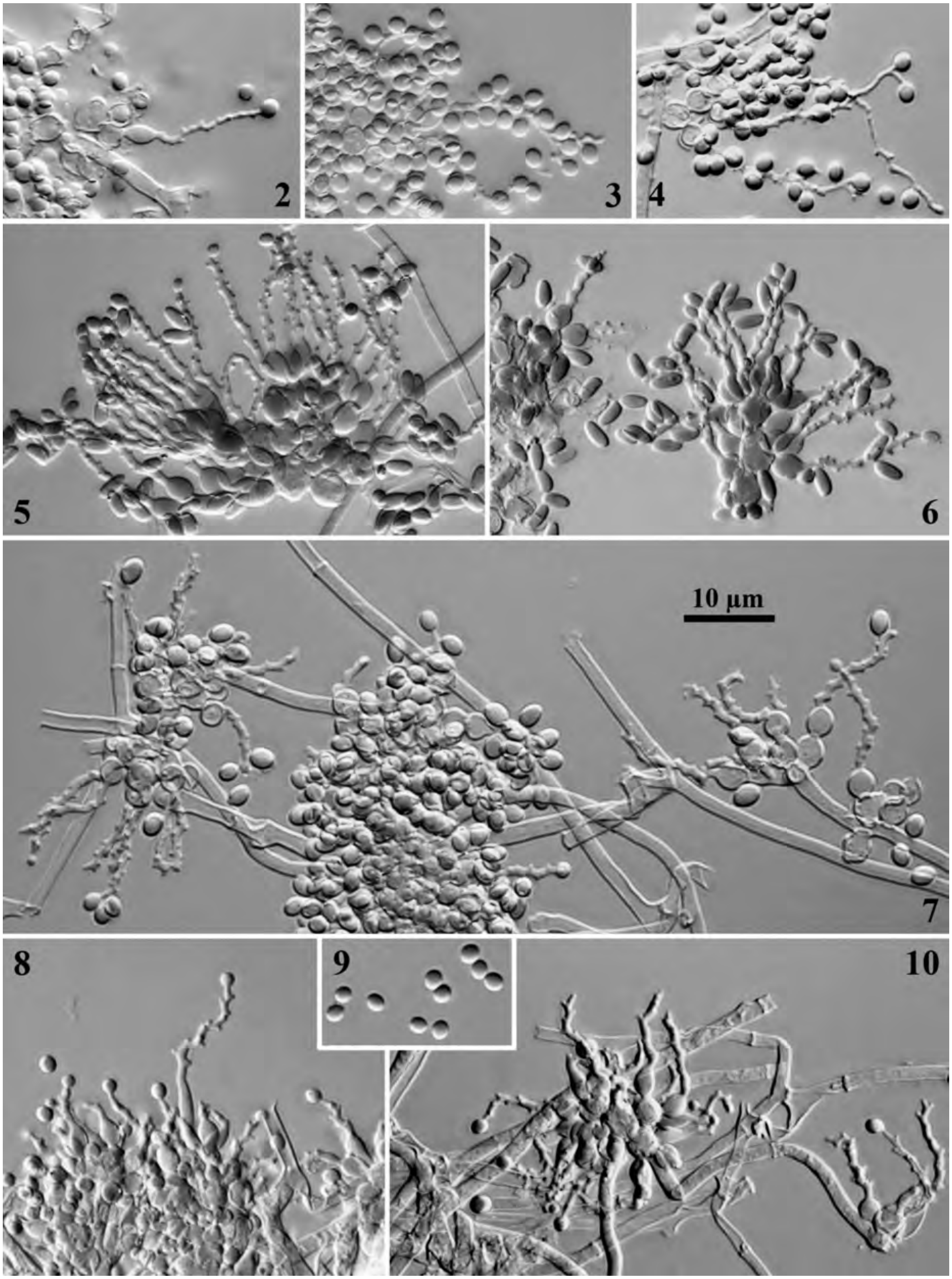
Neotypus of Botrytis bassiana *(hic designatus)*. ITALY. Emilia-Romagna: Villa Cadé, isolated from pupa of *Hyphantria cunea* (Lepidoptera: Arctiidae), Mar 1984, leg. KV Deseö, neotype BPI 880999 is a dried agar culture of ARSEF 1564.

Colony characteristics similar on full strength Sabouraud's dextrose and potato dextrose agar (Difco™), 15–32 mm diam at 10 d at 23 C, appearance densely to loosely lanose, either closely appressed to agar surface or cushion-like and up to 5 mm thick, at first white and often becoming yellowish white (4A2). Conidia aggregated as < 0.1 mm spherical clusters among aerial hyphae and white in mass. Colony reverse uncolored to yellowish white (4A2) or tinted by a diffuse to intense dull red (84C, 84D) soluble pigment that discolors the medium. Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 µm wide. Conidiogenous cells solitary but usually in dense clusters of five or more, base subspherical to ampulliform and 3–6 µm wide, apex with an indeterminate 1 µm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 µm, (FIGS. 2–4). Conidia 2–3 × 2–3 µm, Q = 0.9–1.5 (L^m = 2.7 µm, W^m = 2.2 µm, Q^m = 1.2), globose, subglobose or broadly ellipsoid, rarely ellipsoid, sometimes with inconspicuous hilum at base, hyaline, aseptate, walls thin and smooth.

Habitat and distribution. *B. bassiana* is cosmopolitan and is isolated from soil, insects, phylloplanes and occasionally from living plant tissues.

Additional isolates examined. (TABLE I.) BRAZIL: Mato Grosso do Sul, Mato Grosso, isolated from *Zulia entreriana* (Homoptera: Cercopidae), 26 Jan 1980, leg. RA Daoust (ARSEF 477); Londrina, Paraná, isolated from *Diabrotica speciosa* (Coleoptera: Chrysomelidae), 6 Jan 1983, leg. RA Daoust (ARSEF 958). REPUBLIC OF KOREA: Daeryong Mountain, Kangwon-do, isolated from mantid (Dictyoptera: Mantidae), 16 Sep 1996, leg. JM Sung (culture ARSEF 7238 = EFCC 1037). MOROCCO: Jramma, isolated from *Sitona discoideus* (Coleoptera: Curculionidae), Jan 1984, leg. TJ Poprawski (culture ARSEF 1164); Achouria, isolated from *Sitona discoideus* (Coleoptera: Curculionidae), Feb 1984, leg. TJ Poprawski (culture ARSEF 1174 = A6). HUNGARY: Gödöllö, isolated from unidentified larva (Lepidoptera: Noctuidae), 13 Jun 1984, leg. TJ Poprawski (culture ARSEF 1628). CANADA: Magrath, Alberta, soil, 12 Jul 1991, leg. MS Goettel (culture ARSEF 3462). USA: Randolph, Vermont, isolated from *Paraclemensia acerifoliella* (Lepidoptera: Incurvariidae), 16 May 1997, leg. M Brownbridge (culture ARSEF 5492).

