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Morphological and phylogenetic studies on *Cordyceps sinensis* distributed in southwestern China

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***Cordyceps* is one of the target genera for modern mycological studies. Among them *Cordyceps sinensis* is the most famous but poorly defined species because the fungus is endemic in districted regions of east Eurasia. We have explored the various growing regions and habitats where the fungus grows in the wild. We also examined authentic cultures for the species. We analyzed the sequences of ITS1, 2 and 5.8 S rDNA regions of *C. sinensis* materials collected from 11 localities of southwestern China. Phylogenetic analyses were performed with these sequences and with additional sequences obtained from GenBank. All sequences formed a single cluster, which comprised two subgroups. Our results strongly suggested that intraspecific variation was rather small and that some species that are morphologically similar to *C. sinensis* but with different names might be synonymous with *C. sinensis*. The difference in the pharmaceutical activity among these collected *C. sinensis* from different regions will be studied in the future.**

Key Words——*Cordyceps sinensis*; habitat; intraspecific variation; ITS sequences; teleomorph morphology.

Cordyceps fungi have attracted many mycologists since their discovery because of their peculiar characteristics of fruiting on their host invertebrate animals. The fungi on cicadas had been described in old Chinese medicinal books from ancient times (Li, 1578). From the middle of the Qing dynasty, *Cordyceps sinensis* (Berk.) Sacc. was used as a tonic and supplement for court cuisine (Zang and Kinjo, 1996), and was introduced to Japan through Nagasaki (Ono Ranzan, the Edo era). Nowadays, the fungus has become one of the most famous and esteemed traditional Chinese medicines (Liu, 1978). The fungus has been noted for its potential for medicinal use and hence extensive pharmaceutical studies have been made (e.g., Kinjo et al., 1996).

The name of Dong Chong Xia Cao (in Chinese; Tochu-Kaso in Japanese; catapillar fungus, vegetable wasps and plant worms in English) is used as a general name for most *Cordyceps* species, and also for the specific name referring to *C. sinensis*.

It is believed that *C. sinensis* was introduced into Western scientific society with the specimens purchased in Guangzhou by Reeves in 1842. At first, the fungus was described under the name of *Sphaeria sinensis* Berkeley in 1843 based on these materials. Later the species was accommodated in the genus *Cordyceps* (Fr.) Link by Saccardo (1878). This specimen of the fungus was assumed to have been collected at western Sichuan, China. However, the precise location of the fungus collected was not clear. Thus, the locality name written on the label of the type specimen retained in the Herbarium of the Royal Botanic Gardens, Kew (K. H0221) is only "China" (Zang and Kinjo, 1996).

The fungus is endemic in the alpine shrub-meadow zone of high mountains or highlands of southwestern China, situated in such provinces as Sichuan, Yunnan, Qinghai, Gansu, Hubei, and Xizang (Ying and Zang, 1994), along with northeastern Jilin Province (Gu et al., 1993). The fungus is parasitic on the larvae of Lepidoptera and the host insects have been reported as members of Hepialidae (Lepidoptera, Exoporia; the bat moths), which are distributed in cool weather conditions. They have been variously described: *Hepialus davidi* Poujade, *H. altissima* Daniel (in Sichuan), *H. luteus* Groum-Grshimailo (in Qinghai), *H. nebulosus* Alpheraky, *H. varians* Staudinger (in Xizang), and *H. armorianus* Oberthuer (many collections including Type K. H0221) (Zang and Kinjo, 1996). But identification of the host insects by larvae stage is difficult and identification of the exact host species awaits future determination. Moreover, the commercial commodities of "Dong Chong Xia Cao" available in traditional Chinese medicinal shops have usually no fruiting bodies. Therefore, the identity of these fungi is unclear. The identity of the fungi which are morphologically similar to *C. sinensis*, but having different names, from nearby localities is not fully elucidated, because it is difficult to obtain fresh materials in the wild.

Since 1995, we have attempted to explore some regions where the fungal specimens were collected in the wild. Here we present the results of our studies from field expeditions and morphological observations of *C. sinensis* and our analysis of variation based on rDNA se-

quence analyses. Parts of these results were reported in a previous brief report (Kinjo and Zang, 2001).

Materials and Methods

Investigated localities We visited 11 locations in China listed in Table 1 and collected living fresh materials of *C. sinensis*. These are as follows (name of voucher specimen). Qinghai Province: Yeniushan (Yeniushan), Qingshashan (Qingshashan), Yushu (YUSHU). Gansu Province: Maqu (MAQU). Sichuan Province: Dege (DEGE), Kangding (Kangding), Litang (Litang), Luhuo (LUHUO), Shade (Shade). Xizang Province: Binghu (BINGHU), Nyaramu (Nyaramu) Himalayan north slopes about 37 km to the boundary line for Nepal at 86°E 28°20'N, 4550 m and Rusyasya (Rusyasya) about 80 km east from Nyaramu.

