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A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs

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Abstract: *Beauveria* is a globally distributed genus of soil-borne entomopathogenic hyphomycetes of interest as a model system for the study of entomopathogenesis and the biological control of pest insects. Species recognition in *Beauveria* is difficult due to a lack of taxonomically informative morphology. This has impeded assessment of species diversity in this genus and investigation of their natural history. A gene-genealogical approach was used to investigate molecular phylogenetic diversity of *Beauveria* and several presumptively related *Cordyceps* species. Analyses were based on nuclear ribosomal internal transcribed spacer (ITS) and elongation factor 1-alpha (EF1-α) sequences for 86 exemplar isolates from diverse geographic origins, habitats and insect hosts. Phylogenetic trees were inferred using maximum parsimony and Bayesian likelihood methods. Six well supported clades within *Beauveria,* provisionally designated A–F, were resolved in the EF1-α and combined gene phylogenies. *Beauveria bassiana,* a ubiquitous species that is characterized morphologically by globose to subglobose conidia, was determined to be non-monophyletic and consists of two unrelated lineages, clades A and C. Clade A is globally distributed and includes the Asian teleomorph *Cordyceps staphylinidaecola* and its probable synonym *C. bassiana*. All isolates contained in Clade C are anamorphic and originate from Europe and North America. Clade B includes isolates of *B. brongniartii,* a Eurasian species complex characterized by ellipsoidal conidia. Clade D includes *B. caledonica* and *B. vermiconia,* which produce cylindrical and comma-shaped conidia, respectively. Clade E, from Asia, includes *Beauveria* anamorphs and a *Cordyceps* teleomorph that both produce ellipsoidal conidia. Clade F, the basal branch in the *Beauveria* phylogeny includes the South American species *B. amorpha,* which produces cylindrical conidia. Lineage diversity detected within clades A, B and C suggests that prevailing morphological species concepts underestimate species diversity within these groups. Continental endemism of lineages in *B. bassiana s.l.* (clades A and C) indicates that isolation by distance has been an important factor in the evolutionary diversification of these clades. Permutation tests indicate that host association is essentially random in both *B. bassiana s.l.* clades A and C, supporting past assumptions that this species is not host specific. In contrast, isolates in clades B and D occurred primarily on coleopteran hosts, although sampling in these clades was insufficient to assess host affliation at lower taxonomic ranks. The phylogenetic placement of *Cordyceps staphylinidaecola/bassiana,* and *C. scarabaeicola* within *Beauveria* corroborates prior reports of these anamorph-teleomorph connections. These results establish a phylogenetic framework for further taxonomic, phylogenetic and comparative biological investigations of *Beauveria* and their corresponding *Cordyceps* teleomorphs.

Key words: Ascomycetes, *Beauveria,* Clavicipitaceae, *Cordyceps,* cryptic species, systematics

INTRODUCTION

Beauveria (Bals.) Vuill. (Ascomycota: Hypocreales) is a cosmopolitan genus of haploid, soil-borne hyphomycetes of significance for their role as insect pathogens and the production of biologically active metabolites (Steinhaus 1963, Dunn and Mechalas 1963, Ferron 1978, McCoy 1990, Feng et al 1994, Gillespie and Moorehouse 1989, Ferron et al 1991). Despite long term interest in developing *Beauveria* as a biological alternative to chemically based insecticides, progress toward this goal has been hindered in part by difficulties in recognizing and identifying species in this genus. As a result, little is known about the genetic bases and pattern(s) of variation in the determinants of host range, mode of pathogenesis, virulence and the role of toxic metabolites in entomopathogenesis by individual species of *Beauveria.*

Agostino Bassi (1835) first described *Beauveria* as the causal agent of *mal del segno* or the mark disease, also known as *calcinaccio* or *cannellino* in Italy and white *muscardino* in France, which caused economically devastating epizootics of domestic larval silkworms in southern Europe during the 18th and 19th centuries. In his studies with *Beauveria,* Bassi was the

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first to demonstrate that microbes can act as contagious pathogens of animals, providing an important antecedent to the germ theory of disease (Ainsworth 1973). The first taxonomic recognition of the *muscardino* fungus was proposed by Balsamo-Crivelli (1835a, b) who acknowledged Bassi's discoveries by naming this pathogen *Botrytis bassiana*. The genus *Beauveria,* however, was not formally described until the early 20th century by Vuillemin (1912), who designated *Botrytis bassiana* Bals.-Criv. as the type species.

Beauveria is characterized morphologically by its sympodial to whorled clusters of short-globose to flask-shaped conidiogenous cells, which give rise to a succession of one-celled, hyaline, holoblastic conidia that are borne on a progressively elongating sympodial rachis. Although morphologically distinctive as a genus, species identification in *Beauveria* is difficult because of its structural simplicity and the lack of distinctive phenotypic variation. Conidia are the principal morphological feature used for species identification in *Beauveria*. In shape conidia may be globose, ellipsoidal, reniform to cylindrical, or comma-shaped, and range in size from 1.7 to 5.5 μm. Species identification in *Beauveria* has been complicated by the proliferation of new species described between the late 19th to mid-20th centuries, few of which are morphologically distinct from previously described species.

Several revisionary studies of *Beauveria* have been conducted to evaluate morphological species concepts. Petch (1926) recognized two species, *B. bassiana* and *B. densa* (Link) F. Picard and concluded that cultural data were uninformative for delimiting species. MacLeod (1954) monographed *Beauveria* and, like Petch, recognized only two species, which he classified in *B. bassiana* and *B. brongniartii* (Sacc.) Petch (= *B. densa*). Hoog (1972) concurred with MacLeod but recognized an additional species, *B. alba* (Limber) Saccas, which was later transferred to *Engyodontium* (Limber) Hoog (Hoog 1978). More recently, Hoog and Rao (1975) and Samson and Evans (1982) described several new species. In all, forty-nine species have been placed in *Beauveria* and 22 epithets are currently valid. Today, researchers generally follow Macleod (1954) and Hoog (1972) and classify most environmental isolates of *Beauveria* in either *B. bassiana* or *B. brongniartii,* a practice reflected in contemporary texts and keys to species identification (Humber 1997, Tanada and Kaya 1993).

Ongoing difficulties in applying morphological approaches to species recognition in *Beauveria* have spurred the search for additional sources of taxonomic characters. Alternative character systems that have been investigated include isozymes (St. Leger et al 1992, Poprawski et al 1988), chemotaxonomic characters (Mugnai et al 1989), mitochondrial RFLP (Hegedus et al 1993), immunological approaches (Shimizu and Aizawa 1988; Tan and Ekramoddoullah 1991), rRNA sequencing (Rakotonirainy et al 1991), RFLP (Kosir et al 1991, Maurer et al 1997), introns in the large subunit rDNA (Neuveglise and Brygoo 1994, Neuveglise et al 1996), RFLP and nucleotide sequences of ITS (Neuveglise et al 1994, Coates et al 2002), SSCP analysis of taxon specific markers (Hegedus and Khachatourians 1993, 1996), RAPD markers (Bidochka et al 1994, Cravanzola et al 1997, Maurer et al 1997), and the combined use of morphology and RAPD markers (Glare and Inwood 1998). Although all character systems investigated in these studies were effective in detecting genetic variation within *Beauveria,* none have been applied directly to taxonomic investigations in this genus.

Although biologically relevant species concepts and explicit species recognition criteria have yet to be defined for *Beauveria,* recent molecular and cultural studies have provided insight regarding the phylogenetic position and reproductive biology of several species. An rDNA phylogeny by Sung et al (2001) supports a single evolutionary origin of *Beauveria* within the subfamily Cordycipitoideae of the Clavicipitaceae, and that the teleomorph *C. scarabaeicola* is nested within *Beauveria* and is the sister to *B. caledonica* Bissett & Widden. Second, strains isolated from stromata of several *Cordyceps* species produce *Beauveria* anamorphs, clearly demonstrating that some *Beauveria* species are sexual. These *Cordyceps* species include *C. bassiana* Li, Li, Huang & Fan (Li et al 2001), *C. brongniartii* Shimazu (Shimazu et al 1988), *C. staphylinidaecola* Kobayasi & Shimazu (1982), and *C. sobolifera* Berk. (Liu et al 2001). Together the molecular phylogenetic and cultural data support a *Cordyceps* origin to the *Beauveria* lineage.