Notes. The concept of *B. bassiana* is based on a phylogenetic species that is prevalent in Europe (Meyling et al. 2009) and Asia and which also occurs in Africa and the western hemisphere (Rehner unpubl). A dried herbarium specimen of ARSEF 1564 is designated to neotypify this species because this isolate originated from a location in northern Italy and a lepidopteran host phylogenetically related to silkworms on which *B. bassiana* originally was observed (Bassi 1835; Balsamo-Crivelli 1835a, b). In addition the ex-type of *Cordyceps bassiana* Z.Z. Li, C.R. Li, B. Huang & M.Z. Fan, ARSEF 7047, is placed in the same clade as ARSEF 1564, definitively linking this phylogenetic



FIGS. 2–10. Conidiogenous cells and conidia of *Beauveria* species. 2–4. *B. bassiana* (ARSEF 1564). 5, 6. *B. amorpha* (ARSEF 2641). 7. *B. asiatica* (ARSEF 4850). 8–10. *B. australis* (ARSEF 4598). All images at the same magnification from material mounted in 50% acetic acid plus cotton blue.

species to a teleomorph. *B. bassiana* is hardly distinguishable morphologically from *B. australis*, *B. kipukae*, *B. pseudobassiana* and *B. varroae*, which also produce globose/sublglobose/broadly ellipsoid conidia, but its conidia may be larger than some of these species.

Additional species of *Beauveri*:

Beauveria amorpha (Höhn.) Minnis, S.A. Rehner & Humber, comb. nov. FIGS. 5, 6

MycoBank MB519121

≡ *Isaria amorpha* Höhn., Sitzungsber Kaiserl Akad Wiss, Math-Naturwiss Kl, Abt 1 118:415. 1909 (basionym).

≡ *Beauveria amorpha* (Höhn.) Samson & H.C. Evans, J Invertebr Pathol 39:95. 1982. Nom. inval. via ICBN Art.33.

Holotypus of Isaria amorpha. INDONESIA: Java, Sukabumi, on a large cicada, leg. Major Ouwens, in FH.

Epitypus of Isaria amorpha *(hic designatus)*. BRAZIL: Alambari, São Paulo, on *Solenopsis* sp. (Hymenoptera: Formicidae), received at ARSEF 23 Nov 1988, leg. SB Alves (621), epitype BPI 880996 is a dried agar culture of ARSEF 2641.

Colony growth and appearance on full-strength Sabouraud's dextrose and potato dextrose agars (Difco™) similar, 16–24 mm diam at 10 d at 23 C, velutinous and closely appressed to agar surface and up to 4 mm thick, white, changing to yellowish white (4A2) in older portions of the colony. Conidia aggregated as < 0.1 mm spherical clusters and white in mass. Colony reverse uncolored or yellowish white (4A2) to grayish orange (5B5, 5B6). Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 µm wide. Conidiogenous cells solitary but usually in dense lateral clusters, base subspherical to ampulliform and 3–6 µm wide, apex with an indeterminate 1 µm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 µm (FIGS. 5–6). Conidia 2.5–4 × 1.5–2.5 µm, Q = 1.3–2.2 (L^m = 3.3 µm, W^m = 2.0 µm, Q^m = 1.6), ellipsoid, oblong or cylindrical, at times slightly curved to reniform, occasionally with inconspicuous hilum at base, hyaline, aseptate, walls smooth and thin.

Habitat and distribution. Isolated from *Solenopsis* sp. in São Paulo Brazil. Also known from North America and Australia and to infect Coleoptera.

Additional isolates examined. (TABLE I)

Notes. This species was described originally from material on a cicada found in Java (Höhnel 1909). Samson and Evans (1982) examined the holotype at the Farlow Herbarium (FH) and studied what they believed to be conspecific isolates from South America. Unfortunately they did not validly publish the new combination in *Beauveria*. Thus we have done so here and epitypify the species with an isolate that is similar in appearance to those of Samson and Evans (1982) and the synonym, *Isaria orthopterorum* Petch as described in de Hoog (1972), to stabilize the application of this name. We examined the Samson and Evans (1982) isolates from CBS, but they were not *Beauveria*; thus these isolates might be contaminants. This species is characterized by its versiform, ellipsoid to cylindrical conidia, which may be slightly curved to reniform.

Beauveria asiatica S.A. Rehner & Humber, sp. nov. FIG. 7

Mycobank MB519122

Beauveriae brongniartii similis, sed conidiis majoribus, 2.5–4 × 2–3 µm, et Q minoribus, 1.2–2.0 (late ellipticis, ellipticis vel oblongis; non cylindricis) differt.

Holotypus. REPUBLIC OF KOREA: Kangwon-do, Chiag Mountain, isolated from Coleoptera: Cerambycidae, 14 Jul 1994, leg. JM Sung, holotype BPI 880997 is a dried agar culture of ARSEF 4850.

Etymology. The epithet refers to the Asian origin of the type material.

Colony growth and appearance on full-strength Sabouraud's dextrose and potato dextrose agars (Difco™) similar, 26–30 mm diam at 10 d at 23 C, mycelium dense, velutinous and closely appressed to agar surface or cottony and up to 5 mm thick, white, changing to yellowish white (4A2) in older portions of the colony. Conidia aggregated as < 0.1 mm spherical clusters and white in mass. Colony reverse uncolored or yellowish white (4A2). Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 µm wide. Conidiogenous cells solitary but usually in dense lateral clusters, base subspherical to ampulliform and 3–6 µm wide, apex with an indeterminate 1 µm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 µm (FIG. 7). Conidia 2.5–4 × 2–3 µm, Q = 1.2–2 (L^m = 3.2 µm, W^m = 2.3 µm, Q^m = 1.4), broadly ellipsoid, ellipsoid or oblong, occasionally with inconspicuous hilum at base, hyaline, aseptate, walls smooth and thin.

