Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data

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Wang, L., Zhang, W.M., Hu, B., Chen, Y.Q. and Qu, L.H. (2008). Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. Fungal Diversity 31: 147-155.

Cordyceps militaris is the type species of the genus *Cordyceps* (*Hypocreales*, *Ascomycetes*) and produces bioactive ingredients and exhibits medicinal activities similar to *C. sinensis*, a Chinese herbal medicine. The fruiting bodies of *C. militaris* have now been mass-produced artificially and developed into healthy foods in China as *C. militaris* is much easier to culture than *C. sinensis*. To investigate the genetic variation of *C. militaris* from different regions, wild *C. militaris* strains, strains used for industrial production, and artificially produced fruiting bodies were collected from different regions of China, including Guangdong, Hebei, Liaoning, and Jilin provinces and Seoul, Korea. Twenty-six sequences were used to perform ME, MP, ML and Bayesian analyses. The genetic distance values and phylogenetic trees indicated that genetic variation of *C. militaris* from Britain, China, Japan, Korea and Norway was extremely small (≤ 0.01, K2P model) and did not correlate with geographical origins. Mass production does not affect the genetic stability of *C. militaris*. Its allies, *C. kyushuensis* and *C. roseostromata* had the same distance level as *C. militaris*, and may be synonyms of *C. militaris* based on rDNA ITS sequence data.

Key words: *Cordyceps*, genetic diversity, molecular phylogeny, rDNA ITS

Article Information
Received 31 January 2007
Accepted 4 October 2007
Published online 31 July 2008
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Introduction

Cordyceps militaris (L.) Link is the type species of *Cordyceps* (*Hypocreales*, *Ascomycetes*), which parasitizes larva or pupa of lepidopteran insects generally and has a worldwide distribution. In China *C. militaris* is also named "Bei Dong Chong Xia Cao" or "Bei Chong Cao" and is now used as a substitute for *C. sinensis* in traditional Chinese medicine and health foods. When compared to *C. sinensis*, *C. militaris* contains similar biochemical components but a greater quantity of cordycepin (Li *et al*., 1995). Cordycepin has pharmacological functions such as anti-tumour and immunity regulation (Liu *et al*., 2004). The extracts of *C. militaris* possess extensive pharmacological properties, such as anti-inflammatory, anti-tumor growth, anti-fibrotic, and anti-oxidant activities (Nan *et al*., 2001; Yoo *et al*., 2004, Park *et al*., 2005; Won and Park, 2005). Fruiting bodies of *C. militaris* are relatively easy to obtain in culture since its cultural requirements, including nutrition and controlled environment are not as fastidious as that of *C. sinensis*. Fruiting bodies have been mass-produced as health foods and medicine by some companies in China.

The strains of *C. militaris* used for production are its conidial stage, which has been considered to be *Cephalosporium militaris* (Kobayasi, 1941), *Verticillium militaris* (Gams, 1971), *Paecilomyces militaris* (Liang, 2001; Liu *et al*., 2002) and a *Lecanicillium* species (Gams and Zare, 2001). Different strains of *C. militaris*, however isolated from different places may produce variable cultural characteristics. For example, the size, shape and colour of *C. militaris* fruiting bodies produced by different strains are sometimes quite different and mycelia of some strains on artificial solid media remain white and do not change yellow

