Molecular evidence for the taxonomic status of 
_Metarhizium taii_ and its teleomorph, _Cordyceps taii_  
(Hypocreales, Clavicipitaceae)

Bo Huang, Chunru Li, Richard A. Humber, 
Kathie T. Hodge, Meizhen Fan & Zengzhi Li

Abstract—ITS1-5.8S-ITS2 rDNA was amplified, cloned and sequenced from the 
stroma of _Cordyceps taii_, mycelium of _Metarhizium taii_ and cultures of _M. anisopliae_ var. _anisopliae_ from China. Analysis of integrated data of different strains and varieties 
of _M. anisopliae_ and _M. flavoviride_ from GenBank showed that _Metarhizium taii_ should 
be treated as a synonym of _M. anisopliae_ var. _anisopliae_. The ITS1-5.8S-ITS2 rDNA 
sequences of _C. taii_ and _M. taii_ are identical, indicating that the teleomorph of _M. 
anisopliae_ var. _anisopliae_ is _Cordyceps taii_.

Key words—molecular systematics, anamorph-teleomorph connection, synonymy 
_Cordyceps brittlebankioides_

Introduction

Concern about adverse effects of chemical insecticides in the environment and 
the growing problem of insecticide resistance has led to considerable interest in the 
use of biological agents for control of insect pests (Leal et al. 1997). Many species of 
entomogenous fungi play important roles in pest management in many countries. 
_Metarhizium anisopliae_ var. _anisopliae_ (abbreviation: _M.a. anisopliae_) is one of the 
most important entomogenous fungi, and has been used to control pests in America, 
Brazil, Australia and China, with distinct economic and social benefits.

Green muscardine disease, one of the most common fungal diseases of insects, is 
caused by several taxa in the clavicipitaceous anamorphic genus _Metarhizium_. Since 
this genus was established, several new species or varieties have been described based 
on seemingly minor morphological differences from _M. anisopliae_, the type of the 
genus. These similarities have led some to believe that the taxonomy of this genus was

*Author for correspondence*
badly confused (Milner et al. 1994). Rombach et al. (1986) recognized three species: *M. anisopliae* (Metschn.) Sorokin, *M. flavoviride* W. Gams & Rozsypal, and *M. album* Petch. Tulloch (1976) segregated the type species of the genus into two varieties, *M. a. anisopliae* and *M. a. anisopliae* var. majus (J.R. Johnst.) M.C. Tulloch (abbreviation: *M.a. majus*) primarily based on conidial length, and she accepted *M. flavoviride*. Rombach et al. (1986) segregated *M. flavoviride* into the varieties *M. flavoviride* var. flavoviride (abbreviation: *M.f. flavoviride*) and, by virtue of its smaller conidia, *M. flavoviride* var. minus Rombach et al. (abbreviation: *M.f. minus*). Rath et al. (1995) suggested (but did not validly publish) the 'variety' *M. anisopliae* var. frigidum based on the ability of conidia to germinate at low temperatures and on patterns of hydrocarbon utilization. The isolates assigned to this variety were found by Driver et al. (2000) to be taxonomically heterogeneous and were characterized based on ITS sequences as belonging in two clades of *M. flavoviride*. Guo et al. (1986) described three new species, *M. pingshaense* Q.T. Chen & H.L. Guo, *M. guizhouense* Q.T. Chen & H.L. Guo and *M. cylindrosporae* Q.T. Chen & H.L. Guo. *Metarhizium biformisporae* A.Y. Liu et al. (1989) should be treated as a heterotypic (facultative) synonym of *M. cylindrosporae*. Liu et al. (1989) described two types of conidia being produced by *M. cylindrosporae* – an ability not noticed by Guo et al. (1986) – and it appears that the description of *M. biformisporae* is best treated as an amplified description of *M. cylindrosporae*. In any event, the latter species was transferred into the genus *Nomuraea*, another clavicipitaceous anamorphic genus, by Tzean et al. (1993). Liang et al. (1991) named *M. taii* isolated from the new species, *Cordyceps taii* Z.Q. Liang & A.Y. Liu, and postulated a connection between *C. taii* and *M. taii* based on microcyclic conidiation. Liu et al. (2001) collected a single specimen and described it as a new species, *Cordyceps brittlebankisoides* Zuo, Y. Liu et al., whose anamorph (confirmed by the identical ITS sequences from the teleomorphic stroma and in *vivo* culture) was identified as *M. a. majus*.