Habitat and distribution. On longicorn beetle (Coleoptera: Cerambycidae) in forest habitat, Chiag Mountain, Kangwon-do, Republic of Korea. Also known from *Holotrichia parallela* (Coleoptera: Scarabaeidae), Cangzhou, China.

Additional isolates examined. (TABLE I)

Notes. A member of the *B. brongniartii* complex, *B. asiatica* is characterized by broadly ellipsoid, ellipsoid or oblong conidia that are slightly larger than those of *B. brongniartii*.

Beauveria australis S.A. Rehner & Humber, sp. nov. FIGS. 8–10

Mycobank MB519123

A speciebus in Acrididae et similis Beauveriae bassianae, sed conidiis minoribus, 2–2.5 × 1.5–2.5 μm differt.

Holotypus. AUSTRALIA: Benham, Avoca, Tasmania, soil, 18 Feb 1988, leg. AC Rath, holotype BPI 880998 is a dried agar culture of ARSEF 4598 = F308.

Etymology. The epithet *australis*, meaning southern, refers to the geographic origin of the type in the southern hemisphere.

Colony growth and appearance on full-strength Sabouraud's dextrose and potato dextrose agars similar, 26–30 mm diam at 10 d at 23 C, surface mycelium velutinous and closely appressed to agar surface, becoming yellowish white (4A2) in interior portions of the colony with margin white. Conidia aggregated in < 0.1 mm spherical clusters and white in mass. Colony reverse uncolored to light yellow (4A5) or light orange (5A5). Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense lateral clusters, base subspherical to ampulliform and 3–6 μm wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 μm (FIGS. 8, 10). Conidia 2–2.5 × 1.5–2.5 μm, Q = 1–1.6 (L^m = 2.3 μm, W^m = 1.9 μm, Q^m = 1.2), primarily subglobose, broadly ellipsoid or ellipsoid, less commonly globose, occasionally with inconspicuous hilum at base, hyaline, aseptate, walls smooth and thin.

Habitat and distribution. Isolated from soil and grasshoppers (Orthoptera: Acrididae). Known only from Australia.

Additional isolates examined. (TABLE I)

Notes. The sister lineage to *B. brongniartii*, *B. australis* is differentiated from the former by its conidia with smaller Q values that overlap in size and shape with other *Beauveria* species with subglobose conidia, including *B. bassiana*, *B. kipukae*, *B. pseudobassiana* and *B. varroae.*

Beauveria brongniartii (Sacc.) Petch, Trans Brit Mycol Soc 10:249. 1926. FIGS. 11–13

≡ *Botrytis brongniartii* Sacc., Sylloge Fungorum 10:540. 1982 (basionym).

De Hoog (1972) listed additional synonyms.

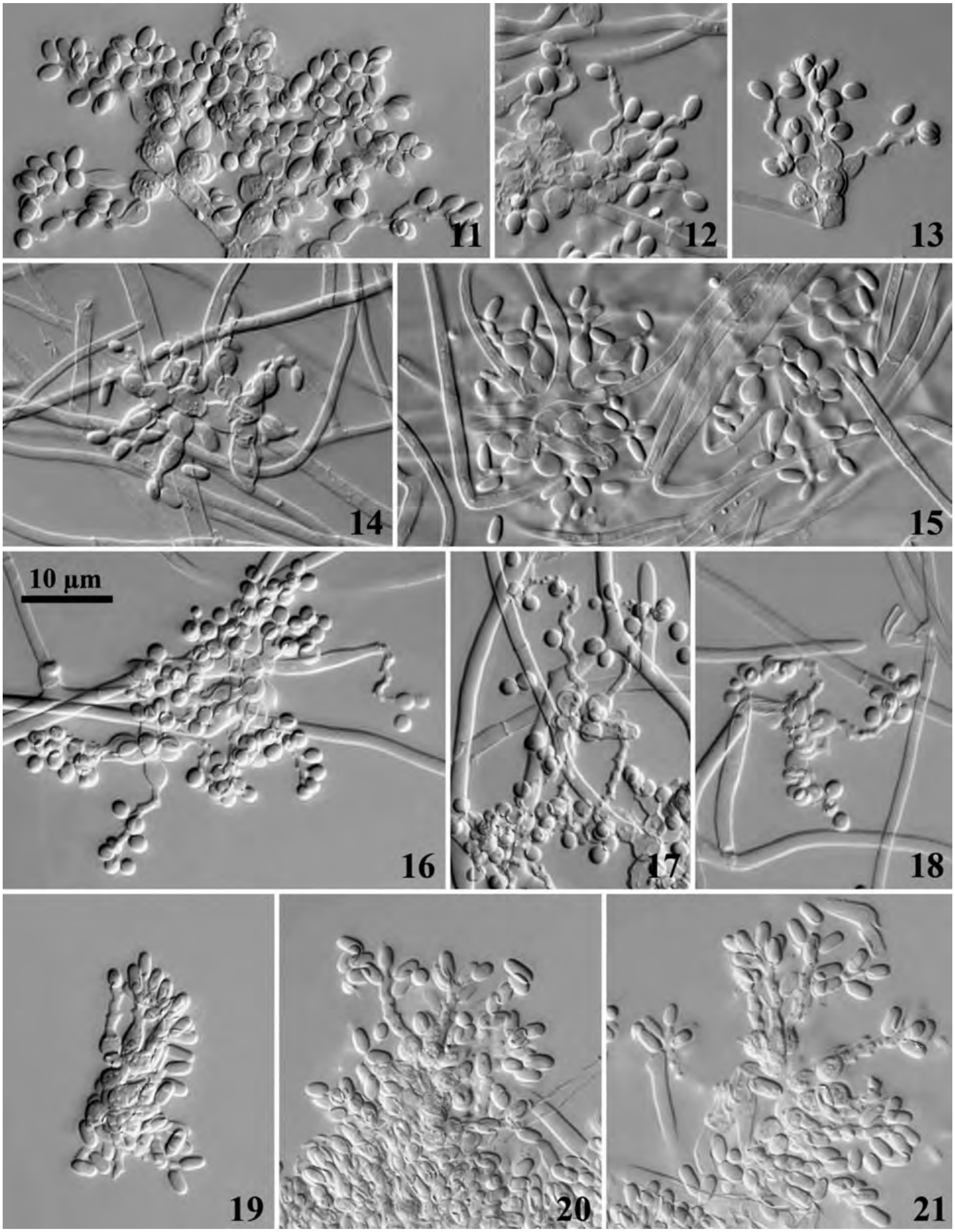
Typus of Botrytis brongniartii. France: On larva of *Melolontha melolontha* [Coleoptera: Scarabaeidae], ARSEF 617 received 27 May 1981, leg. P. Ferron, BPI 881000 (dried culture of ARSEF 617 = ATCC 26156), proposed as a conserved type (Minnis et al. 2011).