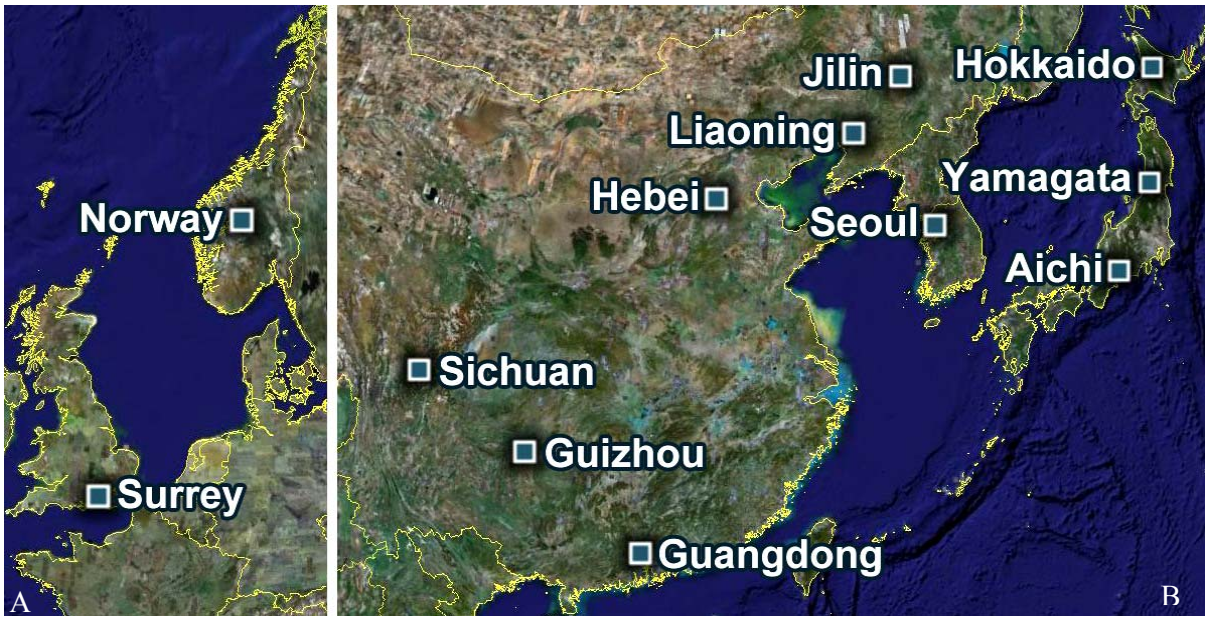

Fig. 1. Localities of *Cordyceps militaris* and *Paecilomyces militaris* used in this study. **A:** northwestern Europe. **B:** northeastern Asia. (by Google™ earth)

or orange with age. As a result, fruiting bodies cannot be formed. Therefore it should be questioned whether these strains are truly the anamorphs of *C. militaris*? It is also important to establish whether there is genetic variation among wild *C. militaris* strains, strains used for industrial production, and fruiting bodies produced from different commercial factories? In the past, people paid more attention to *C. sinensis*. Genetic variation from different geographical regions of the Qinghai-Tibet plateau has been investigated by using randomly amplified polymorphism DNA (RAPD) (Chen *et al*., 1999) and rDNA internal transcribed spacer (ITS) sequence analysis (Chen *et al*., 2004). These studies indicated that *C. sinensis* was genetically diverse and this correlated to biogeography to a certain extent. Sung *et al*. (1999) investigated the genetic variation of *C. militaris* from eleven sites in Korea by RAPD and did not find any correlation between genetic variation and geographical regions. The internal transcribed spacer (ITS) region has been successfully used as a genetic marker to investigate the phylogeny and genetic variation of *Cordyceps* species (Liu *et al*., 2002; Chen *et al*., 2004; Rehner and Buckley, 2005; Stensrud *et al*., 2005). In the present study, we investigate the genetic variation of *C. militaris* from diverse areas of the world, including strains used for industrial production and artificially produced fruiting bodies using sequence analysis of ITS region.

Materials and methods

Materials

Thirteen samples were used to obtain ITS sequences in this study. Samples of *C. militaris* and *P. militaris* were collected from Guangdong (southern China), Hebei (northern China), Liaoning (northeastern China), Jilin (northeastern China) and Seoul (Korea). Specimens were deposited in the Herbarium of Guangdong Institute of Microbiology (HMIGD). The fungal strains used were deposited in Guangdong Institute of Microbiology (GIMYY). Four samples of artificially produced fruiting bodies were obtained from commercial factories from different regions of China. The *C. roseostromata* strain was provided by American Agricultural Research Service, Collection of Entomopathogentic Fungal Cultures (ARSEF). The *C. kyushuensis* strain was provided by the Herbarium of the Institute of Microbiology, Chinese Academia of Sciences (HMAS), which was isolated from the specimens HMAS78115 (Guo and Li, 2000). A further thirteen ITS sequences from GenBank were selected to establish phylogenetic inference efficiently, and included isolates from Britain, southwestern China, Japan

and Norway. The data concerning the sequences used in this study are listed in Table 1 and the localition of the samples are shown in Fig. 1.