Here we present a molecular phylogenetic analysis based on 75 exemplar isolates of *Beauveria* representative of its known taxonomic diversity, geographic distributions and insect host ranges, plus eleven presumptively related *Cordyceps* teleomorph accessions. We compared and combined reconstructed phylogenies of two nuclear loci, the ribosomal internal transcribed spacer (ITS) and elongation factor 1-alpha (EF1-α), to infer an organismal phylogeny. We then used the combined gene phylogeny to address the following questions: 1) What is the pattern of morphological variation with respect to this phylogeny and how do phylogenetic groupings correspond to prevailing morphological species concepts in *Beauveria*? 2) Are morphological species in *Beauveria* cryptically diverse? 3) What are the geographic dis-

tributions of species lineages within this genus? 4) What is the coevolutionary pattern of association between *Beauveria* and its insect hosts? 5) What are the phylogenetic affinities of *C. bassiana, C. scarabaeicola, C. sobolifera* and *C. staphylinidaecola,* each of which has been directly linked to *Beauveria*?

MATERIALS AND METHODS

Biological materials.—Isolates of *Beauveria* and *Cordyceps* were obtained from the USDA-ARS Entomopathogenic Fungus Collection (ARSEF), Ithaca, New York (Humber 2001), and are listed in TABLE I. *Beauveria* species sampled in this study include *Beauveria amorpha* (Höhn.) Samson & H. C. Evans, *B. bassiana, B. brongniartii* (Sacc.) Petch, *B. caledonica* Bissett & Widden, *B. vermiconia* de Hoog & V. Rao, and multiple unidentified *Beauveria* accessions. Isolates were selected to represent diverse agricultural and non-agricultural habitats and different geographic regions including North, South and Central America, Europe, North Africa, Asia and Australia, and from different insect orders, including Coleoptera, Dermaptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanoptera (TABLE I). Nine living isolates of *Cordyceps,* accessioned as *C. bassiana, C. staphylinidaecola,* and *C. scarabaeicola,* were also included. Additionally, portions of stromata from two dried specimens of C, *scarabaeicola,* originating from the Entomopathogenic Fungal Culture Collection (EFCC, Korea), were provided by J. Spatafora and G.-H. Sung (TABLE I). Cultures of the outgroup taxa, *Cordyceps militaris* (L.) Link (JWS 00-293) and *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm. (JWS 00-224) were provided by J. Spatafora. Isolates were stored in 10% glycerol at −70 C. Isolates were grown on quarter strength SDY medium (Goettel and Inglis 1997). Mycelium for DNA extraction was produced by culturing in quarter strength SDY broth at 100 rpm on a rotary shaker for 2–3 days at 25 C. Mycelium was harvested from broth cultures by centrifugation, washed twice with sterile distilled water, then lyophilized and stored at −20 C.

DNA extraction, PCR and sequencing.—Lyophilized mycelium was ground under liquid nitrogen in microcentrifuge tubes and the DNA extracted by a modification of a method described by Cambareri and Kinsey (1993). The ground mycelium was suspended in a detergent solution composed of 2 M NaCl, 0.4% w/v deoxycholic acid, 1.0% w/v polyoxyethylene 20 cetyl ether, and incubated at 55 C for 15–30 min. The lysate was extracted with an equal volume of CIA (24:1 chloroform : isoamyl alcohol) and the cellular debris pelleted by centrifugation at 14 000 × *g* for 15 min. The supernatant was transferred to a clean tube, mixed with an equal volume of 6 M guanidinium thiocyanate, and total nucleic acids were precipitated with the addition of 0.6 volumes of isopropanol. Nucleic acids were spooled onto a bent pipet tip and dissolved in 300 μL TE buffer (10 mM Tris pH 7.5, 0.1 mM EDTA). RNA was digested with 5 μL of a 10 mg per ml solution of RNAse A (Amresco, Solon OH) for 30 min at 37 C. Following RNA digestion the supernatant was extracted with an equal volume of CIA. The supernatant was adjusted to 2.5 M lithium chloride and incubated at −20 C for 30 min to overnight followed by a 15 min centrifugation at 14 000 × *g* to remove carbohydrate precipitate. The supernatant was transferred to a clean tube and nucleic acids were precipitated with 2.5 volumes of 95% ethanol and pelleted by centrifugation at 14 000 × *g*. The DNA pellets were washed twice in 70% ethanol, air-dried, and then resuspended in sterile distilled water to a final concentration of 1–2 ng per μL and stored at −20 C.

Two nuclear gene regions, ITS and EF1-α, were sequenced and analyzed. The ITS was amplified and sequenced with primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC) (White et al 1990). The nearly complete coding region of EF1-α was amplified and sequenced using a combination of primers designed in our laboratory using the computer program Oligo 6 (MBI, Cascade, Colorado). An ~1200 bp segment spanning the 5′ ⅔ of EF1-α was amplified with primers EF1T (5′-ATGGGTAAGGARGACAAGAC) and 1567R (5′-ACHGTRCCRATACCACCSATCTT). The EF1T × 1567R fragments were sequenced with the amplification primers and two internal primers, EFjR (5′-TGYTCNCGRGTYTGNCCRTCYTT) and 983F (5′-GCYCCYGGHCAYCGTGAYTTYAT). An overlapping fragment of approximately 1000 bp that extends nearly to the 3′ end of EF1-α was amplified with primers 983F and 2218R (5′-ATGACACCRACRGCRACRGTYTG). The amplification primers and three additional internal primers, 1577F (5′- CARGAYGTBTACAAGATYGGTGG), 1567RintB (5′-ACHGTRCCRATACCACCRAT) and 2212R (5′-CCRAACRGCRACRGTYYGTCTCAT) were used for sequencing the 983F × 228R amplicon.

PCR amplifications were performed in a total volume of 50 μL, which included 5μL of 10× PCR buffer (10 mM Tris/HCl pH 8.0, 50 mM KCl, 1.5–2.0 mM $MgCl_2$), 4 μL of dNTP mix (1.25 mM each dATP, dCTP, dGTP, and dTTP), 10 pmol each of the opposing amplification primers, 0.5 ul *Taq* polymerase (Promega, Madison WI), and 5–20 ng genomic DNA. PCR for both loci was performed using a touchdown PCR procedure (Don et al 1991). Touchdown PCR amplifications were initiated with a 2 min denaturation at 94 C. The annealing temperature in the first amplification cycle was 66 C, which was subsequently incrementally reduced by 1 C per cycle over the next 9 cycles. An additional 36 amplification cycles were then performed, each consisting of 30 s denaturation at 94 C, a 30 s annealing step at 56 C, and a 1 min extension at 72 C, concluding with a 10 min incubation at 72 C. PCR reaction volumes were reduced to approximately 10 μL by lyophilization, then separated on a 1.5% NuSieve agarose gel (BioWhittaker, Rockland, Maine) in a low EDTA Tris-acetate buffer (40 mM Tris-acetate, 0.1 mM EDTA). PCR products were cut from the gel, frozen and thawed and the DNA extruded from the gel slice by centrifugation for 10 min at 20 000 × *g*.

Miniaturized sequencing reactions were performed with ABI BigDye 2.0 (Applied Biosystems, Foster City, CA) using 0.5 μL BigDye diluted in 1.5 μL dilution buffer (400 mM Tris/HCl pH 9.0, 10 mM $MgCl_2$), 3 pmol primer, 75–100 ng gel-purified PCR template in a total volume of 5 μL.