Colony characteristics on full-strength Sabouraud's dextrose and potato dextrose agars (Difco™) similar, 20–30 mm diam at 10 d at 23 C, appearance densely to loosely lanose, either closely appressed to agar surface or cushion-like and up to 7 mm thick, color white and often becoming yellowish white (4A2, 4A3) to pale yellow (4A4). Conidia aggregated as < 0.1 mm spherical clusters and white in mass. Colony reverse off-white to pale yellow (4A4) and in some isolates discolored by a diffuse to strongly dull red (84C, 84D) soluble pigment. Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense lateral clusters, base subspherical to ampulliform and 3–6 wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 μm (FIGS. 11–13). Conidia 2.5–3.5 × 1–2 μm, Q = 1.6–2.4 (L^m = 3 μm, W^m = 1.6 μm, Q^m = 1.9), primarily oblong or cylindrical, occasionally with inconspicuous hilum at base, hyaline, aseptate, walls thin and smooth.

Habitat and distribution. Occurring in soils in natural and agricultural habitats, pathogen principally of Coleoptera but also infects other insect taxa. Known geographic range includes Asia, Europe and North America.

Additional isolates examined. (TABLE I) MOROCCO: Achouria, isolated from adult *Sitona discoideus* (Coleoptera: Curculionidae), Feb 1984, leg. TJ Poprawski (culture ARSEF 1174). FRANCE: isolated from *Melolontha melolontha* (Coleoptera: Scarabaeidae), 17 Oct 1983, leg. RA Hall and J Fargues (culture ARSEF 979). HUNGARY: Gödöllö, isolated from larva, (Lepidoptera: Noctuidae), 13 Jun 1984, leg. TJ Poprawski (culture ARSEF 1628). CANADA: Magrath, Alberta, soil, 12 Jul 1991, leg. MS Goettel (culture ARSEF 3462).

Notes. The original concept of *B. brongniartii* was based on material isolated from locusts in North Africa (Saccardo 1892). However in Europe, where *B. brongniartii* frequently occurs, the prevailing concept of this species corresponds to the principal *Beauveria* pathogen of the European cockchafer, *Melolontha melolontha.* Although the host sources for isolates examined here indicate a wider host range for this species, there is no evidence that *B. brongniartii* occurs specifically on Orthoptera. In addition conidia in the original material were described as being oval or ellipsoid with a smaller Q value (1.6) than the prevailing concept. Only a single clade is known to exist in Europe. Because there is a conflict between the prevailing, economically important concept of this species and the application of this species dictated by the protolog this name has been proposed for conservation with a conserved type to preserve current usage (Minnis et al. 2011). The known species range includes the Holarctic and southeastern Asia. Its colony growth characteristics are similar to other *Beauveria*



FIGS. 11–21. Conidiogenous cells and conidia of *Beauveria* species. 11–13. *B. brongniartii* (ARSEF 617). 14, 15. *B. caledonica* (ARSEF 2567 = DAOM 191855). 16–18. *B. kipukae* (ARSEF 7032). 19–21. *B. malawiensis* (ARSEF 7760 = IMI 228343). All images at the same magnification from material mounted in 50% acetic acid plus cotton blue.

species including other members of both the *B. bassiana* and *B. brongniartii* morphological complexes. Most isolates have oblong or cylindrical conidia and can be distinguished by having among (see *B. caledonica* and *B. malawiensis*) the largest Q value of species with ellipsoid/oblong/cylindrical conidia.

Beauveria caledonica Bissett & Widden, Can J Bot 66:361. 1988. FIGS. 14, 15

Comments. See Bissett and Widden (1988) for a description and Rehner and Buckley (2005) for additional discussion of this species.

Beauveria kipukae S.A. Rehner & Humber, sp. nov. FIGS. 16–18

Mycobank MB519124

Similis Beauveriae bassianae, sed ab eo propter sequentia Bloc dicta (GenBank HQ880734) differt.

Holotypus. USA. HAWAII: Bird Park (Kipuka Puaulu, Mauna Loa, Hawaii, isolated from unidentified Homoptera (Delphacidae), 26 Jan 2002, leg. RG Hollingsworth, holotype BPI 881001 is a dried culture of ARSEF 7032.

Etymology. Hawaiian, from kipuka, which refers to native montane Hawaiian forests isolated by recent lava flows.

Colony growth and appearance on full-strength Sabouraud's dextrose and potato dextrose agars (Difco™) similar, 21–26 mm diam at 10 d at 23 C, velutinous, closely appressed to agar surface, margin white and colony interior white or changing to yellowish white (4A2). Conidia aggregated in < 0.1 mm spherical clusters and white in mass. Colony reverse uncolored to yellowish white (4A2). Colony odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense clusters of five or more, base subspherical to ampulliform and 3–6 μm wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subterminal cells mostly 2.5–6 × 3–6 μm, (FIGS. 16–18). Conidia 2–3.5 × 1.5–3 μm, Q = 0.9–1.4 (L^m = 2.5 μm, W^m = 2.2 μm, Q^m = 1.2), globose, subglobose or broadly ellipsoid, rarely ellipsoid, sometimes with inconspicuous hilum at base, hyaline, aseptate, walls smooth and thin.

Habitat and distribution. Known from a single isolate from an unidentified homopteran in remnant native montane forest surrounded by recent lava flows in Bird Park, Mauna Loa, Hawaii.