Isolation of the fungus Isolation was made on a Sabouraud glucose agar (SGA). Individual specimens were cleaned using a calligraphic brush under running tap water and distilled water. Superfluous water was removed with sterile filter paper. The stromatal head was dissected longitudinally using a razor blade. Some pieces of the stromatal sections were transferred into sterile water in a small sterile petri dish, and were dissected with sterile fine needles. Bundled asci were pulled from the peridium and were transferred to sterile water using fine needles. After thoroughly cleansing the specimens with sterile water several times, bundled asci were picked up on the tip of a fine needle and transferred and spread to the surface of a SGA plate. Primary isolation was made after confirmation of germination with the aid of the microscope. Germinating single ascospore was picked up and transferred to a fresh SGA slant. Thus, several monoascosporic cultures were established for each specimen.

When the ascospores were immature, isolation was made aseptically by placing small pieces of tissue from sclerotia or stroma on SGA. Colonies from these pieces were checked and colonies which were identical with those from monoascosporic origins were transferred to fresh SGA slants.

Cultures were kept at 18–20°C and they produced *Hirsutella* type conidia on SGA slant. The DNA sequences of these cultures were compared with those of corresponding original fruit body specimens.

Morphological observations Morphological characteristics of teleomorphic stages of the fungi were examined for each of the samples collected and also the materials obtained from herbarium including the type specimens (K. H.0221) of the Herbarium K. Colors designated were from the Kornerup and Wanscher (1978) Color Standard and the Rayner (1970) color chart, and were referred to the letters M. and R., respectively.

DNA extraction, amplification and sequencing Small pieces of fruit bodies or debris of cultured strains were crushed in liquid nitrogen. DNA was extracted from them with DNeasy Plant mini Kit (QIAGEN GmbH, Germany). The ITS 1, 2 and 5.8 S rDNA regions were amplified with the ITS7 (5'-GGCCG GGAAG CTCTC CAAAC TCGGT CATT-3') and ITS8 (5'-CCTCT GCAAA TTACA ACTCG GGCC-3') primers. Each PCR was used Ready-To-Go PCR Beads (Amersham Pharmacia Biotech Inc., NJ) and GeneAmp PCR System 9600 (Applied Biosystems, CA, USA). The PCR products were purified with QIAquick PCR Purification Kit (QIAGEN) and cloned with TOPO TA cloning Kit (Invitrogen Corp., CA, USA). The Plasmid were purified with QIAprep Spin Miniprep Kit (QIAGEN GmbH, Germany). They were sequenced using a ABI PRISM 377 DNA sequencer. The sequencing reaction were used the BigDye Terminator Kit (Applied Biosystems, CA., USA), using primers T7P (5'-TAATA CGACT CACTA TAGGG-3'), M13Rev (5'-CAAGGA AACAG CTATG AC-3'), ITS4 (5'-TCCTC CGCTT ATTGA TATGC-3'), and ITS5 (5'-GGAAG TAAAA GTCGT TAACA AGG-3'). The sequence data were assembled with AutoAssembler (Applied Biosystems, CA, USA). The 18S rDNA regions were amplified with SSJ (5'-CTGGT TGATC CTGCC AGTAG-3') and SST (5'-ACGGA ACCTT GTTAC GACT-3') primers. The purified PCR products were sequenced using primers, SSJ, NS2 (5'-GGCTG CTGGC ACCAG ACTTG C-3'), NS3 (5'-GCAAG TCTGG TGCCA GCAGC C-3'), NS4 (5'-CTTCC GTCAA TTCCT TTAAG-3'), NS5 (5'-AACTT AAAGG AATTG ACGGA AG-3'), NS6 (5'-GCATC ACAGA CCTGT TATTG CCTC-3'), NS7 (5'-GAGGC AATAA CAGGT CTGTG ATGC-3'), P (5'-GCCTC CTGCC TTCCT TG-3'), Q (5'-CAAGG AAGGC AGCAG GC-3'), R (5'-AAACC AACAA AATAG AA-3'), or S (5'-CGGCC ATGCA CCACC-3'). The 28 S rDNA regions were amplified with LS1 and LSD primers. The accession numbers of these sequences were AB067735–49. Vouchers were deposited at the Natural History Museum and Institute, Chiba (CBM).