DNA extraction and amplification

Total genomic nucleic acids were extracted from both fruiting bodies and fresh mycelia. The surfaces of fruiting bodies were removed carefully and the inner segments were sliced into Eppendorf tubes. Mycelia of strains were scraped from the surface of PDA slopes and placed in Eppendorf tubes. After this, 300 µl DNA extraction buffer (1% SDS, 10 mM EDTA, pH 8.0, 10 mM Tris-HCl, pH 7.5, 10 mM NaCl) was added to Eppendorf tubes, followed by 3-5 freeze-thaw cycles in liquid nitrogen and water at 65°C. The phenol-chloroform-isoamyl alcohol (25:24:1) was added to extract nucleic acids and the residual phenol was removed with chloroform-isoamyl alcohol (24:1). After alcohol precipitation, nucleic acid extracts were checked by agarose gel electrophoresis, and then samples were stored at -20°C.

The PCR amplification was performed in a Biometra T gradient PCR machine, using primer A (forward), 5'-CGTAGGTGAACCT GCGGAAGGATCA-3' and primer B (reverse), 5'-TTCCCTGTTCACTCGCCGTTACT-3' (Bachellerie and Qu, 1993). Target DNA was produced by EX Taq DNA polymerase (TaKaRa) under a temperature profile of 94°C for 3 minutes, followed by 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes, and final, extension at 72°C for 10 minutes. The amplification products were purified with Qiaquick PCR Purification kits (QIAGEN, Germany), and then used directly for sequencing.

DNA sequencing and phylogenetic analysis

Sequencing was performed using ABI PRISM 377 DNA sequencer (Perkin Elmer). All sequences were aligned using MEGA version 3.1 (Kumar *et al*., 2004). Alignments were manually corrected and then saved as fasta file. All sequences of *C. militaris* and *P. militaris* were submitted to RDP v3 beta 05 (Martin and Rybicki, 2000) and TOPALi v2 (Milne *et al*., 2004) to detect recombination by six automated methods and DSS method. Pairwise distance matrix was generated with Kimura two-parameter model (K2P) (Kimura, 1980) by using MEGA version 3.1 (Kumar *et al*., 2004). Minimum Evolution (ME) tree and Maximum Parsimony (MP) trees were searched by Close-Neighbour- Interchange (CNI) and tested by bootstrap analyses (Felsenstein, 1985) with 1000 search replicates. ME tree was generated with K2P model and MP consensus tree was constructed. ModelGenerator v0.7 (Keane, 2004) was used to fit the model for Maximum likelihood (ML) analyses and Bayesian analyses. ML tree was then built by PAUP version 4.0 Beta 10 (Swofford, 2003), using a heuristic search with Tree-Bisection-Reconnection (TBR) algorithm, TrN + G model suggested by ModelGenerator and bootstrap analyses with 100 search replicates. Bayesian posterior probabilities (PP) were calculated using MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). 1,000,000 generations, GTR + G model, temp set to 0.1 and former 50% generations that were discarded as burn-in phase were used for calculating PP in the majority rule consensus tree.

Twenty-six sequences including thirteen reference sequences from GenBank were aligned and used to perform phylogenetic analyses. The alignments were submitted to TreeBase and are available as SN3215. Pairwise distance matrix generated with K2P model was presented in Table 2.

Results

Seventeen sequences were used to detect recombination. No potential recombination was detected by the two kinds of software used. There were no problems with underestimation of the time to most recent common ancestor and the amount of recent divergence in phylogenetic analysis (Schierup and Hein, 2000). All distance values among samples of *C. militaris* (bold) were no more than 0.01, while the minimum distance value among *C. militaris* and its allies (except *C. kyushuensis* and *C. roseostromata*) was 0.082. *Cordycpes militaris* from Norway had no genetic distance with those from Yamagata, Hokkaido, Sichuan and Guizhou, and *C. militaris* from Jilin had the largest genetic distance (0.010) with that from Seoul although the geographical distance between Jilin and Seoul was much less than those between China, Japan and Norway (Fig. 1).

Table 1. Strains used in this study.