Recently developed molecular techniques, and particularly DNA sequencing, provide valuable tools for studying phylogenetic relationships and clarifying the taxonomic position of confusing species. Molecular techniques have been used by several authors to study genetic variation and taxonomy of *Metarhizium*. Liu et al. (1994) determined partial sequences for selected regions from small (18S) and large (28S) subunit rRNAs of species of *Metarhizium*. Curran et al. (1994) sequenced PCR products from ITS1-5.8S-ITS2 rDNA regions of 31 strains of *Metarhizium*, and found 19 kinds of ITS1-5.8S-ITS2 rDNA regions. Rakotonirainy et al. (1994) sequenced the D1 and D2 expansion region of 28S rDNA from 42 strains of *Metarhizium*. The above results collectively confirmed (i) the monophyly of the genus *Metarhizium*; (ii) the separation of *M. anisopliae* and *M. flavoviride*; and (iii) the need to subdivide *M. anisopliae* into several varieties or species.

Driver et al. (2000) partially resolved the taxonomy of *Metarhizium* using sequence data and RAPD patterns; ITS sequence data were recognized as the most important taxonomic criterion. Based on distinctive ITS sequences, *M. flavoviride* and *M. anisopliae* were recognized to include five and four clades, respectively. In addition to recognizing *M. album* as a distinct species, the classification of Driver et al. (2000) divided *M. anisopliae* into four varieties and *M. flavoviride* into four named varieties plus one other clade not treated as taxonomically distinct. The four species of *Metarhizium* described from China (Guo et al. 1986, Liang et al. 1991) have apparently not been deposited in...
recognized three species: *M. ozyspal,* and *M. album* into two varieties, *M. a. loch* (abbreviation: *M.a. loch*). Rombach et al. (1995) suggested *frigidum* based on the patterns of hydrocarbon products from *M. jlavoviride* and *M. anisopliae* (Driver et al. 2000) to divide *M. jlavoviride* and *M. anisopliae* into several varieties or species from the teleomorphic stage. In any event, the latter species is a connection between *Cordyceps* and *Metarhizium* originally determined by comparison of microcyclic conidiogenesis and ascospores (Li et al. 1991), but this important result needed more proof. The aim of this study was to provide genetic verification of the connection between *C. taii* and *M. taii* and to clarify the taxonomic status of *M. taii* by comparison of its ITS sequences with those of other *Metarhizium* isolates already included in GenBank.

**Materials and methods**

**Fungal isolates**—A specimen of *M. taii* (HS00060501) occurring as a pathogen of a noctuid (lepidopteran) larva was collected from Huoshan County, Western Anhui, China. A culture originally identified as *M. taii* (RCEF0772) was isolated from its ascospores discharged onto glass slides. A culture of *M.a. anisopliae* (RCEF0386) was isolated from a noctuid larval cadaver collected in Yuxi County, Western Anhui, China. Those isolates that are included in this study only by means of sequence data obtained from GenBank are listed in Table 1.

**DNA preparation and amplification**—A portion of a stroma from the field-collected specimen and from cultured mycelia of *M. taii* (isolated from discharged ascospores) were each finely ground under liquid nitrogen, and genomic DNA was extracted by use of benzyl chloride according to the method of Zhu et al. (1994). The extracted DNA was stored in 100 μl TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) at 4°C, and DNA solutions were diluted 10-fold with TE for use in PCR reaction.

The nuclear ITS1-5.8S-ITS2 regions were amplified with the primer pair described by Mavridou & Tzypas (1998). The PCR reactions were performed in 50 μl volume with the following components: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 0.001% gelatin, 200 μM of each dNTP, 20-100 ng genomic DNA, 12 pmole of each primer, and 2.5 units Taq DNA polymerase (Sangon, China). The reactions were prepared on ice in 500 μl microcentrifuge tubes, overlaid with 20 μl mineral oil, and placed in a thermal cycler (Techne, UK). Cycling parameters were programmed as follows: an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 10 min. Efficiency of amplification was monitored by running 8 μl of each product through 1.2% agarose gels using TAE buffer and visualizing PCR products with ethidium bromide.