The sequences were aligned with CLUSTAL W (Thompson et al., 1994). Phylogenetic trees were constructed by a neighbour-joining method (Saitou and Nei, 1987). The distance matrix was calculated using DNADIST with the Kimura 2-parameter method with NEIGHBOR (transition to transversion rate: 2.0). One thousand replicate bootstrap samplings were carried out with the software package PHYLIP (version 3.72; Felsenstein, 1993), using SEQBOOT, NEIGHBOR, and CONSENSE.

Results

Habitat of *Cordyceps sinensis* As shown in Table 1, the collecting sites were high mountain areas, at least 3,600 m above sea level, with plains of alpine meadow subdominant with graminea grasses, smart weeds and other herbaceous plants. For example, such places as Bei-Ma Shan are filled with *Saussurea*, *Myconopsis*, *Kobresia* and *Primula*.

Pedicularis and *Potentilla* are commonly associated with shrubby rhododendrons (Zang et al., 1990). In winter the grounds of these plains are frozen and covered with snow more than 120 days in a year. Average annual temperature is said to be near 0°C (Yang et al., 1994). A typical scene of alpine meadow without woody plants is shown in Fig. 1. Figure 2 illustrates

Table 1. Specimens and cultural isolates of *Cordyceps sinensis* examined.

Vouchers	Locality	Province	Geographic location			Collection date
Specimen						
Yeniushan	Yeniushan	Qinghai	101°E	36°30′N	4200 m	2000.5.
Qingshashan	Qingshashan	Qinghai	102°40′E	36°20′N	3650 m	2000.5.
YUSHU	Yushu	Qinghai	97°E	33°N	5000 m	1998.6.
MAQU	Maqu	Gansu	102°40′E	34°40′N	3780 m	1998.5.
DEGE	Dege	Sichuan	99°10′E	32°N	4200 m	1998.6.
Kangding	Kangding	Sichuan	102°E	29°N	4100 m	2000.5.
LUHUO	Luhuo	Sichuan	100°E	32°N	4000 m	1998.6.
Litang	Litang	Sichuan	100°E	30°N	4100 m	2000.6.
Shade	Shade	Sichuan	102°E	31°N	4200 m	1999.6.
BINGHU	Binghu	Xizang	100°E	30°N	3800 m	1999.6.
Nyaramu-1	Himalayan north slope		86°E	28°20′N	4550 m	2000.6.
Nyaramu-2	Himalayan north slope		86°E	28°20′N	4550 m	2000.6.
Rusyasya	Himalayan north slope		85°50′ E	28°20′N	4550 m	2000.6.
Ascospore isolate						
Kangding A-1	From Kangding collection of 2000.5.					2000.6.
Kangding A-2	From Kangding collection of 2000.5.					2000.6.
Kangding A-4	From Kangding collection of 2000.5.					2000.6.
Kangding B-3	From Kangding collection of 2000.5.					2000.6.
Kangding B-6	From Kangding collection of 2000.5.					2000.6.
SHANGHAI	Provided by Y. Pan, Shanghai Agricultural Sciences					1994.6.

workers collecting *C. sinensis*. When the fungus is growing up from the ground after snow melt, it is easy to find the growing stipe of the fruiting body, since obstructive plants are not present (Figs. 3, 4). Below the growing fungus, a gallery hole approximately 16 cm deep by 2 cm wide was usually found (Fig. 5). These galleries were considered the remains of tunnels through which worms which may serve as fungal hosts attacked the roots or rhizomes of graminea, polygonum, cyperaceous or other herbaceous plants. This is the first demonstration of such a relationship.

Morphology of the fruiting ascostromata

1. Type study: The holotype (K. H0221) of the fungus kept in the Herbarium of the Royal Botanic Gardens, Kew, was labeled as *Sphaeria sinensis* Berk., Stroma, China.

The holotype material with a few wormholes left in ascocarps damaged by worms. The stromata single, cylindrical 1.7–2.2 cm, 2.4–4 mm diam, with sterile acuminate apex. Ascogeniuous portion glabrous or punctate with ostioles of the perithecia. Stipes 2–2.6 cm, 2–2.6 mm diam with silky surface. Perithecia globoid or vase-shaped, 150–380(–550) × 110–240 μm. The cortex usually consisting of one layer of closely interwoven hyphae, pseudoparenchymatous 80–170 μm thick. Asci cylindrical, narrowing below, 160–240(–400) × 5.2–6.5(–12) μm with a hemispherical thickening of the wall at the apex, asci not fully mature and hence thinner.