Notes. *B. kipukae* has conidia that are smaller, based on means, than *B. bassiana* but cannot be distinguished from *B. australis*, *B. pseudobassiana* and *B. varroae* in the absence of phylogenetically informative nucleotide characters. Known only from a single specimen; it is not known whether *B. kipukae* is a Hawaiian endemic.

Beauveria malawiensis S.A. Rehner & Aquino de Muro, Mycotaxon 98:140. 2007 [2006]. FIGS. 19–21

Notes. See Rehner et al. (2006b) for a description and discussion of this taxon.

Beauveria pseudobassiana S.A. Rehner & Humber, sp. nov. FIGS. 22–24

Mycobank MB519125

Similis Beauveriae bassianae, sed conidiis minoribus, 2–3 × 1.5–2.5 μm, et ab eo propter sequentias Bloc dictas (GenBank HQ880723) differt.

Holotypus. USA Virginia: Page County, Koontz Lokey, isolated from larva of *Lymantria dispar* (Lepidoptera: Lymantriidae), 10 Jun 1991, leg. AE Hajek, holotype BPI 881002 is a dried culture of ARSEF 3405.

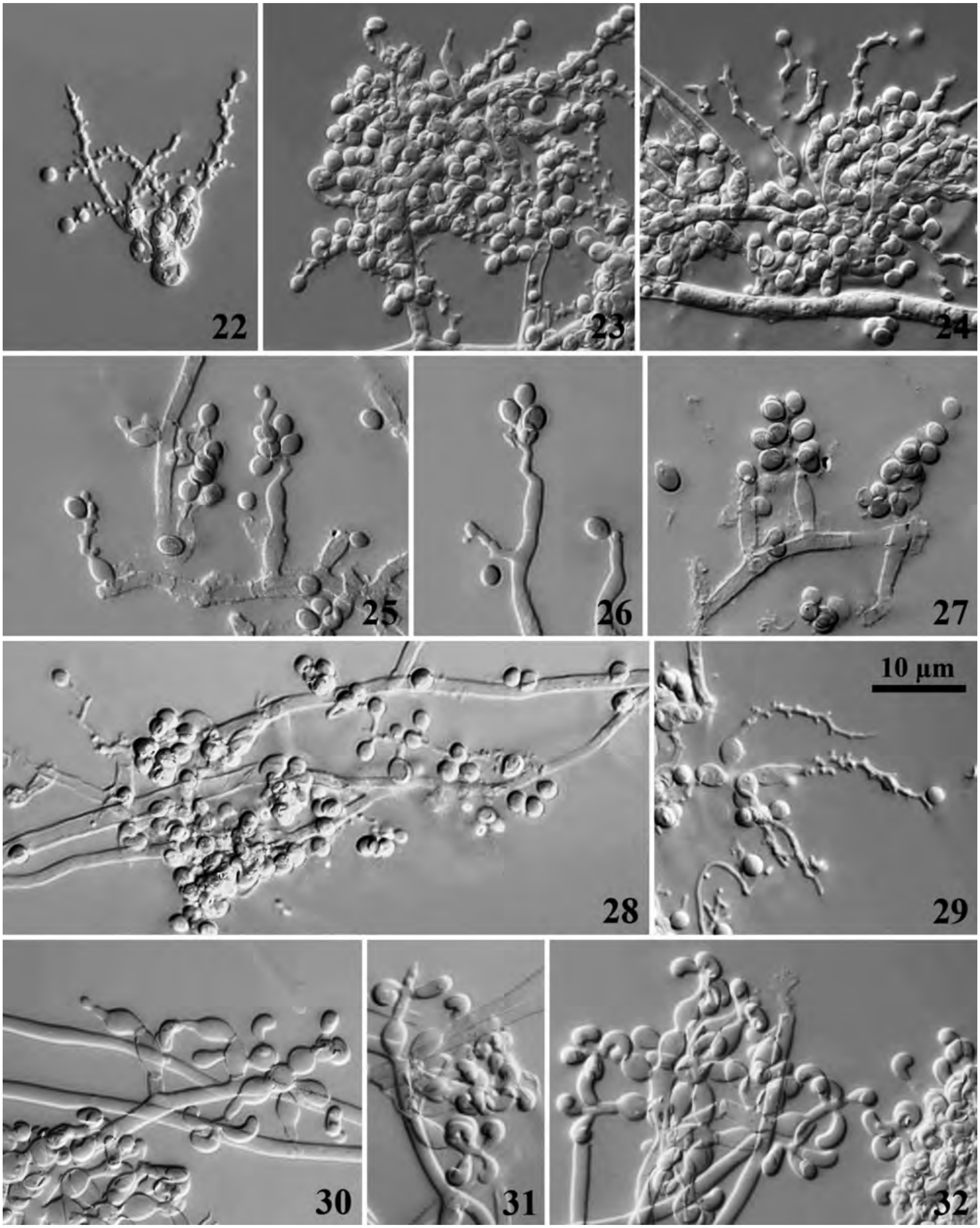
Etymology. *Pseudobassiana* refers to the close phenotypic similarity of this species to *B. bassiana.*

Colony growth and appearance on full-strength Sabouraud's dextrose and potato dextrose agars (Difco™) similar, 21–34 mm diam at 10 d at 23 C. Surface mycelium subvelutinous, velutinous to cottony, closely appressed to agar surface or greater than 5 mm thick; margin white with colony interior white or changing to yellowish white (4A2) to pale yellow (4A3). Conidia aggregated as < 0.1 mm spherical clusters, white in mass, conidia occasionally forming a farinaceous surface layer in older cultures. Colony reverse either uncolored or yellowish white (4A2, 4A3). Odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense lateral clusters, base subspherical to ampulliform and 3–6 wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 μm (FIGS. 22–24). Conidia 2–3 × 1.5–2.5 μm, Q = 1.1–1.4 (L^m = 2.3 μm, W^m = 2 μm, Q^m = 1.2), primarily subglobose or broadly ellipsoid, rarely ellipsoid, occasionally with inconspicuous hilum at base, hyaline, aseptate, walls thin and smooth.

Habitat and distribution. *B. pseudobassiana* is a globally distributed soilborne, broad host-range generalist entomopathogen that occurs in a wide range of habitats. Its conidia are generally smaller than *B. bassiana* but it is indistinguishable from *B. varroae*, *B. kipukae* and *B. australis*, which also have globose/subglobose/broadly ellipsoid conidia.