Amplification products were purified by use of a Wizard™ PCR Prep DNA Purification System Kit (Promega Co., France) and methods provided by the company.

**DNA cloning and sequencing of amplified its region**—Purified ITS PCR products were cloned into T-Vectors, which we prepared from SK plasmids. These vectors were used to transform *Escherichia coli* XL1-Blue, and the plasmid DNA was extracted using an alkaline lysis method (Sambrook et al. 1989).

The primers T7/T3 (forward and reverse) were used to sequence both strands using the dideoxy-nucleotide chain termination on an ABI 3700 automated sequencer at
Shanghai Genecore Biotechnologies Company. Sequence data of C. taii (HS00060501), M. taii (RCEF0772) and M.a. anisopliae (RCEF0386) have been deposited in the GenBank genome sequence database with accession numbers AF348393, AF348394 and AF348395, respectively.

Table 1. Previously deposited GenBank sequences used in this study.*

<table>
<thead>
<tr>
<th>GenBank sequence</th>
<th>Fl #</th>
<th>Fungal taxon</th>
<th>Host insect / order (or other source substrate)</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFI34150</td>
<td>1027</td>
<td>M. a. anisopliae</td>
<td>Oxya multidentata /Orthopt.</td>
<td>Pakistan</td>
</tr>
<tr>
<td>AFI35210</td>
<td>1029</td>
<td>M. a. anisopliae</td>
<td>Schistocerca gregaria /Orthopt.</td>
<td>Eritrea</td>
</tr>
<tr>
<td>AFI35211</td>
<td>1031</td>
<td>M. a. anisopliae</td>
<td>Teleogryllus commodus /Orthopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI35212</td>
<td>1034</td>
<td>M. a. anisopliae</td>
<td>Psammosuccinea /Orthopt.</td>
<td>Thailand</td>
</tr>
<tr>
<td>AFI35213</td>
<td>1045</td>
<td>M. a. anisopliae</td>
<td>Dermestidae albhorbitum / Coleopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI35214</td>
<td>1091</td>
<td>M. a. anisopliae</td>
<td>Teleogryllus commodus /Orthopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI35215</td>
<td>1114</td>
<td>M. a. anisopliae</td>
<td>Soil, Maquarie Island</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI35216</td>
<td>1156</td>
<td>M. a. anisopliae</td>
<td>[immunoocompromised human]</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI35617</td>
<td>163</td>
<td>M. a. anisopliae</td>
<td>Kalotermes sp. / Isopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI36376</td>
<td>203</td>
<td>M. a. anisopliae</td>
<td>Inopus rubripes /Dipt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI36928</td>
<td>208</td>
<td>M. a. anisopliae</td>
<td>Phasalacridium viitatum /Orthopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37054</td>
<td>114</td>
<td>M. a. anisopliae</td>
<td>Antigerus parvalus / Coleopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37055</td>
<td>23</td>
<td>M. a. anisopliae</td>
<td>Aeromia albofasciata /Hemipt.</td>
<td>Mexico</td>
</tr>
<tr>
<td>AFI37056</td>
<td>700</td>
<td>M. a. anisopliae</td>
<td>Costelytra zealandica /Coleopt.</td>
<td>New Zealand</td>
</tr>
<tr>
<td>AFI37057</td>
<td>328</td>
<td>M. a. anisopliae</td>
<td>Inopus rubriceps /Dipt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37058</td>
<td>379</td>
<td>M. a. anisopliae</td>
<td>Inopus rubriceps /Dipt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37059</td>
<td>775</td>
<td>M. a. anisopliae</td>
<td>Soil (near moud of Captotermes lacteus / Isoptera)</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37062</td>
<td>987</td>
<td>M. a. acridum</td>
<td>Ornithacris coerulii / Orthopt.</td>
<td>Niger</td>
</tr>
<tr>
<td>AFI37065</td>
<td>147</td>
<td>M. a. lepidiotae</td>
<td>Lepidiota contorta / Coleopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI37061</td>
<td>589</td>
<td>M. a. miagius</td>
<td>Oryctes rhinocerus / Coleopt.</td>
<td>Indonesia</td>
</tr>
<tr>
<td>AFI38270</td>
<td>28</td>
<td>M.f. flavoviride</td>
<td>Adoryphora couloni / Coleopt.</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI38372</td>
<td>1172</td>
<td>M.f. minus</td>
<td>Niliparvula laevis / Homopt.</td>
<td>Philippines</td>
</tr>
<tr>
<td>AFI39853</td>
<td>1123</td>
<td>M.f. new-zealandicum</td>
<td>Soil</td>
<td>Australia</td>
</tr>
<tr>
<td>AFI39850</td>
<td>72</td>
<td>M.f. pemphigi</td>
<td>Pemphigus treherri / Homopt.</td>
<td>UK</td>
</tr>
<tr>
<td>AFI397057</td>
<td>Mf</td>
<td>M. album</td>
<td>Nephrotettix virescens / Homopt.</td>
<td>Philippines</td>
</tr>
<tr>
<td>AFI397238</td>
<td>MaF</td>
<td>Cordyceps breitlaukousidae</td>
<td>Scarabaeidae / Coleoptera</td>
<td>China</td>
</tr>
</tbody>
</table>