2. Fresh materials: Morphological measurements were carried out with materials collected at Kangding, Sichuan Province and those from Himalayan north slopes in Xizang Province. The morphological characteristics of Kangding materials are as follows (Fig. 6A–L). Stroma arising from the head of the host, solitary, simple, Dark Brown (M. 7F6 to 8F4) or Dark Brick to Sepia (R.), straight, cylindrical, –80-mm long, 5–6 mm thick; fertile part middle to terminal clearly defined from the stalk, –35-mm long, 5–6 mm thick; top of the stromata sterile shortly –3.5-mm aculate; stalk cylindrical, smooth, somewhat rigid fibrous, –3-mm in diam; cortex layer 0.5 mm thick, medullar layer white, composed of the tissue of textura porrecta, longitudinally and densely arranged, at the base connected with the host directly and more darker. Perithecia superficial to faintly immersed into cortex at the base, ovoid to oblate 360–440 × 240–280(–320) μm, densely, pressed together neighbouring; peridium 40–65 μm thick, Brownish Yellow (M. 5C8) to Yellowish Brown (M. 5D8) or Ochraceous (R.), several-layered with the tissue of textura angularis. Asci hyaline, (2)4-spored, cylindrical, 350–380 × 9.5–13 μm, with a 4.8–5.6 μm thick perforated depressed globose cap; axial canal 0.8–1 μm in diam. Ascospores hyaline, filiform, smooth, multiseptate, 200–320 × 4.6–6.4 μm, 36–40 cells, never breaking up into part spores; faintly tapered to both ends. Ascospores germed in 3–4 days at 20°C on SGA medium.

Morphological data on Himalayan materials are as follows. Nyaramu-1: 67–74 × 3.5–4.0 mm in sizes, clavate with sterile acuminate apex 3.0–3.6 × 1.8–2.0 mm, stipe hard and glabrous, Light Brown (M. 7D6) or Fawn (R.), broad at the base, 45–47 × 3.0–3.3 mm, cortex 0.4–0.5 mm thick, metulae white.

Rusyasya-1: 44–60 × 2.7–3.6 mm in sizes with sterile acuminate apex 1.8–2.8 × 1.5–1.8 mm, clavate, stipe

Fig. 1. Typical scene of alpine shrub-meadow of *Cordyceps sinensis* growth area (Xizang Province; 2000.6).
Fig. 2. Typical scene searching and collecting *Cordyceps sinensis* (Yushu, Qinghai Province, 1998.6).
Fig. 3. Apical part of *Cordyceps sinensis* stipe protruding among *Kobresia robusta* Maxim. Ascostmatal part is not produced. (Nyaramu; 2000.6.). Scale bar = 1 cm.
Fig. 4. Close up of apical part of *Cordyceps sinensis* stipe protruding from the ground (Nyaramu; 2000.6.). Scale bar = 1 cm.
Fig. 5. Gallery hole of bat moth with *Cordyceps sinensis* (Nyaramu; 2000.6.). Scale bar = 1 cm.