TABLE I. List of specimens sequenced, geographic origins, host or substrate, conidial size, and GenBank accession numbers. Culture accession numbers are those of the USDA-ARS Entomopathogenic Fungus Culture Collection unless otherwise noted

ARSEF #	Identification	Country	Host class: order	Conidial size (μm)	ITS GenBank	EF1-α GenBank
32	*Beauveria* sp.	USA	Orthoptera: Acrididae	2.3–5.0 × 2.3–3.1	AY532017	AY531930
152	*B. bassiana*	Commonwealth of Independent States	Hymenoptera: Pamphiliidae	2.9–3.5 × 2.5–3.1	AY531983	AY531892
156	*B. bassiana*	Poland	Hymenoptera: Ichneumonidae	1.7–2.3 × 1.5–1.9	AY531985	AY531895
292	*B. bassiana*	Commonwealth of Independent States	NA	2.5–3.1 × 2.3–2.9	AY532011	AY531921
296	*B. bassiana*	USA	NA	2.1–3.6 × 1.9–2.4	AY532013	AY531922
299	*B. bassiana*	Rumania	Lepidoptera: Lymantriidae	2.5–2.9 × 2.3–2.6	AY532014	AY531923
300	*B. bassiana*	Australia	Hemiptera: Lygaeidae	2.1–2.7 × 1.9–2.5	AY532015	AY531924
326	*B. bassiana*	Australia	Lepidoptera: Pyralidae	2.1–4.0 × 2.1–2.9	AY532021	AY531929
344	*B. bassiana*	USA	Coleoptera: Chrysomelidae	2.3–3.1 × 2.3–2.9	AY532023	AY531932
652	*B. bassiana*	China	Lepidoptera: Pyralidae	2.7–3.4 × 2.5–2.9	AY532032	AY531941
656	*B. amorpha*	People's Republic of China	Homoptera: Cicadellidae	2.9–4.2 × 1.8–2.3	AY532033	AY531942
678	*B. brongniartii*	People's Republic of China	Homoptera: Cicadellidae	3.3–4.2 × 2.1–2.5	AY532037	AY531946
681	*B. bassiana*	Commonwealth of Independent States	NA	2.2–2.7 × 2.1–2.6	AY532038	AY531947
714	*B. bassiana*	People's Republic of China	Homoptera: Delphacidae	2.1–2.9 × 1.7–2.6	AY532042	AY531951
730	*B. bassiana*	Brazil	Hymenoptera: Vespidae	2.1–2.8 × 1.7–2.3	AY532043	AY531952
733	*B. bassiana*	Brazil	Coleoptera: Curculionidae	1.9–2.7 × 1.7–2.2	AY532044	AY531953
751	*B. bassiana*	Vietnam	Coleoptera: Chrysomelidae	2.3–3.1 × 2.3–2.9	AY532045	AY531954
753	*B. bassiana*	Brazil	Coleoptera: Curculionidae	1.7–3.0 × 1.6–2.6	AY532046	AY531955
788	*B. bassiana*	Brazil	Coleoptera: Chrysomelidae	2.3–3.2 × 2.0–2.9	AY532047	AY531956
792	*B. bassiana*	USA	Hemiptera: Lygaeidae	2.3–3.6 × 2.2–2.7	AY532048	AY531957
793	*B. bassiana*	USA	Soil	2.5–3.8 × 2.1–3.6	AY532049	AY531958
796	*B. bassiana*	Colombia	Dermaptera	2.4–2.9 × 1.9–2.6	AY532050	AY531959
812	*B. bassiana*	France	Hemiptera: Tingidae	2.3–3.1 × 2.3–3.1	AY532051	AY531960
813	*B. bassiana*	France	Coleoptera: Curculionidae	2.9–3.7 × 2.1–2.7	AY532052	AY531961
816	*B. bassiana*	France	Coleoptera: Curculionidae	2.3–3.3 × 2.3–3.2	AY532053	AY531962
842	*B. bassiana*	Colombia	Coleoptera: Curculionidae	1.8–2.5 × 1.7–2.2	AY532054	AY531963
843	*B. bassiana*	Costa Rica	Lepidoptera: Saturniidae	1.9–3.8 × 1.9–2.9	AY532055	AY531964
937	*B. bassiana*	Brazil	Coleoptera: Chrysomelidae	1.9–2.5 × 1.7–2.1	AY532056	AY531965
1040	*Beauveria* sp.	Japan	Lepidoptera: Bombycidae	4.2–7.1 × 2.1–2.7	AY531972	AY531881
1041	*B. bassiana*	Japan	Coleoptera: Cerambycidae	4.2–4.8 × 3.5–4.0	AY531973	AY531882
1053	*B. bassiana*	Brazil	Orthoptera: Acrididae	2.4–3.1 × 2.1–2.5	AY531974	AY531883
1153	*B. bassiana*	Morocco	Coleoptera: Curculionidae	2.3–2.9 × 2.1–2.7	AY531975	AY531884
1156	*B. bassiana*	France	Coleoptera: Curculionidae	1.9–2.3 × 1.5–2.1	AY531976	AY531885
1185	*B. bassiana*	France	Coleoptera: Curculionidae	2.5–3.1 × 2.3	AY531977	AY531886
1359	*B. bassiana*	Poland	NA	NA	AY531978	AY531887
1398	*B. bassiana*	France	Coleoptera: Curculionidae	1.9–2.9 × 1.9–2.5	AY531979	AY531888
1431	*B. brongniartii*	Philippines	Coleoptera: Cerambycidae	3.1–3.8 × 2.1–2.5	AY531980	AY531889