Alignments and analysis—DNA sequences generated by us and downloaded from GenBank were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignment was refined by eye. Beauveria bassiana (Bals.-Criv) Vuil, a related clavicipitacean anamorph, was used as an outgroup. Parsimony analysis was performed in PAUP* version b10 (Swofford 2002) using a heuristic search. A starting tree was obtained via a "closest" addition sequence, tree-bisection-reconnection was used as the branch-swapping algorithm, and MULTREES was off. Gaps were treated as missing data, and 112 ambiguously aligned characters were excluded from the analysis. Bootstrap values were calculated based on 100 replicates of a heuristic fast stepwise addition search.
Bayesian analysis was carried out using MrBayes 3.0b4 (Huelsenbeck 2000, Huelsenbeck et al 2001). We used a six parameter model to run four chains for 500,000 generations, saving a tree every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree (Fig. 3) was then calculated using PAUP*.

Results

Characterization of specimen HS00060501 and its isolate, RCEF0772—The host is covered with yellow mycelium. Three stromata emerge from the head of the host, they are cylindric, 38 x 3.5-6 mm broad at stalk, with a yellowish fertile head of 20-35 x 2.5 mm. Perithecia flask-shaped with curved neck, obliquely immersed, 810-(950)-1120 x 230-380 μm. Ascii cylindric, 300-490 x 3.4-5.7 μm, with ascus cap 3.1 μm in diameter. Part ascospores cylindrical, 20.0-(28.6)-34.6 x 1.4-(2.5)-3.1 μm.

Colonies on Czapek’s agar attaining a diameter of 35-43 mm with 14 days at 25°C, flat, with little aerial mycelium, dull gray green, reverse center deep green, subcenter to margin light sandy yellow. Cylindrical conidiogenous cells with short neck, 7.2-(14.4)-21.6 x 1-(1.4)-1.8 μm, borne in a palisade on mycelium or conidiophore. Conidia cylindric, (5.0-)7.0-9.0(10.0) x 2.4-(2.8)-3.2 μm, two-celled conidia absent.

Based on the long part spores and other important morphological characters, the Cordyceps specimen (HS00060501) from Huoshan County was identified as Cordyceps.
taii. The morphological characters of the conidiogenous structures and conidia arising from the part spores agree with the original description of M. taii (Liang et al. 1991). Two more recently collected specimens of Cordyceps taii collected in Anhui Province are illustrated in Figures 1 and 2.

Clarification of taxonomic position of Metarhizium taii—Regions of the 5.8S rRNA and the complete internal transcribed spacer (ITS) regions from M. taii (GenBank AF348394) and M. a. anisopliae from China were sequenced; their total sizes were 449 and 451 nucleotides, respectively. These sequences and ITS sequences obtained from GenBank for 20 strains and 4 varieties of M. anisopliae were subjected to phylogenetic analysis (Fig. 3). The aligned sequences of the new Chinese isolates of M. taii and M. anisopliae showed a high degree of similarity with those of the 17 strains of M. a. anisopliae from Driver et al. (2000). There are 22 mutations in total, half of which were transitions (7 C/T and 4 A/G); the remaining mutations included three C/A, two T/G, one A/T as well as five putative insertions or deletions. The nature of these mutations suggests that these sequences may have diverged only recently. Among these mutations, 11 occurred in ITS1 and 11 in ITS2; the sequences of the 5.8S rRNA gene among these 18 isolates of M. a. anisopliae (a set of sequences including that from FI-1029, which was derived from the IMI ex neotype culture for M. anisopliae) and that of M. taii were identical. Divergence in 5.8S rRNA gene sequences between M. taii and other Metarhizium species or varieties varies from 0–1.8% (Tab. 2).