Additional isolates examined. (TABLE I)

Notes. *B. pseudobassiana* is similar phenotypically to *B. bassiana* in all respects except for its slightly smaller



FIGS. 22–32. Conidiogenous cells and conidia of *Beauveria* species. 22–24. *B. pseudobassiana* (ARSEF 3405). 25–27. *B. sungii* (ARSEF 1685). 28, 29. *B. varroae* (ARSEF 8257). 30–32. *B. vermiconia* (ARSEF 2922 = CBS 645.74). All images at the same magnification from material mounted in 50% acetic acid plus cotton blue.

conidia. Nucleotide characters are necessary to identify *B. pseudobassiana* with confidence.

Beauveria sungii S.A. Rehner & Humber, sp. nov. FIGS. 25–27

Mycobank MB519126

Similis Beauveriae brongniartii, sed conidiis latioribus, 1.5–2.5 μm, et Q minoribus, 1.2–1.8 (late ellipticis, ellipticis vel oblongis; non cylindricis) differt.

Holotypus. JAPAN. Ibaraki Prefecture: *Lachnosterna morosa* (Coleoptera: Scarabidae), 1984, leg. M. Shimazu, holotype BPI 881003 is a dried culture of ARSEF 1685.

Etymology. *Sungii* is named in honor of Jae-Mo Sung in recognition of his contributions to knowledge of the biodiversity and life histories of clavicipitaceous fungi.

Colony growth and appearance similar on both full-strength Sabouraud's dextrose agar and potato dextrose agar (Difco™), 32–41 mm diam at 10 d at 23 C, mycelium dense, cottony, often 5 mm or more thick, colony growth initially white, remaining white or changing to yellowish white (4A2), pale yellow (4A3) or light yellow (4A4) in older portions of the colony. Conidia not readily observed in most cultures. Colony reverse pale yellow (4A3), light yellow (4A5) or shades of orange to deep orange (5A3–5A7). Colony odor indistinct. Vegetative hyphae septate, branched, hyaline or translucent pale yellow, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense clusters of five or more, base subspherical to ampulliform and 3–6 μm wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 μm (FIGS. 25–27). Conidia 2.5–3.5 × 1.5–2.5 μm, Q = 1.2–1.8 (L^m = 3 μm, W^m = 2.1 μm, Q^m = 1.5), broadly ellipsoid, ellipsoid or oblong, sometimes with inconspicuous hilum at base, hyaline, aseptate, walls thin and smooth.

Habitat and distribution. Known from Japan and Korea. Isolated from coleopteran hosts from forest habitats.

Additional isolates examined. (TABLE I)

Notes. *B. sungii* is characterized via in vitro cultures by its distinctly yellow colony pigmentation and ellipsoid or oblong conidia. It is distinguished from other species of *Beauveria* with ellipsoid conidia, including *B. amorpha*, *B. asiatica*, *B. brongniartii* and *B. caledonica*, by its shorter conidia and more intense yellow pigmentation in mature colonies. All but one of the *B. sungii* strains examined here were isolated from *Cordyceps* collections identified either as *Cordyceps staphylinidicola* Kobayasi & Shimizu or *C. scarabaeicola* Kobayasi, indicating the need for taxonomic studies of these and other yellow-pigmented *Cordyceps* species that also produce *Beauveria* anamorphs.

Beauveria varroae S.A. Rehner & Humber, sp. nov. FIGS. 28, 29

Mycobank MB519127

Similis Beauveriae bassianae, sed ab eo propter sequentias Bloc dictas (Genbank HQ880733) differt.

Holotypus. FRANCE. Montardier Gard: isolated from *Varroa destructor* (Acari: Varroidae), 29 Jun 2005, leg. WG Meikle and G Mercadier, holotype BPI 881004 is a dried culture of ARSEF 8257.

Etymology. V*arroae* refers to the host genus from which this species was first isolated.

Colony growth and appearance on full-strength Sabouraud's dextrose agar and potato dextrose agar (Difco™) similar to that of *B. bassiana*, 26–30 mm diam at 10 d at 23 C, velutinous, closely appressed to agar surface, margin white with interior of colony becoming yellowish white (4A2) to pale yellow (4A3). Conidia aggregating as < 0.1 mm spherical clusters and white in mass. Colony reverse uncolored to light yellow (4A4, 4A5). Colony odor indistinct. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–2 μm wide. Conidiogenous cells solitary but usually in dense clusters of five or more, base subspherical to ampulliform and 3–6 μm wide, apex with an indeterminate 1 μm wide geniculate, denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly 2.5–6 × 3–6 μm, (FIGS. 28, 29). Conidia 2–3.5 × 2–3 μm, Q = 1–1.3 (L^m = 2.5 μm, W^m = 2.2 μm, Q^m = 1.1), globose, subglobose or broadly ellipsoid, sometimes with inconspicuous hilum at base, hyaline, aseptate, walls thin and smooth.

Habitat and distribution. Isolated from *Varroa destructor* mite ectoparasites of honeybee (*Apis mellifera*) in France. Also infecting *Larinus* sp. (Coleoptera), Switzerland.

Additional isolates examined. (TABLE I)

Notes. *B. varroae* is not morphologically distinguishable from other *Beauveria* species that produce globose/subglobose/broadly ellipsoid conidia. *B. varroae* and *B. kipukae* form a sister clade to the *B. bassiana* complex.

Beauveria vermiconia de Hoog & V. Rao, Persoonia 8:209. 1975. FIGS. 30–32

Comments. See de Hoog and Rao (1975) for a description and Rehner and Buckley (2005) for additional remarks on this species.

DISCUSSION

Genetic diversity surveys of *Beauveria* in relation to insect host, habitat and geography report their occurrence in a wide range of terrestrial habitats, principally as pathogens of insects and arthropods and from soil, but also from phylloplanes and as endophytes (Aquino de Muro et al. 2003, 2005; Rehner et al. 2006a; Glare et al. 2008; Vega et al. 2008; Meyling et al. 2009; Ghikas et al. 2010 and references cited therein). These studies reported high genotypic diversity, however species identities and species boundaries often were not explicitly defined, in part because comparable genotypic information from taxonomically authenticated specimens was not included or was unavailable. In addition objective comparisons among a majority of diversity surveys is difficult or impossible due to the use of dissimilar types of genetic markers. To improve understanding of species diversity and species limits and to develop essential data for the molecular identification of *Beauveria* species we conducted a taxonomic appraisal of the genus based on a multilocus phylogenetic analysis that included ex-type strains for currently recognized species and new species lineages discovered in a globally comprehensive screening of *Beauveria* isolates.