TABLE I. Continued

ARSEF #	Identification	Country	Host class: order	Conidial size (μm)	ITS GenBank	EF1-α GenBank
1478	*B. bassiana*	Brazil	Hemiptera: Pentatomidae	2.1–2.5 × 1.9–2.3	AY531981	AY531890
1479	*B. bassiana*	Brazil	Lepidoptera: Stenomatinae	1.9–2.7 × 1.6–2.0	AY531982	AY531891
1558	*B. bassiana*	Italy	Lepidoptera: Cossidae	4.4–4.8 × 3.1–3.5	AY531984	AY531893
1567	*B. bassiana*	Switzerland	Coleoptera: Scolytidae	3.7–5.2 × 1.9–2.3	AY531986	AY531894
1628	*B. bassiana*	Hungary	Lepidoptera: Noctuidae	2.3–3.3 × 2.2–3.1	AY531987	AY531896
1678	*B. bassiana*	Japan	Coleoptera: Cerambycidae	3.8–4.2 × 2.7–3.3	AY531988	AY531897
1679	*B. bassiana*	Japan	Coleoptera: Cerambycidae	2.1–2.9 × 1.9–2.7	AY531989	AY531898
1685	*Beauveria* sp.	Japan	Coleoptera: Scarabaeidae	3.1–4.4 × 2.5–3.1	AY531990	AY531899
1802	*B. bassiana*	Greece	Hemiptera: Miridae	2.2–2.9 × 2.0–2.5	AY531991	AY531900
1811	*B. bassiana*	Morocco	Coleoptera: Curculionidae	2.1–2.5 × 1.9–2.1	AY531992	AY531901
1829	*B. brongniartii*	Brazil	Lepidoptera: Castniidae	2.3–2.9 × 2.2–2.4	AY531993	AY531902
1830	*B. brongniartii*	Brazil	Lepidoptera: Pyralidae	2.3–3.8 × 2.1–3.3	AY531994	AY531903
1848	*B. brongniartii*	Belgium	Coleoptera: Rhizophagidae	2.1–2.9 × 1.9–2.3	AY531995	AY531904
1953	*B. bassiana*	Brazil	Hymenoptera: Vespidae	1.9–2.3 × 1.6–2.0	AY531996	AY531905
1959	*B. bassiana*	Brazil	Orthoptera: Acrididae	2.1–3.1 × 1.9–2.5	AY531997	AY531906
1969	*B. bassiana*	Peru	Coleoptera: Curculionidae	NA	AY531998	AY531907
1975	*B. bassiana*	France	Coleoptera: Carabidaee	2.0–2.5 × 1.9–2.3	AY531999	AY531908
1988	*B. bassiana*	Austria	Hemiptera: Nabidae	2.2–2.9 × 2.1–2.7	AY532000	AY531909
2040	*B. bassiana*	Korea	Coleoptera: Curculionidae	2.2–2.5 × 2.0–2.5	AY532001	AY531910
2054	*B. bassiana*	USA	Lepidoptera: Lymantriidae	2.5–2.9 × 2.5–2.7	AY532002	AY531911
2251	*B. amorpha*	Brazil	Coleoptera	3.5–4.2 × 2.1–2.5	AY532003	AY531912
2544	*B. bassiana*	Mexico	Homoptera: Cicadidae	2.7–3.1 × 2.5–2.9	AY532005	AY531914
2567	*B. caledonica*	Scotland	Soil		AY532006	AY531915
2579	*B. bassiana*	USA	Coleoptera: Chrysomelidae	2.1–3.3 × 2.1–2.7	AY532007	AY531916
2641	*B. amorpha*	Brazil	Hymenoptera: Formicidae	3.8–5.2 × 1.7–2.5	AY532008	AY531917
2857	*B. bassiana*	Mexico	Lepidoptera	2.5–3.2 × 2.5–2.9	AY532009	AY531918
2883	*B. bassiana*	USA	Homoptera: Aphididae	2.5–3.1 × 2.3–2.9	AY532010	AY531919
2922	*B. vermiconia*	Chile	Soil	2.1–2.9 × 1.7–2.7	AY532012	AY531920
3097	*B. bassiana*	NA	NA	NA	AY532016	AY531925
3216	*B. bassiana*	USA	Thysanoptera: Thripidae	2.3–3.5 × 2.1–2.9	AY532019	AY531927
3220	*B. bassiana*	Portugal	Lepidoptera: Tortricidae	2.5–3.8 × 2.5–2.7	AY532020	AY531928
3405	*B. bassiana*	USA	Lepidoptera: Lymantriidae	2.3–3.7 × 2.1–2.9	AY532022	AY531931
4021	*B. bassiana*	Denmark	Coleoptera: Curculionidae	2.1–2.5 × 2.1–2.5	AY532024	AY531933
4362	*Beauveria* sp.	Japan	Soil	3.5–3.9 × 2.1–2.5	AY532025	AY531934
4384	*B. brongniartii*	China	Coleoptera: Scarabidae	4.2–4.6 × 2.3–2.5	AY532026	AY531935
4474	*B. brongniartii*	China	Coleoptera: Scarabidae	4.2–4.8 × 2.3–2.5	AY532027	AY531936
4850	*B. brongniartii*	Korea	Coleoptera: Cerambycidae	4.2–5.9 × 2.7–3.8	AY532028	AY531937
			Isolated from Biolisa™, Nitto		AY532060	AY531968
ND1[1]	*B. brongniartii*	Japan	Denko	4.2–5.8 × 2.7–2.9		
4933	*B. bassiana*	France	Coleoptera: Scolytidae	2.3–3.1 × 2.1–2.9	AY532029	AY531938
7047	*Cordyceps bassiana*	China	Lepidoptera: Cossidae	1.9–2.3 × 1.6–2.0	AY532041	AY531950

TABLE I. Continued

ARSEF #	Identification	Country	Host class: order	Conidial size (μm)	ITS GenBank	EF1-α GenBank
EFCC 2533[2]	*Cordyceps* cf. *scarabaeicola*	Nepal	NA	NA	AY532058	AY531967
EFCC 252[2]	*Cordyceps* cf. *scarabaeicola*	S. Korea	NA	NA	AY532057	AY531966
5689	*Cordyceps scarabaeicola*	China	Coleoptera: Scarabaeidae	3.0–4.4 × 2.5–3.2	AY532030	AY531939
5718	*Cordyceps staphylinidaecola*	Korea	Unidentified larva	2.0–2.3 × 2.0–2.3	AY532031	AY531940
6721	*Cordyceps staphylinidaecola*	Korea	Coleoptera: Cerambycidae	2.1–2.4 × 2.0–2.3	AY532034	AY531943
6722	*Cordyceps staphylinidaecola*	Korea	Hemiptera: Pentatomidae	2.1–2.3 × 2.0–2.3	AY532035	AY531944
6723	*Cordyceps staphylinidaecola*	Korea	Homoptera: Coccidae	2.0–2.3 × 2.0–2.3	AY532036	AY531945
7043	*Cordyceps staphylinidaecola*	Korea	NA	3.1–4.3 × 2.5–3.2	AY532039	AY531948
7044	*Cordyceps staphylinidaecola*	Korea	NA	3.0–4.4 × 2.5–3.2	AY532040	AY531949
JWS 00-293[2]	*Cordyceps militaris*	USA	NA	NA	NA	AY531969
JWS 00-224[2]	*Paecilomyces farinosus*	USA	NA	NA	NA	AY531970

[1] EFCC, Entomopathogenic Fungus Culture Collection, Korea.
[2] Joseph Spatafora, Oregon State University, Corvallis, OR.
[3] Ann Hajek, Cornell University, Ithaca, NY.
NA, not available.

Cycle sequencing was performed in 96-well microtiter plates according to the manufacturer's instructions except that the total number of cycles was increased to 35. Cycle sequencing products were separated from residual reaction components by ethanol precipitation. The sequencing reactions were suspended in deionized formamide, heat denatured, and run on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

DNA sequences were assembled and edited using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan) and multiple sequence alignments were constructed with the MegAlign module of DNASTAR 5 (LaserGene, Madison, Wisconsin) and output in Nexus format for phylogenetic analysis. Multiple sequence alignments for ITS and EF1-α were concatenated into a single file using MacClade 4.0 (Maddison and Maddison 2000).

Phylogenetic analysis.—ITS and EF1-α data sets were analyzed separately and in combination under maximum parsimony (MP) and Bayesian-likelihood criteria. Bayesian inference was used because it enables relatively rapid analysis (as compared to maximum likelihood) of large data sets under complex evolutionary models of nucleotide substitution (Larget and Simon 1999) and yields posterior probabilities supporting phylogenetic hypotheses (Lewis 2001).

Parsimony analyses were implemented in PAUP 4.0b10 (Swofford 2001) using the heuristic search option with TBR branch swapping under equal character weighting, excluding both gapped and uninformative characters. To increase the probability that all islands of most-parsimonious trees were identified (Maddison 1991, Stewart 1993, Swofford et al 1996), 500 random-addition replicate analyses were executed. A heuristic MP bootstrap analysis (Felsenstein 1985) consisted of 1000 pseudoreplicates (TBR branch swapping), with 10 random-addition replicates per pseudoreplicate, and with gapped and parsimony-uninformative characters excluded. Clades with bootstrap values ≥70% were considered strongly supported by the data.

Bayesian analyses, started from a random tree using the program's default values for the prior probabilities, consisted of four simultaneous Markov chains, three heated and one cold, which were run for 10^6 generations. The Bayesian analyses were repeated four times and a single tree was sampled randomly every 100th generation. The log-likelihood scores for all generations were examined to identify the burn-in phase, or those initial generations in which likelihood scores progressively improve until they fluctuate narrowly around a stable value. In all Bayesian analyses, the latter 5000 sampled trees from each analysis were pooled (after confirming they had converged on similar log-likelihood values) and imported into PAUP 4.0b and a 50% consensus tree computed, with the support values for each branch constituting their posterior probability. Clades with posterior probabilities ≥95% were considered as significantly supported by the data (Huelsenbeck et al 2002).

Host association.—We performed a PTP test (Archie 1989, Faith and Cranston 1991) in PAUP 4.0b10 as described by Kelley and Farrell (1998) and Farrell et al (2001) to determine whether the pattern of fungus-insect associations differed significantly from the expectations of a randomly dis-

tributed character. Insect host order was coded as an unweighted character and randomly permuted 1000 times while maintaining the original character frequencies. The observed number of changes for the original data was compared to the distribution of reconstructed changes determined for the permuted data mapped on a single representative most parsimonious tree (MPT) and the P value of the original data computed. A score of $P < 0.05$ would indicate the actual data lie outside the test distribution, leading to rejection of the null hypothesis that host association is random.