The parsimony search found 2864800 equally parsimonious trees, each of 376 steps and with CI=0.811 and RI=0.808. Of the 683 included characters, 465 were constant, and 106 were parsimony-informative. The strict consensus tree is shown in Fig. 3. The tree resulting from the Bayesian analysis has an identical topology.

The large number of trees obtained via the parsimony approach reflects a lack of variation among isolates of a single variety. In general, the ITS locus provided little resolution at the varietal level, but did provide support for the recognition of all but one of the varieties (M. a. majus). The Bayesian analysis generally agreed with the parsimony consensus tree in that each of the varieties described by Driver et al. (2000) was supported by tree topology except for M. a. majus. Metarhizium taii groups with all strains of M. a. anisopliae with 99% bootstrap support; if the 85th character of the alignment is excluded, then strain FI379 is included in this clade with 99% bootstrap support. Furthermore, all varieties of M. anisopliae recognized by Driver et al. (2000)–M. a. anisopliae, M. a. majus, M. a. lepidotae Driver & Milner, and M. a. acridum Driver & Milner–cluster together in a clade with 100% bootstrap support and differ distinctly from M. album and from all described varieties of M. flavoviride. These results suggest that M. taii should properly be treated either as a variety of M. anisopliae or as being taxonomically indistinguishable from M. a. anisopliae.

Table 2 shows that the range of variation in nucleotide divergence among M. taii and 18 isolates of M. a. anisopliae from ITS1, ITS2 and both combined falls within the scope of variation among M. a. anisopliae isolates, and is distinctly greater than that among M. taii, M. a. lepidotae and M. a. acridum. The range of variation among M. taii and 18 isolates of M. a. anisopliae from ITS1+ITS2 regions is a little less than among M. a. majus and 18 isolates of M. a. anisopliae.

Table 2. Nucleotide divergence among isolates of M. taii and M. a. anisopliae

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence length (nucleotides)</th>
<th>ITS1 divergence (nucleotides)</th>
<th>ITS2 divergence (nucleotides)</th>
<th>Both combined divergence (nucleotides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. taii</td>
<td>449</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. a. anisopliae</td>
<td>451</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The new variety M. taii was described on the basis of its morphology until morphological means to identify, sequence data and the ITS2 sequence of M. taii an identical to that of M. a. anisopliae. The 20 strains of M. a. anisopliae and M. flavoviride, originally ide varieties, were genetically-biologically distinct from the taxa unverified since the ex type variety in a different genus.
regions of the 5.8S rRNA gene from M. taii (GenBank AF348393) were subjected to phylogenetic analysis. Isolates of M. taii and M. anisopliae and the 17 strains of M. anisopliae were sequenced. These mutations included three C/A, two T/G, and one A/G. The ITS1-5.8S-ITS2 region (449 bp) for C. taii (GenBank AF348393) was identical to that of M. anisopliae (RCEF0772). The sizes of ITS1, 5.8S, and ITS2 in the ITS1-5.8S-ITS2 region are 134 bp, 158 bp, and 167 bp, respectively. The alignment showed that C. taii and M. anisopliae isolated from this Cordyceps specimen had identical (completely congruent) ITS1-5.8S-ITS2 sequences. This suggests that C. taii and M. anisopliae are genetically identical. We suggest that Cordyceps taii and M. anisopliae are the teleomorphic and anamorphic phases of a single organism.

Discussion

The new varieties M. acridum and M. lepidiotum Driver & Milner were recognized on the basis of their distinctive ITS sequence data (Driver et al. 2000). The ITS1 and ITS2 sequence data were the only characters included in their Latin diagnoses; therefore, until morphological or other characters are correlated with these genotypes, the only means to identify varieties of Metarhizium anisopliae within the classification is with sequence data (Driver et al. 2000).