Beauveria comprises 12 well supported terminal lineages (FIG. 1), as revealed by the multilocus phylogeny. Six terminal clades correspond to previously recognized species including *B. amorpha*, *B. bassiana*, *B. brongniartii*, *B. caledonica*, *B. malawiensis* and *B. vermiconia*, and six additional clades are recognized as new species including *B. asiatica*, *B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. sungii* and *B. varroae*. Morphological examinations of these species, including the new species described here, serve to confirm that cultural morphology and conidial characteristics represent the principal visible extrinsic features that vary among species of *Beauveria*. However extensive overlap in these characters limits their utility as diagnostic taxonomic characters for species identification and only a minority of species can be positively identified on the basis of phenotype alone. In contrast to the limited number and established limitations of available morphological characters, each of the four loci used here to reconstruct the phylogeny of *Beauveria* are effective for accurate diagnosis of all 12 species on the basis of multiple species-specific phylogenetically informative nucleotide characters. These datasets accordingly can form the basis for the future development of sequence-, PCR-RFLP- or SNP-based identification systems to aid accurate taxonomic assignments of *Beauveria* species in epidemiological and ecological investigations.

The majority of strains in this study were isolated as anamorphs from insects and soil. However several strains were isolated from *Cordyceps* specimens either from stromatal tissues or ascospores. These teleomorph-derived strains were assigned phylogenetically either to *B. bassiana*, *B. brongniartii* or *B. sungii*. Teleomorphs for *B. bassiana* (Li et al. 2001) and *B. brongniartii* (Shimazu et al. 1988) have been described, whereas the anamorph-teleomorph connection for *B. sungii* reported here represents a third *Beauveri*a species whose life cycle is confirmed to include a sexual state. The teleomorph specimens for the strains included in this study were not available for examination, but collection data describe all three teleomorphs as having yellow-pigmented stromata. Additional morphological and cultural investigations are needed to characterize these teleomorphs and to determine whether they are synonyms of previously described *Cordyceps* species, such as *C. staphylinidicola* Kobayasi & Shimizu (1982), *C. sulfurea* Kobayasi & Shimizu (1983) and *C. scarabaeicola* Kobayasi & Shimizu (1976), and possibly additional *Cordyceps* species that produce yellow-pigmented stromata. Recent progress in methods for in vitro fruiting of *Cordyceps* species with *Beauveria* anamorphs (Sung et al. 2006; Lee et al. 2007, 2010) are promising developments with potential to inform systematic and life history studies of *Beauveria* and its *Cordyceps* teleomorphs through integrated phylogenetic, developmental and mating studies.

ACKNOWLEDGMENTS

We are extremely grateful for the help of Veronica Martins, Ellen P. Buckley, Karen Hansen, Michael M. Wheeler and Clare Whittaker who provided invaluable laboratory support throughout this investigation. In addition we thank Christian Feuillet for his assistance in editing the Latin diagnoses, Genevieve Lewis-Gentry at FH for providing information on the holotype of *Isaria amorpha* and Ted Schultz for advice and assistance with the Bayesian analysis. The mention of trade products or firm names does not imply that the U.S. Department of Agriculture recommends them over similar products or firms not mentioned.

LITERATURE CITED

Aquino de Muro M, Elliott S, Moore D, Parker BL, Skinner M, Reid W, Bouhssini M El. 2005. Molecular characterization of *Beauveria bassiana* isolates obtained from overwintering sites of Sunn Pest (*Eurygaster* and *Aelia* species). Mycol Res 109:294–306, doi:10.1017/S0953756204001832

———, Mehta S, Moore D. 2003. The use of amplified fragment length polymorphism for molecular analysis of *Beauveria bassiana* isolates from Kenya and other countries and their correlation with host and geographical origin. FEMS Microbiol Lett 229:249–257, doi:10.1016/S0378-1097(03)00841-3

Balsamo-Crivelli G. 1835a. Ossevazione sopra una nuova specie di Mucedinea del genere *Botrytis*, etc. Bibl Ital 79:125.

———. 1835b. Zwei neuen Arten Mucedineen, *Botrytis bassiana* und *Mucor radicans*, und über die Entickelung der ersteren Art im Seidenwurme. Linneae 10: 609–618.

Bassi A. 1835. Del mal del segno, calcinaccio o moscardino, malattia che affligge I bachi da seta. Lodi: Tipografia Orcesi [English translation by PJ Yarrow: 1958. On the mark disease, calcinacccio or muscardine, a disease that affects silk worms. Ainsworth GC, Yarrow PJ, eds. APS Phytopathol. Classics 10. p 1–49].

Bissett J, Widden P. 1986. A new species of *Beauveria* from Scottish moorland soil. Can J Bot 66:361–362, doi:10.1139/b88-057

de Hoog GS. 1972. The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. Stud Mycol 1:1–41.

Faria MR, Wraight SP. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43:237–256, doi:10.1016/j.biocontrol.2007.08.001

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791, doi:10.2307/2408678

———, Churchill GA. 1996. A hidden Markov model approach to variation among sites in rate of evolution. Mol Biol Evol 13:93–104.

Ghikas DV, Kouvelis VN, Typas MA. 2010. Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii*. BMC Microbiol 10:174, doi:10.1186/1471-2180-10-174

Glare TR, Reay SD, Nelson TL, Moore R. 2008. *Beauveria caledonica* is a naturally occurring pathogen of forest beetles. Mycol Res 112:352–360, doi:10.1016/j.mycres.2007.10.015

Goettel MS, Eilenberg J, Glare TR. 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Iatrou K, Gill S, eds. Elsevier: Compr Mol Insect Sci 6:361–406, doi:10.1016/B0-44-451924-6/00088-0

Hofstetter V. 1978. Notes on some fungicolous hyphomycetes and their relatives. Persoonia 10:33–81.