Conidial measurements.—Between twenty to thirty conidia from 10–20 day old cultures were suspended in 0.01% Tween 40 and mixed with an equal volume of molten (70 C) 0.1% Nusieve GTG agarose (BioWhittaker, Rockland, ME). Conidia were observed using a Nikon E600 microscope equipped with Nikon DXM 1200 digital camera and Nikon ACT-1 image capture software. Conidia size measurements are given in TABLE I.

RESULTS

Molecular data sets.—ITS and EF1-α sequence data sets were constructed for 75 isolates of *Beauveria* spp. and eleven *Cordyceps* accessions identified as *C. bassiana, C. scarabaeicola* and *C. staphylinidaecola* (TABLE I). The ITS and EF1-α sequence data sets consisted of 605 and 1729 aligned positions, respectively. Gapped and uninformative positions were excluded from all parsimony analyses and the final data set contained 198 parsimony-informative characters, with ITS and EF1-α contributing 35 and 163 informative sites, respectively. EF1-α contained three closely spaced introns at the 5′ end of the gene whose combined length was approximately 400 bp. The EF1-α intron regions yielded nearly 66% of the informative sites obtained from this locus, with the remaining variable and informative sites occurring at 3rd codon positions in the exons. ITS and EF1-α sequences were also determined for *Cordyceps militaris* (JS 00-224) and *Paecilomyces farinosus* (JS 00-293). GenBank accession numbers for all sequence data generated in this study are listed in TABLE I.

Rooting the Beauveria *phylogeny.*—The root of the *Beauveria* phylogeny was inferred from an initial parsimony analysis that included all *Beauveria* isolates plus *C. scarabaeicola* accessions EFCC 2533 and EFCC 252, and single exemplar isolates of *C. militaris* (JS 00-224) and *Paecilomyces farinosus* (JS 00-293). These latter two species were previously determined to be closely related to but distinct from *Beauveria* in an 18S SSU rDNA phylogeny (Sung et al 2001). Nucleotide data for this analysis were obtained from the PCR fragment 983F × 2218R of EF1-α, which spans the latter ⅔ of the gene. This region of EF1-α lacks introns in the Hypocreales and, except for a unique fifteen base pair insertion (5 codons) in the outgroup *P. farinosus,* is colinear in *Beauveria,* thus facilitating sequence alignment and bolstering confidence in underlying assumptions of positional homology. In contrast, both the 5′ portion of EF1-α, which consists primarily of intron sequences, and the ITS spacers were unsuitable for this particular analysis because of extensive alignment ambiguities between *C. militaris, P. farinosus* and the *Beauveria* ingroup.

The EF1-α exon data set used to infer the root for *Beauveria* consisted of 87 3rd position parsimony-informative characters. Both *C. militaris* and *P. farinosus* were nearly equally divergent from *Beauveria* and from each other (data not shown). Regardless of which taxon was used as outgroup, the resulting phylogenetic analyses yielded topologically and statistically comparable sets of trees (data not shown). The parsimony analysis using *C. militaris* as outgroup yielded 120 equally parsimonious trees (MPT) of 191 steps with a rescaled consistency index (RC) of 0.4558 and 94% bootstrap support for *Beauveria* monophyly. In both analyses, *C. scarabaeicola* EFCC 2533 was basal to a monophyletic *Beauveria* (data not shown). Based on this result, the ITS and EF1-α sequences from *C. scarabaeicola* EFCC 2533 were used to root all subsequent analyses.

EF1-α phylogeny.—Parsimony analysis of EF1-α was terminated after 24 hours of CPU time. This analysis had progressed only to the 107th replicate and yielded 17 975 MPT of 353 steps with a character rescaled consistency index (RC) = 0.5751. The analysis was repeated, restricting the search to the first 100 trees encountered per replicate, for a total of 2000 replicates. The resulting trees had the same statistics as discovered in the original heuristic search. For the Bayesian analysis, four independent Markov-Chain Monte Carlo (MCMC) generations were run, each with a burn-in of 500K generations. All runs converged on approximately the same likelihood score and 5000 post burn-in trees from all four analyses were pooled. Trees inferred in both analyses were topologically compatible and a 50% consensus from the Bayesian analysis is shown in FIG. 1. In all, 30 internal branches in the parsimony analysis received bootstrap support greater than 70% and 38 branches in the Bayesian analysis had posterior probabilities equal to or greater than 95% (FIG. 1).

ITS phylogeny.—Maximum parsimony analysis of the ITS data set yielded 52 MPT of 60 steps with RC of 0.6001. A 50% consensus tree from the Bayesian likelihood analysis is presented in FIG. 1. Branch resolution and support in the ITS phylogeny was low, and

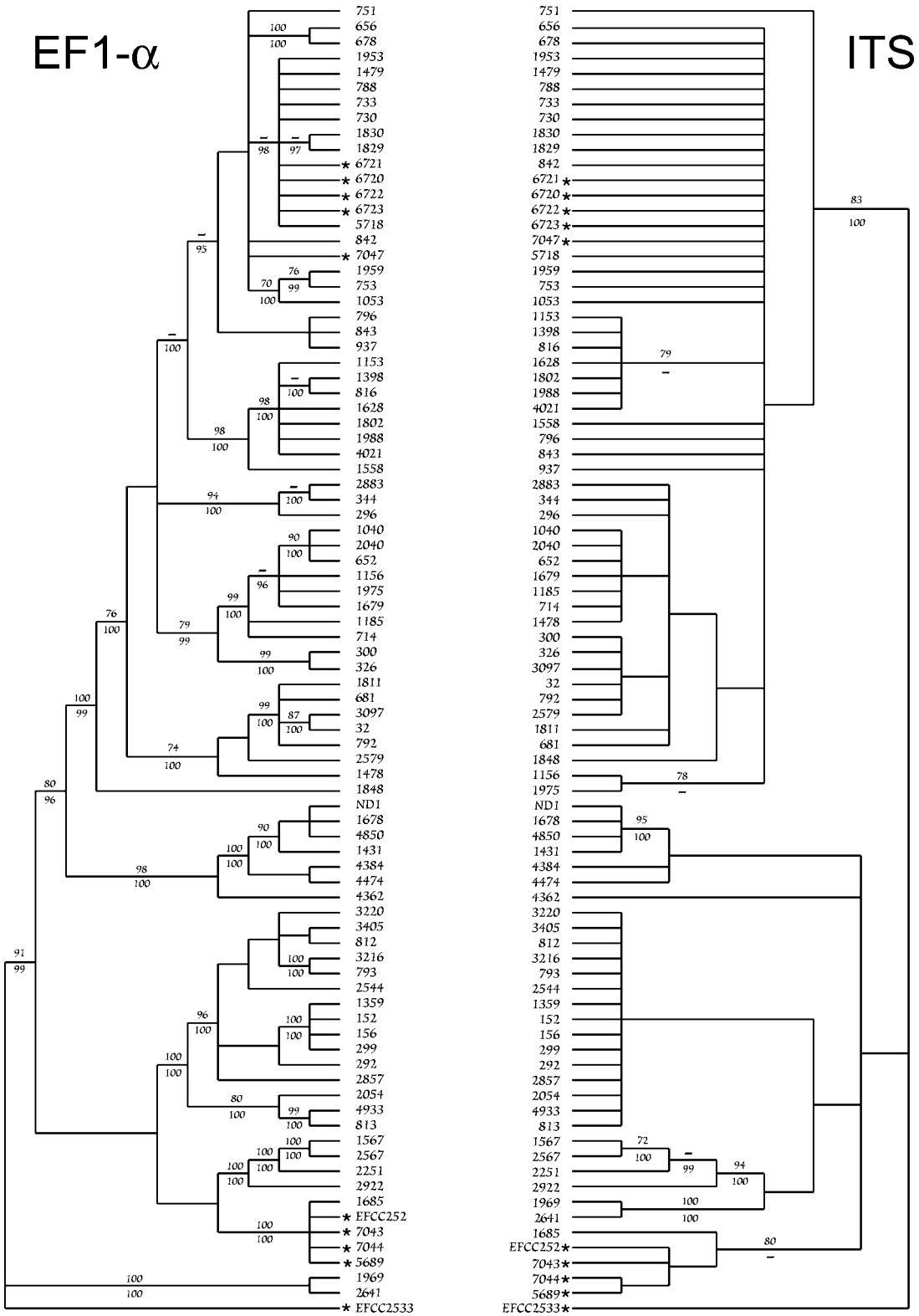

FIG. 1. Strict (50%) consensus tree of the EF1-α and ITS phylogenies determined in the Bayesian likelihood analysis. Bootstrap values ≥70% and posterior ≥95%, are labeled above and below appropriate internodes, respectively. Branch termini are labeled according to ARSEF accession numbers of individual isolates; asterisked accessions indicate *Cordyceps* teleomorphs.