In the phylogenetic tree, Cordyceps brittlebankisoides falls in the Metarhizium flavoviride clade. However, its purported anamorph M. majus belongs to the Metarhizium anisopliae clade. Liu et al. (2001) concluded that C. brittlebankisoides is the teleomorph of M. majus based on the morphological characteristics of its phialides and conidia; they did not compare the ITS sequences of their fungus with those of other Metarhizium taxa. These authors also noted that the spore sizes and cultural color of their fungus were similar to those of M. flavoviride (Liu et al. 2001). Based on the overall genetic evidence, we believe that C. brittlebankisoides is not the teleomorph of M. majus, but may instead be the teleomorph of a still undescribed variety of M. flavoviride. Such a genetically-based reclassification within Metarhizium echoes the genetically-based decision to transfer grasshopper- and locust-pathogenic isolates originally identified on morphological and cultural bases as M. flavoviride to a new variety in a different species as M. acridum (Driver et al. 2000).

The taxonomic status of M. taii as a species has remained both uncertain and unverified since its description. Because the nucleotide divergence between M. taii and the ex type isolate for M. anisopliae falls within the range of variation among M. anisopliae isolates, and very little divergence was found in the ITS1 and ITS2 sequences

### Table 2. Nucleotide divergence within and among species for isolates of M. anisopliae

<table>
<thead>
<tr>
<th>Isolates being compared</th>
<th>5.8S</th>
<th>ITS1</th>
<th>ITS2</th>
<th>ITS1+ITS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. taii, 18 isolates of M. anisopliae</td>
<td>0</td>
<td>0.7-3.0</td>
<td>1.1-2.8</td>
<td>1.0-2.3</td>
</tr>
<tr>
<td>M. anisopliae isolates</td>
<td>0</td>
<td>0-4.4</td>
<td>0-2.8</td>
<td>0.3-2.6</td>
</tr>
<tr>
<td>M. taii, FI-1029 (ex type of M. anisopliae)</td>
<td>0</td>
<td>1.6</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>M. taii, M. lepidiotae, M. a. acridum</td>
<td>0-0.6</td>
<td>9.6-20.5</td>
<td>9.1-10.6</td>
<td>9.3-15.1</td>
</tr>
<tr>
<td>M. taii, M. majus</td>
<td>0</td>
<td>3.0</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>M. a. majus, 18 isolates of M. a. anisopliae</td>
<td>0</td>
<td>2.2-4.4</td>
<td>0.6-2.3</td>
<td>1.3-2.5</td>
</tr>
</tbody>
</table>

*Genetic divergence (%) = (number of substitutions/total base pairs) x 100.
between *M. taii* and 18 isolates of *M. a. anisopliae*, we propose that *M. taii* is a synonym of *M. a. anisopliae*; there is not even enough distinctive genetic variation between *M. taii* and *M. a. anisopliae* to justify treating *M. taii* as a new variety of *M. anisopliae*. Tulloch (1976) separated the varieties of *M. a. anisopliae* and *M. a. majus* mainly by the shape and lengths of their conidia, (3.3-)5.0–8.0(-9.0) μm and (9.0-)10.0–14.0(-18.0) μm, respectively. The anamorph of *Cordyceps taii* was recognized as a new species mainly based on the dimensions of its conidia [(4.8-)7.8–9.6(-14) μm] and the (apparently very infrequent) formation of two-celled conidia (Liang et al. 1991). In this study, the conidia of our *M. taii* strain (RCEF0772) are clearly within the limits of *M. a. anisopliae* defined by Tulloch: the conidia of the strain isolated by Liang fell partly in the range of *M. a. anisopliae*. Longer than usual conidia in *M. a. anisopliae* have been described previously in strains from China with lengths of 7.5–8.0 μm (Guo et al. 1986) and in strains from Australia at 8–9 μm (Yip et al. 1992). The lengths of conidia from *M. taii* fall between those of *M. a. anisopliae* and *M. a. majus*, but in view of the high genetic similarity demonstrated here the slight difference between *M. a. anisopliae* and *M. taii*, and in conidial length between *M. a. anisopliae* and *M. a. majus*, can be regarded as differences among strains. Liang et al. (1991) found differences in the ability to form synnemata and two-celled conidia compared with other species or varieties of *Metarhizium*. Comparable morphological differences among isolates of other fungal entomopathogens have not been treated as taxonomically significant. For example, isolates of many diverse entomopathogenic species of *Paecilomyces* may or may not form synnemata, with the ability to do so varying on an isolate-by-isolate basis. Similarly, Li et al. (2001) reported on an isolate of *Beauveria bassiana* able to produce large synnemata, a behavior not generally known from other isolates of this globally distributed species. The combined molecular and morphological evidence presents compelling support for the conclusion that *M. taii* cannot be retained as an independent taxon at either the specific or varietal level and that it must be synonymized with *M. a. anisopliae*.