———, Miadlikowska J, Kauff F, Lutzoni F. 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). Mol Phylogenet Evol 44:412–426, doi:10.1016/j.ympev.2006.10.016

———, Rao V. 1975. Some new hyphomycetes. Persoonia 8: 207–212.

Höhnel F Von. 1909. Fragmente zur Mykologie (VI Mitteilung, Nr. 182 bis 288). S&r Akud Wiss Wien 118:275–452.

Huang B, Li CR, Li ZG, Fan MZ, Li ZZ. 2002. Molecular identification of the teleomorph of *Beauveria bassiana*. Mycotaxon 81:229–236.

Katoh K, Kuma K-I, Toh H, Miyata T. 2005. MAFFT 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511–518, doi:10.1093/nar/gki198

———, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 94:286–298, doi:10.1093/bib/bbn013

Kobayasi Y, Shimizu D. 1976. The genus *Cordyceps* and its allies from New Guinea. Bull Nat Sci Mus, Ser. B (Tokyo) 2:133–151.

———, ———. 1982. *Cordyceps* species from Japan 4. Bull Nat Sci Mus, Ser. B (Tokyo) 83:79–91.

———, ———. 1983. *Cordyceps* species from Japan 6. Bull Nat Sci Mus, Ser. B (Tokyo) 9:1–21.

Kornerup A, Wansher AJ. 1961. Methuen Handbook of Color. London: Methuen & Co. 243 p.

Lee J-O, Shrestha B, Kim T-W, Sung G-H, Sung J-M. 2007. Stable formation of fruiting body in *Cordyceps bassiana*. Mycobiology 35:230–234, doi:10.4489/MYCO.2007.35.4.230

———, ———, Sung G-H, Han S-K, Kim T-W, Sung J-M. 2010. Cultural characteristics and fruiting body production in *Cordyceps bassiana*. Mycobiology 38:118–121, doi:10.4489/MYCO.2010.38.2.118

Li Z, Li C, Huang B, Fan M. 2001. Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. Chin Sci Bull 46:751–753, doi:10.1007/BF03187215

Liu Y-J, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular datasets in the tribe Triticeae (Gramineae). Syst Biol 45:524–545, doi:10.1093/sysbio/45.4.524

Matheny PB, Liu Y-J, Ammirati JF, Hall BD. 2002. Using RPB1 to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). Am J Bot 89:688–698, doi:10.3732/ajb.89.4.688

Meyling NV, Lübeck M, Buckley EP, Eilenberg J, Rehner SA. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. Mol Ecol 18:1282–1293, doi:10.1111/j.1365-294X.2009.04095.x

Minnis AM, Rehner SA, Humber RA. 2011. Proposal to conserve the name *Botrytis brongniartii* (*Beauveria brongniartii*) with a conserved type (Ascomycota). Taxon 60:233.

Posada D, Crandall K. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818, doi:10.1093/bioinformatics/14.9.817

Reeb V, Lutzoni F, Roux C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Mol Phylogenet Evol 32:1036–1060, doi:10.1016/j.ympev.2004.04.012

Rehner SA, Buckley EP. 2005. A *Beauveria* phylogeny inferred from ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98, doi:10.3852/mycologia.97.1.84

———, Posada F, Buckley EP, Infante F, Castillo A, Vega FE. 2006a. Phylogenetic origins of African and Neotropical

Beauveria bassiana s.l. pathogens of the coffee berry borer, *Hypothenemus hampei*. J Invert Pathol 93:11–23, doi:10.1016/j.jip.2006.04.005

———, Aquino de Muro M, Bischoff JF. 2006b. Description and phylogenetic placement of *Beauveria malawiensis* sp. nov. (Clavicipitaceae, Hypocreales). Mycotaxon 98: 137–145.

Roberts DW, Hajek AE. 1992. Entomopathogenic fungi as bioinsecticides. In: Leatham GF, ed. Frontiers in industrial mycology. New York: Chapman & Hall. p 144–159.

Rojas EI, Rehner SA, van Bael SA, Herre EA, Cannon P, Chen R, Pang J, Wang R, Zhang Y, Peng Y-Q, Sha T. 2010. *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panamá: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102: 1318–1338, doi:10.3852/09-244

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

Saccardo PA. 1892. Sylloge Fungorum. 10:1–964.

Samson RA, Evans HC. 1982. Two new *Beauveria* spp. from South America. J Invert Pathol 39:93–97, doi:10.1016/0022-2011(82)90162-8

Shimazu M, Mitsuhashashi W, Hashimoto H. 1988. *Cordyceps brongniartii* sp. nov., the teleomorph of *Beauveria brongniartii*. Trans Mycol Soc Japan 29:323–330.

Stiller JW, Hall BD. 1997. The origin of red algae: implications for plastid evolution. Proc Natl Acad Sci USA 94:4250–4255, doi:10.1073/pnas.94.9.4520

Sung J-M, Lee J-O, Humber RA, Sung G-H, Shrestha B. 2006. *Cordyceps bassiana* and production of stromata in vitro showing *Beauveria* anamorph in Korea. Mycobiology 34:1–6, doi:10.4489/MYCO.2006.34.1.001

Sung G-H, Hywel-Jones NL, Sung J-M, Luangsa-ard JJ, Shrestha B, Spatafora JW. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Stud Mycol 57:5–59, doi:10.3114/sim.2007.57.01

Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). 4. Sunderland, Massachusetts: Sinauer Associates.

Vega FE, Posada F, Aime MC, Pava-Ripoli M, Infante F, Rehner SA. 2008. Entomopathogenic fungal endophytes. Biol Control 46:72–82, doi:10.1016/j.biocontrol.2008.01.008

Vellinga EC. 1988. Glossary. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC, eds. Flora Agaracina Neerl 1:54–64.

Vey A, Hoagland RE, Butt TM. 2001. Toxic metabolites of fungal biocontrol agents. In: Butt TM, Jackson C, Magan H, eds. Fungi as bioiocontrol agents: progress, problems and potential. Wallingford, UK: CAB International. p 311–346.

von Höhnel F. 1909. Fragmente zur Mikologie (VI Mitteilung, Nr. 182 bis 288). Sitzungsber Akad Wiss Wien 118:275–452.

White TJ, Bruns TD, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Inis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [doctoral dissertation]. Austin: Univ Texas Press. 115 p.