only five branches were supported by both the bootstrap and Bayesian analyses (FIG. 1).

Combined ITS and EF1-α phylogeny.—No significant topological conflicts were noted between the EF1-α and ITS tree topologies and the data sets were combined and analyzed together. To reduce search time, only the first 100 trees encountered in 2000 replicate searches were swapped to completion. All trees from this search had a length of 429 steps and RC = 0.5595. A single exemplar tree from the parsimony analysis, which was also present among the trees from the Bayesian analysis, is given in FIG. 2.

Geographic origin and host affiliation.—The geographic origins and host association of individual *Beauveria* isolates are summarized in the consensus tree in FIG. 3. Insect-fungal associations were particularly heterogeneous in clades A and C (i.e. *B. bassiana s.l.*), which were obtained from insect species classified in seven and six classes, respectively. A permutation test of host affiliation performed separately for both clades A and C yielded the test statistics $P = 0.40$ and $P = 0.46$, respectively, indicating that the observed pattern of host association was indistinguishable from a random distribution. Isolates in clades B, D, E and F were isolated primarily from coleopteran hosts or from soil. However, the taxon sampling in these clades was too limited to perform meaningful statistical tests of host associations.

Conidia morphology.—Conidia shape among the isolates examined in this study varied between globose, ellipsoidal, cylindrical and comma-shaped (TABLE I). In general, isolates within each major clade (FIG. 2) had a similar conidial morphology. In clade A, the majority of isolates had globose to subglobose conidia 2.3–3.2 um in diameter. One notable exception within clade A was a monophyletic pair of Chinese isolates accessioned under *B. amorpha* and *B. brongniartii* (ARSEF 656 and 678, respectively), which had ellipsoidal conidia, 2.9–4.2 × 1.8–2.5 μm. Isolates in Clade C also produced globose to subglobose conidia similar to those produced by isolates in clade A, except they were slightly smaller and measured 2.1–2.9 μm. Clade B included isolates with ellipsoidal to subcylindrical conidia that ranged from 3.3–4.8 × 2.1–2.5 μm. Clade D isolates had either cylindrical conidia (ARSEF 1567, 2251, 2567), 3.8–5.2 × 1.9–2.3 μm, or comma-shaped conidia (ARSEF 2922), measuring 1.9–2.5 μm at their largest dimension. Isolates in clade E produced ellipsoidal conidia 3.0–4.4 × 2.5–3.2 μm. In clade F, only isolate ARSEF 2641 produced conidia and these were cylindrical in shape and 4.2–5.2 × 1.7–2.1 μm.

DISCUSSION

We conducted a phylogenetic analysis of the genus *Beauveria* and several *Cordyceps* species based on historical reconstructions of EF1-α and ITS. EF1-α was much more informative for inference of relationships in *Beauveria* than ITS. The obtained phylogeny supports the monophyly of *Beauveria* and six principal clades within the genus, several of which encompass additional lineage diversity. We used this phylogeny as a basis to consider the taxonomy of *Beauveria* and to discuss patterns of variation in morphology, geographic distribution and host range in this genus.

Beauveria *phylogeny.*—The clades resolved within *Beauveria,* with one exception, correspond closely to species previously defined on the basis of conidial morphology and are provisionally referred to here as clades A–F.

Clade A constitutes a globally distributed set of isolates that were accessioned primarily as *B. bassiana*. The majority of isolates in this clade produce globose to subglobose conidia 2.3–3.2 μm in diameter, which is consistent with the traditional morphological diagnosis of *B. bassiana*. However, the convergent morphology of conidia produced by members of clade C, which is phylogenetically distinct from clade A, exposes a previously unrecognized complication in the taxonomic circumscription of this widespread and important morphological species complex. The taxonomic recognition of each of these two clades is thus minimally required to formalize their distinct status. However, a type specimen for *B. bassiana* is not known to exist. Thus, a neotype for *B. bassiana* needs to be selected from either clade A or C and a second species described to accomodate the alternate clade. Currently, an isolate from clade A is being designated the isoneotype of *B. bassiana* (Humber pers comm). The designation of clade A as the source of a type for *B. bassiana* appears to be the better of the two available options because, as a species that is recognized throughout the world, only clade A has been shown to have a global distribution. Moreover, several commercially registered biocontrol strains and numerous research strains received from laboratories throughout the world have been placed phylogenetically in clade A (Rehner and Buckley unpubl), indicating that clade A most probably embodies what most researchers consider to be *B. bassiana*.

The monophyly of Clade A was strongly supported in the single and combined gene phylogenies (FIGS. 1–3), however, phylogenetic inference within this clade was determined almost entirely from nucleotide variation at EF1-α. Several deep lineages were resolved, each of which included isolates from different continents (FIGS. 1–3). We believe that this intri-