Species	Locality	Specimen or Strain*	Sequence Source	Accession Number
C. militaris	Guangdong, China	A	This study	DQ342251
P. militaris	Guangdong, China	GIMYY 0201	This study	DQ342250
C. militaris	Guizhou, China	G97034-2	Liu *et al*., 2002	AJ309329
C. militaris	Sichuan, China	G97034	Liu *et al*., 2002	AJ309328
C. militaris	Hebei, China	HMIGD 20945	This study	DQ342246
C. militaris	Hebei, China	A	This study	DQ342244
P. militaris	Hebei, China	GIMYY 0202	This study	DQ342245
C. militaris	Liaoning, China	A	This study	DQ342249
C. militaris	Jilin, China	HMIGD 20870	This study	DQ342243
C. militaris	Jilin, China	A	This study	DQ342247
P. militaris	Jilin, China	GIMYY 0203	This study	DQ342252
P. militaris	Seoul, Korea	GIMYY 9805	This study	DQ342248
C. militaris	Aichi, Japan	BCMU CM16	Yokoyama and Hara, Unpubl.	AB233336
C. militaris	Yamagata, Japan	----	Nikoh and Fukatsu, 2000	AB027379
C. militaris	Hokkaido, Japan	BCMU CM03	Yokoyama *et al*., 2004	AB084156
C. militaris	Surrey, Britain	K(M) 73501	Liu *et al*., 2002	AJ309331
C. militaris	Norway	ARON 2725.H	Stensrud *et al*., 2005	AJ786572
C. kyushuensis	Liaoning, China	HMAS78115**	This study	EF368021
C. roseostromata	Kangwon-do,Korea	ARSEF 4870	This study	EF368022
C. pruinosa	Guangdong, China	HMIGD 20930	This study	DQ342253
C. pseudomilitaris	Thailand	BIOTEC NHJ6	Stensrud *et al*., 2005	AJ786589
C. bassiana	Anhui, China	ST000903-02	Huang *et al*., 2002	AF347612
C. brongniartii	Yunnan, China	G97030	Liu *et al*., 2002	AJ309348
C. takaomontana	Japan	NIFTS HF774	Yokoyama *et al*., 2005	AB189444
Hypocrea rufa	----	GJS 97-243	Lieckfeldt *et al*., 1999	AJ230667
Hypocrea lutea	----	IFO 9061	Nikoh and Fukatsu, 2000	AB027384

*"A" means samples of artificial fruiting bodies. ** The strain was isolated from HMAS78115
C. = *Cordyceps*, *P.* = *Paecilomyces*

It was concluded, therefore, that the genetic variation of *C. militaris* was extremely small and did not correlate with geographical origins. Furthermore, the distance values among *C. kyushuensis*, *C. roseostromata* and *C. militaris* were less than 0.01, the same as those among samples of *C. militaris*, which suggested they are conspecific.

The topological structures of all trees generated from ME, MP, ML and Bayesian analyses were similar. The ME tree with bootstrap and PP value is presented in Fig. 2. All samples of *C. militaris* generated from different regions, *C. kyushuensis* and *C. roseostromata* were in the same clade with 98-100% bootstrap and PP support. All branches from this clade were very short and showed that all samples of *C. militaris* from different regions were genetically stable. There was, however, a low supported subclade in this clade, but it was not related to regions, distances and types of samples. For example, the samples from Aichi, Hebei and Guangdong were in this subclade, while those from Yamagata, Hokkaido, Sichuan and Guizhou were not despite the fact that Aichi is nearer to Yamagata and Hokkaido than to Hebei and Guangdong (Fig. 1). Moreover, there was no recombination detected in *C. militaris*. The phylogenetic tree coincides with the result above that genetic diversity of *C. militaris* was extremely small and did not correlate with geographical origins.

Discussion

Genetic stability of Cordyceps militaris

In our previous publication concerning *C. sinensis* (Chen *et al*., 2004) we described the genetic distance (*K* values) generated using the K2P model. The *K* values within a Province and among provinces of Tibet, Qinghai and Sichuan were 0.01 to 0.02, and the *K* values between Gansu and Yunnan were 0.02 to 0.03. In this study, *K* values from *C. militaris*