Some studies have characterized the range of variation in nucleotide divergence in ITS1 and ITS2 regions from different strains in the other fungal groups. *Epicoccum nigrum* and *Phoma episcopalina* are synanamorphs of the same biological species, and but the ranges of divergences in the sequences of their ITS1 and ITS2 genes have been found to be 0–2.8% and 0–2.6%, respectively (Arenal et al. 2000). As a standard for comparison, other studies have found infraspecific variability in ITS1 sequences for *Hymenoscyphus ericae*, *Trichoderma harzianum*, and *Ganoderma lucidum*, to be 1.2%–3.5%, 0–2%, and 0–4%, respectively (Egger & Sigler 1993, Grondona et al. 1997). Even higher infraspecific divergence has been found for other fungi such as *Gaumannomyces graminis*, with 1.5–12.0% for ITS1 and 0–2.9% for ITS2 (Bryan et al. 1995); and *Colletotrichum acutatum* with 0–6% in the ITS1 (Sreenivasaprassad et al. 1994).

*M. a. anisopliae* is one of the most common and important species of entomopathogenic fungi. Like other such globally distributed and very important entomopathogens as *Beauveria bassiana* and *Beauveria brongniartii*, the teleomorph of this hyphomycete has remained uncertain or unknown. Schaerffenberg (1959) claimed that the teleomorph of *M. anisopliae* belonged to the order Sphaeriales (Ascomycota), but this hypothesis was never well supported either by Schaerffenberg or by any subsequent workers. Liang et al. (1991) provided the first credible evidence that the teleomorph of any *Metarhizium* species is a clavicipitaceous fungus belonging in the genus *Cordyceps*;
M. taii is a synonym of M. anisopliae. Tulloch as mainly by the shape \((10.0-14.0(-18.0)) \mu m\), a new species mainly nd the (apparently very n this study, the conidia M.a. anisopliae defined ly in the range of M.a. n described previously 86) and in strains from m M. taii fall between high genetic similarity ae and M. taii, and in regarded as differences ity to form synnemata ieties of Metarhizium.

![Figure 3. Strict consensus of 2864800 equally parsimonious trees based on ITS region sequence data from Metarhizium spp. Values above the branches indicate bootstrap support; posterior probabilities resulting from the Bayesian analysis are shown below the branches.](image-url)
this connection was based on studies of secondary (microcyclic) conidiogenesis by discharged part spores. The data presented here show that *M. taii* cannot be maintained as an independent species and that the most reasonable treatment for this species is as a facultative (heterotypic) synonym of *M. a. anisopliae*:

*Metarhizium anisopliae* (Metschn.) Sorokin var. *anisopliae*, [Plant Parasites of Man and Animals as Causes of Infectious Diseases] 2: 267 (1883) [in Russian]


Because the present molecular and cultural evidence confirms unambiguously that the teleomorph of *Metarhizium taii* is *Cordyceps taii*, it must also now be recognized that the teleomorph of *M. anisopliae* var. *anisopliae* is *C. taii*.

**Acknowledgments**

We are grateful to Drs. Wenying Zhuang and Yijian Yao for reviewing the manuscript. This work was supported partly by the National Natural Science Foundation of China (Grant No. 30300004, 30330500), the Key Laboratory of Systematic Mycology and Lichenology (Grant No. 912.), the Anhui Provincial Science Foundation for Excellent Youth and program for "NCET".

**Literature cited**


oniidiogenesis by not be maintained this species is as a parasites of Man and 260 (1991). biguously that the ecognized that the manuscript. This work Grant No. 30300004, Grant No. 912.), the ICET*.