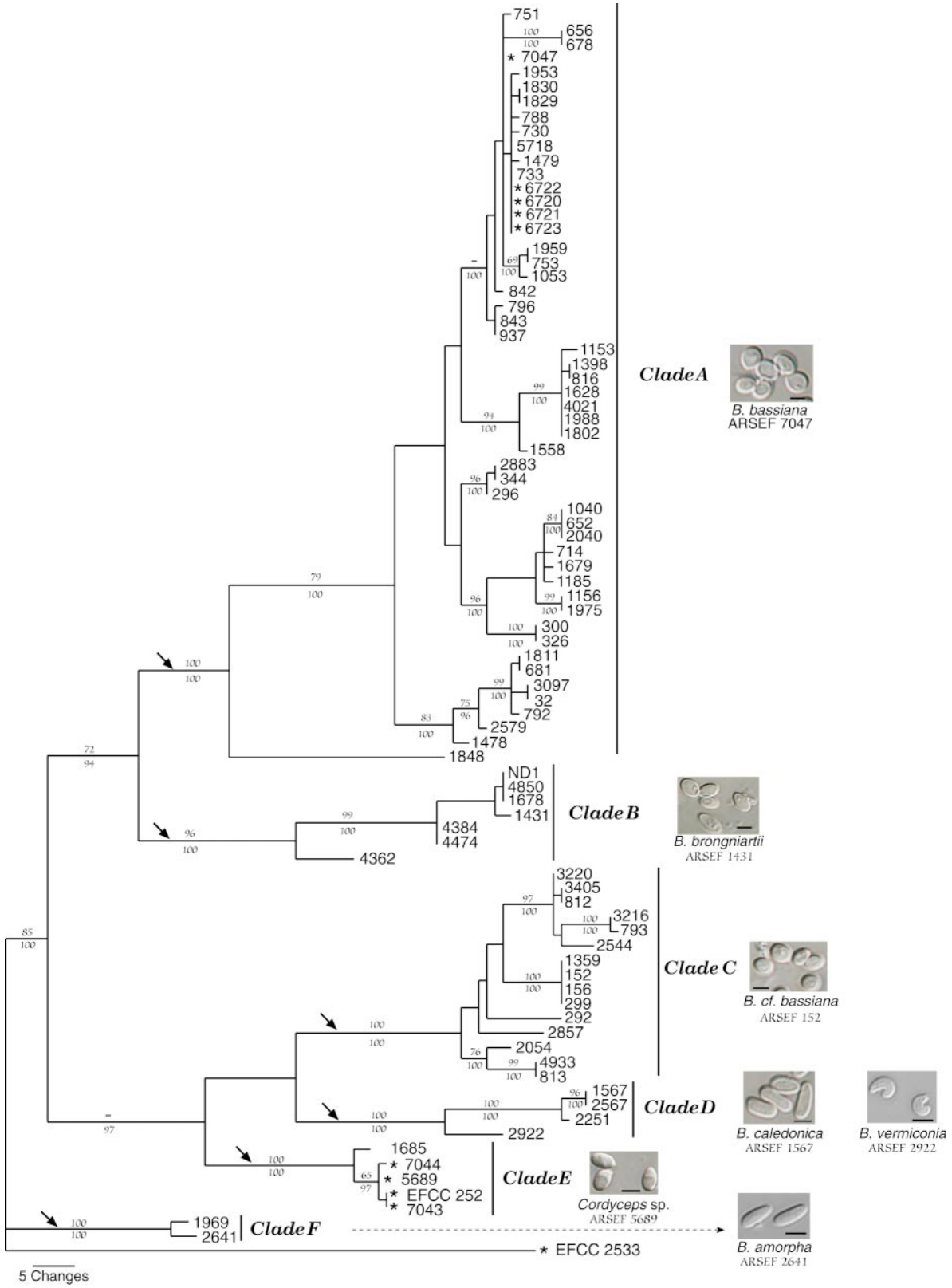

FIG. 2. A single tree from the combined analysis of EF1-α and ITS phylogeny that was present among the shortest trees in both the parsimony and Bayesian likelihood analyses. Bootstrap values ≥70% and posterior probabilities ≥95% are labeled above and below appropriate internodes, respectively. Branch termini are labeled according to ARSEF accession numbers of individual isolates; asterisked accessions indicate *Cordyceps* teleomorphs. Photomicrographs of conidia from representative isolates are illustrated adjacent to each clade. Scale bars are equal to 2 μm.

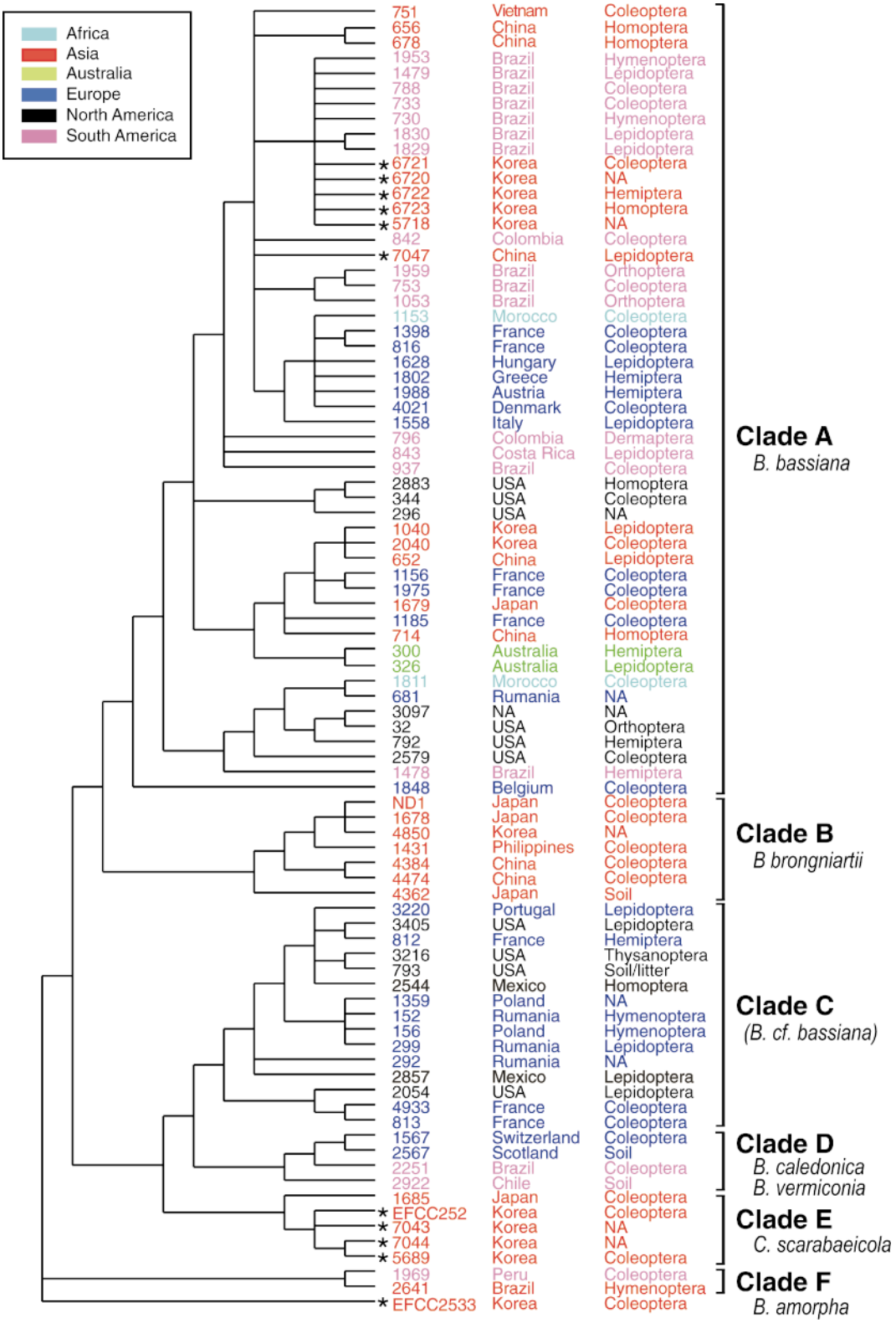

FIG. 3. Strict (50%) consensus tree of the Bayesian likelihood analysis of the combined ITS and EF1-α data. Terminal branches are labeled according to isolate accession number, continent of origin and the taxonomic class of insect species from which they were isolated. *Cordyceps* teleomorphs are indicated by an asterisk.

cate phylogeographic partitioning of clade A reflects past intercontinental dispersal events and that allopatric divergence has played a significant role in its phylogenetic diversification. The complex phylogenetic structure further suggests that clade A may contain multiple phylogenetic species, although this hypothesis requires further investigation. The co-occurrence of different terminal lineages, which was observed in all of the geographic regions sampled (FIG. 3), raises the interesting question of how these closely related species co-exist in sympatry. The development of methods to differentiate among cryptic sympatric lineages will be an essential first step toward elucidating the community structure of *B. bassiana* and the population biology of individual species.

Isolates within clade A were cultured from a wide range of insect species classified in seven insect classes (TABLE I). A permutation test of host affiliation yielded the result that the observed pattern of host associations was indistinguishable from a random distribution. This result support the longstanding view that *B. bassiana s.l.* is not host specific but an opportunistic entomopathogen capable of attacking a wide range of insect taxa. This conclusion is reinforced by evidence that closely related isolates originating from the same geographic region were isolated from taxonomically distant insect hosts (FIG. 3). We find no evidence to support the view that lineage diversification in clade A is due to phylogenetic tracking or host jumping, which both require a prior history of host specialization.