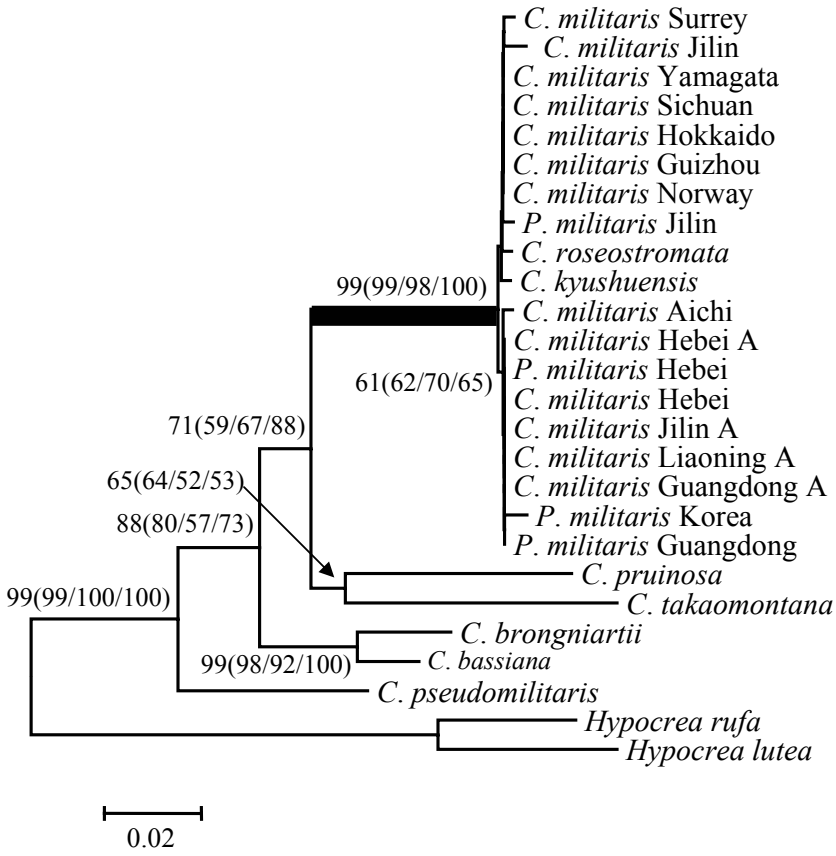

Fig. 2. ME tree of *C. militaris* and related taxa based on ITS sequence analysis. The numbers on each branch are the bootstrap values and Posterior Probabilities (%) (shown >50%) obtained by ME (MP/ML/Bayesian) analysis. "A" = samples of artificially produced fruiting bodies, *C.* = *Cordyceps*, *P.* = *Paecilomyces*.

samples were less than 0.01, although their origins were distant (Fig.1) and samples contain three types of material. The RAPD analysis of *C. sinensis* showed that *C. sinensis* has obvious genetic variation and can be divided into three geographical groups (Chen *et al*., 1999). ITS sequences analysis of *C. sinensis* also supported this geographical genetic variation (Kinjo and Zhang, 2001). In this study, no genetic variation of *C. militaris* from different regions of the world was found. An investigation of the genetic variation of *C. militaris* from eleven sites in South Korea by RAPD analysis also found no connection between genetic variation and geographical regions (Sung *et al*., 1999). Obviously, the genetic variation of *C. militaris* from China, South Korea, Japan and Europe is less than that of *C. sinensis* from different areas of China and did not correlate with geographical origins and types. Thus, *C. militaris* distributed in the world can be regarded as a uniform population.

The differences in genetic variation within *C. militaris* and *C. sinensis* possibly occur because of their different natural habitats and conditions. *Cordyceps sinensis* has fastidious nutritional and environmental requirements. It only parasitizes larva of *Hepialus* spp. which live underground and is only distributed in many small areas of Qinghai-Tibet Plateau from 3000 to 5000 m above sea level. Additionally, its ascospores (140-245 μm long) do not break into secondary ascospores and its conidia (*Hirsutella*) are less amounts and wrapped up in slime. These biological characteristics of *C. sinensis* may restrict its spread in Qinghai-Tibet Plateau. On the contrary, *C. militaris* parasitizes larva or pupa of many kinds of insects (*Lepidoptera* mainly) which live above the ground and its ascospores break

Table 2. Pairwise distance matrix generated with Kimura two-parameter model.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*C. militaris* Yamagata	--												
2	*C. militaris* Hokkaido	**0.000**	--											
3	*C. militaris* Aichi	**0.004**	**0.004**	--										
4	*C. militaris* Sichuan	**0.000**	**0.000**	**0.004**	--									
5	*C. militaris* Guizhou	**0.000**	**0.000**	**0.004**	**0.000**	--								
6	*C. militaris* Surrey	**0.002**	**0.002**	**0.006**	**0.002**	**0.002**	--							
7	*C. militaris* Norway	**0.000**	**0.000**	**0.004**	**0.000**	**0.000**	**0.002**	--						
8	*C. roseostromata*	**0.002**	**0.002**	**0.006**	**0.002**	**0.002**	**0.004**	**0.002**	--					
9	*C. kyushuensis*	**0.002**	**0.002**	**0.006**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	--				
10	*C. militaris* Jilin	**0.004**	**0.004**	**0.008**	**0.004**	**0.004**	**0.006**	**0.004**	**0.006**	**0.006**	--			
11	*C. militaris* Hebei A	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	--		
12	*P. militaris* Hebei	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	--	
13	*C. militaris* Heibei	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	**0.000**	--
14	*C. militaris* Jilin A	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	**0.000**	**0.000**
15	*P. militaris* Korea	**0.006**	**0.006**	**0.006**	**0.006**	**0.006**	**0.008**	**0.006**	**0.008**	**0.008**	**0.010**	**0.004**	**0.004**	**0.004**
16	*C. militaris* Liaoning A	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	**0.000**	**0.000**
17	*C. militaris* Guangdong A	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	**0.000**	**0.000**
18	*P. militaris* Guangdong	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.000**	**0.000**	**0.000**
19	*P. militaris* Jilin	**0.002**	**0.002**	**0.006**	**0.002**	**0.002**	**0.004**	**0.002**	**0.004**	**0.004**	**0.006**	**0.004**	**0.004**	**0.004**
20	*C. bassiana*	0.084	0.084	0.087	0.087	0.084	0.087	0.084	0.084	0.087	0.089	0.084	0.084	0.084
21	*C. pruinosa*	0.111	0.111	0.116	0.113	0.111	0.113	0.111	0.113	0.111	0.116	0.113	0.113	0.113
22	*C. pseudomilitaris*	0.128	0.128	0.130	0.130	0.128	0.130	0.128	0.130	0.130	0.133	0.130	0.130	0.130
23	*C. takaomontana*	0.100	0.100	0.100	0.102	0.100	0.102	0.100	0.100	0.097	0.105	0.100	0.100	0.100
24	*C. brongniartii*	0.092	0.092	0.097	0.094	0.092	0.094	0.092	0.092	0.094	0.097	0.094	0.094	0.094
25	*Hypocrea lutea*	0.229	0.229	0.223	0.231	0.229	0.232	0.229	0.229	0.229	0.235	0.226	0.226	0.226
26	*Hypocrea rufa*	0.226	0.226	0.226	0.228	0.226	0.229	0.226	0.229	0.226	0.232	0.223	0.223	0.223

		14	15	16	17	18	19	20	21	22	23	24	25	26
14	*C. militaris* Jilin A	--												
15	*P. militaris* Korea	**0.004**	--											
16	*C. militaris* Liaoning A	**0.000**	**0.004**	--										
17	*C. militaris* Guangdong A	**0.000**	**0.004**	**0.000**	--									
18	*P. militaris* Guangdong	**0.000**	**0.004**	**0.000**	**0.000**	--								
19	*P. militaris* Jilin	**0.004**	**0.008**	**0.004**	**0.004**	**0.004**	--							
20	*C. bassiana*	0.084	0.090	0.084	0.082	0.084	0.087	--						
21	*C. pruinosa*	0.113	0.114	0.113	0.111	0.113	0.111	0.116	--					
22	*C. pseudomilitaris*	0.130	0.134	0.130	0.128	0.130	0.131	0.096	0.166	--				
23	*C. takaomontana*	0.100	0.106	0.100	0.098	0.100	0.100	0.126	0.115	0.188	--			
24	*C. brongniartii*	0.094	0.100	0.094	0.092	0.094	0.095	0.036	0.113	0.098	0.119	--		
25	*Hypocrea lutea*	0.226	0.231	0.226	0.227	0.226	0.233	0.203	0.245	0.231	0.264	0.229	--	
26	*Hypocrea rufa*	0.223	0.224	0.223	0.223	0.223	0.229	0.200	0.228	0.229	0.258	0.220	0.080	--

*"A" means samples of artificial fruiting bodies.
Values ≤ 0.01 are in bold. *C.* = *Cordyceps*, *P.* = *Paecilomyces*

into numerous smaller secondary ascospores (2-.3.5 μm long) (Kobayasi and Shimizu, 1983). Furthermore, it grows much faster than *C. sinensis* and its conidia are nemerous and more easily dispersed in the air. Therefore, we speculate that *C. militaris* spreads more extensively and survives more easily than *C. sinensis*. There also appears to be no barriers for high gene flow among *C. militaris* strains from different geographical groups, which results in *C. militaris* being less divergent than *C. sinensis*.