Clade B, the sister to clade A, includes isolates of *B. brongniartii* (Sacc.), a species characterized by ellipsoidal to sub-cylindrical conidia, 3.3–4.8 × 2.1–2.5 μm in size. Originally described from Europe (Petch 1926), *B. brongniartii* is commonly associated with Coleoptera and is used as a biocontrol agent against the European cockchafer, *Melolantha melolantha* (Keller et al 1989). In this study, only Asian material was available for analysis. A Japanese *B. brongniartii* isolate (ND1), formulated by the Nitto Denko Corporation as the mycoinsecticide Biolisa™ for control of beetles, was identical in sequence to two Asian isolates ARSEF 1678 and 4850. Together these isolates form a monophyletic, and possibly conspecific, group. Isolates of European *B. brongniartii* received from J. Enkerli (Enkerli et al 2001) after the present analyses were conducted, grouped in clade B but are phylogenetically distinct from the Asian isolates (Rehner and Buckley data not shown). This suggests that *B. brongniartii* constitutes a complex of several or more cryptic species, which available evidence suggests is distributed across Eurasia. Where host information was available, all clade B isolates were isolated from Coleoptera.

Clade C isolates examined in this study originate from North America and Europe and were identified by their collectors primarily as *B. bassiana* (Humber 2001). As discussed previously, apart from having slightly smaller conidia, clade C is morphologically indistinguishable from clade A. A second point of similarity between these two clades is that clade C has a wide host range, which suggests that it too is a generalist entomopathogen. Although no conspicuous morphological or cultural characteristics have been identified that consistently differentiates clades A and C, fixed nucleotide differences at both ITS and EF1-α, and other genes, may provide the most direct means of differentiating isolates from these two clades (Rehner and Buckley unpubl).

Clade D includes isolates that have either cylindrical or comma-shaped conidia. A Brazilian isolate, ARSEF 2251, accessioned under the name *B. amorpha,* produces cylindrical conidia 3.5–4.2 × 2.1–2.5 μm. These conidial dimensions are shorter and broader than those described for *B. amorpha* by Samson and Evans (1982), which they reported as 3.5–5.0 × 1.5–2.0 μm. For this reason we suspect that ARSEF 2251 is misidentified and may represent an undescribed species. *B. caledonica,* represented here by European isolates ARSEF 2567 and 1567 produce larger cylindrical conidia, 3.7–5.2 × 1.9–2.3 μm, consistent with the conidia described for this species (Bissett and Widden 1986). By contrast, *B. vermiconia* produced distinctive comma-shaped conidia 2.1–2.9 × 2.3–2.9 μm (Hoog and Rao 1975).

Clade E contains isolates from northeast Asia and includes one unidentified *Beauveria* isolate (ARSEF 1685) and four *Cordyceps* individuals, which were identified as either *C. scarabaeicola* (ARSEF 5689) or *C. staphylinidaecola* (ARSEF 7043, 7044; EFCC 252). All live isolates from this clade produced broadly ellipsoidal conidia in culture, measuring 3.1–4.4 × 2.5–3.1 μm. The close similarities in DNA sequence, conidial morphology and geographic origin suggest that these isolates may be conspecific.

Clade F includes two South American isolates, ARSEF 2641 and 1969. ARSEF 2641 was accessioned as *B. amorpha.* The conidia produced by this isolate were 4.5–5.2 × 1.7–2.1 μm, which closely match the narrowly cylindrical conidia described in the amended description of *B. amorpha* by Samson and Evans (1982). Clade F is presently the basal-most lineage in the *Beauveria* phylogeny in which the sympodial conidiogenous cells that characterize *Beauveria* have been documented. The second isolate in clade F, ARSEF 1969, was sterile in culture.

Teleomorph connections to Beauveria.—Four Asian *Cordyceps* teleomorphs identified as *C. basssiana, C. scar-*

abaeicola, and *C. staphylinidaecola* were linked phylogenetically to *Beauveria* at three discrete points in the present analysis: as the sister to *Beauveria s.l.,* and within clade E and within clade A (FIGS. 1–3). Thus, sexual reproduction is confirmed to occur in both basal and derived lineages of *Beauveria*. From these results we believe it to be likely that most if not all lineages in *Beauveria* maintain the potential for sexual reproduction.

EFCC 2533, a fruiting body identified as *C. scarabaeicola,* was placed as the basal branch in the *Beauveria s.l.* clade. Cultures for this collection were not available, thus its mode of conidiogenesis could not be determined. Additional data are needed to determine whether EFCC 2533 is a divergent basal lineage within *Beauveria* or a related genus.

Clade E included four teleomorphs (ARSEF 5689, 7043, 7044; EFCC 252) and one anamorph (ARSEF 1685) isolates. Due to the close genetic relationship of these isolates and their similar anamorphs, it is probably that these isolates are conspecific.

Five teleomorph collections grouped phylogenetically in clade A and are considered to represent the sexual stage of *B. bassiana* (FIGS. 1–3). These collections include the ex-type culture of *C. bassiana* from China, ARSEF 7047, and four Korean isolates identified as *C. staphylinidaecola,* ARSEF 5718, 6721, 6722, and 6723. This finding corroborates the *Beauveria bassiana-Cordyceps* anamorph-teleomorph link proposed by Li et al (2001). The close genetic relationship of these five individuals suggests that they are conspecific, with *C. staphylinidaecola* possibly an older synonym of *C. bassiana* (R. Humber pers comm). Interestingly, these teleomorphs were isolated from three insect classes (Coleoptera, Homoptera and Hemiptera; TABLE I), a pattern that matches the diverse host associations observed among the anamorph isolates that constitute the bulk of clade A. It appears then that neither sexual nor asexual reproduction within clade A requires infection of a specific host.

Teleomorph connections have also been proposed to two additional species of *Beauveria* including *B. brongniartii* (Shimazu 1988) and *C. sobolifera* (Liu et al 2001). However, cultures and specimens were not available for these species at the time of this study. ITS sequences from *C. sobolifera* (GenBank AJ309325 and AJ309326) were reported by Liu et al (2001), which enabled comparison to sequence data determined here. These *C. sobolifera* ITS sequences aligned poorly with data from our *Beauveria* isolates nor did they nest within *Beauveria* when analyzed phylogenetically (Rehner and Buckley unpubl). Further examination of the form and development of conidiogenesis and a more detailed phylogenetic placement of *C. sobolifera* is needed to determine the status of the *C. sobolifera* anamorph.

Despite the long-held view that *Beauveria* is strictly mitosporic and presumably clonal (but see Paccola-Meirelles and Azevedo 1991, Bello and Paccola-Meirelles 1998, for discussion of parasexuality in *B. bassiana*), the phylogenetic connection between *Cordyceps* and *Beauveria* demonstrated here suggests that many, if not all, species of *Beauveria* are sexual. Why sexual reproduction by *Beauveria* has not previously been observed is curious in view of extensive history of research for both *Beauveria* and *Cordyceps*. Nonetheless, the present finding should stimulate efforts to integrate the collection, culturing and phylogenetic analysis of *Cordyceps* teleomorphs and *Beauveria* anamorphs wherever possible. The accumulating evidence that *Beauveria* is sexual suggests the potential for developing conventional approaches to genetic analysis and genetic improvement of this important genus of entomopathogens.

Conclusions.—The molecular phylogeny inferred for *Beauveria* provides a perspective on the current taxonomic understanding of this genus and a foundation for future revisionary systematic studies. With only one exception (i.e. clades A and C), phylogenetic terminals resolved in the present analysis correspond to species previously described on the basis of morphology. Thus, the broad patterns of diversity in *Beauveria* have been accurately predicted by prior morphological studies. However, for groups scrutinized in some detail, e.g. *B. bassiana s.l.* (clades A and C), the inferred patterns of underlying phylogenetic diversity indicate a history of cryptic diversification, possibly signifying these lineages consist of multiple cryptic species. These results demonstrate that deep sampling of globally distributed species complexes, coupled with molecular phylogenetic analyses, is an expedient strategy for assessing species diversity, and a necessary first step to detailing their evolutionary history and historical ecology. The discovery of *Cordyceps* teleomorphs associated with *Beauveria* contradicts earlier assumptions that *Beauveria* is strictly asexual. Appreciation of this reproductive option will expand the scope and character of future investigations of this widespread and important group of entomopathogenic fungi.

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