Cordyceps militaris has been used in Traditional Chinese Medicine and health foods over a long period. Now, it is mass-produced in some provinces of China such as Guangdong,

Table 3. Morphological comparison of *C. militaris* with *C. kyushuensis* and *C. roseostromata*.

Species	Host	Stromata; Stipes; Fertile Parts(mm);color	Perithecium (μm)	Secondary spore (μm)
C. militaris	Pupa or larva of Lepidoptera	13-65 × *; 10-50 × 1-6; 2-30 × *; orange yellow	450-670 × 230-370	2-3.5 × 1
C. kyushuensis	Larva of Lepidoptera	15-20 × *; 5-8 × 2; *; yellow	410-580 × 210-330	4-5 × 1
C. roseostromata	Larva of Coleoptera	*; * × 2.3-3; 1.2-5 × 0.4-2.2; red	280-300 × 140-160	4-5 × 1

* Information was not provided in original morphological description.

Hebei, Jilin and Liaoning as artificially produced fruiting bodies. This is because *C. militaris* isolates are relatively easy to culture. Our results show that these strains are the anamorph of *C. militaris* and mass production does not affect their genetic stability. Differences in cultural characters observed in factories may be due to isolate degeneration or different cultural practices and not differences in genetic characteristics of strains.

Cordyceps militaris and its closely related species, C. kyushuensis and C. roseostromata

Cordyceps militaris is similar to *C. kyushuensis*, *C. roseostromata*, *C. pruinosa*, *C. pseudomilitaris*, *C. cardinalis*, *C. bassiana*, *C. brongniartii* and *C. takaomontana* in morphology. Among them, *C. kyushuensis* and *C. roseostromata* (Kobayasi and Shimizu, 1983) are most similar to *C. militaris*. In this study *C. kyushuensis* and *C. roseostromata* had the same distance levels (≤ 0.01) as all *C. militaris* samples, and far less than that among *C. militaris* and the other taxa used in this study. *Cordyceps kyushuensis*, *C. roseostromata* and *C. militaris* clustered within a clade with strong bootstrap and PP support (≥ 98%) in the phylogenetic tree. The molecular evidence indicated that *C. militaris*, *C. kyushuensis* and *C. roseostromata* may be conspecific. Sung and Spatafora (2004) also found that *C. roseostromata* and *C. militaris* clustered together with 100% bootstrap support and had almost no nucleotide differences within them (based on analysis of SSU rDNA and LSU rDNA data). According to Kobayasi and Shimizu (1983), no distinct morphological differences are found among these three species and a comparison of their morphological characters are shown in Table 3. *Cordyceps kyushuensis* is almost identical to *C. militaris* except that the former has somewhat longer secondary ascospores (4-5 μm). However, longer secondary ascospores (3-6 μm) can be also observed in some specimens of *C. militaris* from China. The main difference between *C. roseostromata* and *C. militaris* is not the size of secondary spores, but that the former parasitizes larva of Coleoptera and its stromata and perithecia are smaller. However, the difference in hosts may not be a good character to differenttiate these species as *C. militaris* can infect a wide range of insect hosts. Additionally, the size of stromata in many *Cordyceps* species is generally related to that of the host and the amount of stromata growing on it. This indicates that *C. kyushuensis* is a synonym of *C. militaris* based on their molecular data and morphological characters. *Cordyceps roseostromata* is also potentially conspecific with *C. militaris*, but with some variations in the size of stromata and perithecia. However, the isolate used in the analysis of *C. roseostromata* is problematic as Sung and Spatafora (2004) have explained in their paper.

Acknowledgements

This work was supported by the National Science Foundation of China (No. 30270009), the Natural Science Foundation of Guangdong (No. 20010844, No.05000603) and the Program for New Century Excellent Talents in University (NCET-04-0788). We thank ARSEF and HMAS for providing strains of *C. roseostromata* and *C. kyushuensis*, J.Y. Chen and J.F. Wu, Yiyuan *Cordyceps* Cultivation Plant, Jiangmen city of Guangdong for their providing some samples of *C. militaris*.

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