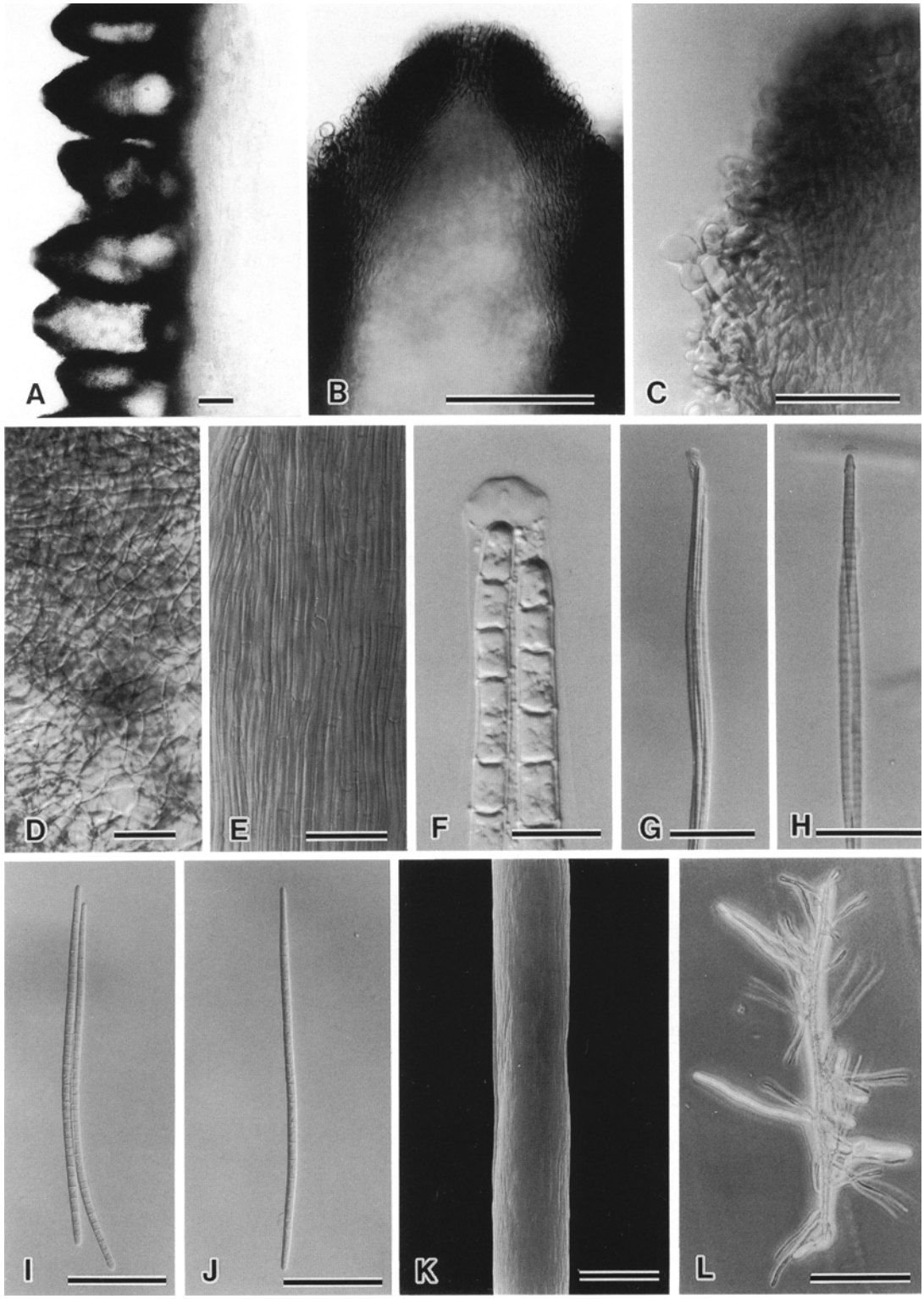

Fig. 6. Morphological characteristics of mature fruit body of *Cordyceps sinensis* collected in Kangding. A. Transverse section through the perithecial clava. B. Perithecium. C. Peridial wall. D. Peridial cells. E. Pararelly arranged cells of medullae of stalk. F. Apical structure of an ascus with septated ascospores. G. 4 ascospores parallely arranged in an ascus. H. 2 ascospores in an ascus. I. 2 filamentous scoleco-ascospores with defined septa. J. Single ascospore released from ascus. K. Surface ornamentation of an ascospore. L. Germinating asocospore without fragmentation to part spores. Scale bars: A, B=100 μm; C, G–J, L=50 μm; D, E=20 μm; F=10 μm; K=5 μm.

Dark Brown (M. 7F6 to 8F4), 32–34 × 2.4–3.0 mm. Cortex 0.4–0.5 mm thick, metulae white. Perithecia ovoid to ellipsoidal, 360–420 × 240–320 μm with 40–65 μm thick peridial walls. Their bases are embedded in the cortex layer of the storoma, 68–120 μm thick. The ostioles 0.9–1 μm diam, projecting above the surface. Asci cylindrical, 350–380 × 10–13 μm with a hemispherical thickening of the wall at the apex. Two to 4 ascospores, hyaline, filiform tapered at both ends, 310–350 × 4.0–7.0 μm are parallely arranged in the ascus. Ascospores are 51–93 septated with irregular distances, but not constricted nor separated at the septa.

These characteristics agreed with those of previous reports (e.g., Chaudhuri, 1931; Kobayasi, 1941; Liang et al., 1995; Chen et al., 1999). Other materials used in this study were unfortunately immature, but their external morphology and partial inner morphology were within the range of variation of *C. sinensis*.

The host insect of the fungus in Nyaramu and Rusyasya at the Himalayan north slopes situated in the Xizang highland was identified as the larvae of *Thitarodes* sp. by its external morphology. Host species of other collections were not determined.

Phylogenetical analyses The sequence data of cultured strains were completely identical with those of fruiting bodies in each case. Hence, here, we denoted only the name of the locality where specimens were collected except Kangding isolates (Table 1). In this case, 5 isolated strains were used and no difference was detected between them and fruiting body specimens. The phylogenetical tree based on ITS1, ITS2 and 5.8S rDNA regions constructed with our sequencing data and DNA GenBank data (Table 2) is shown in Fig. 7 (28S and 18S r DNA trees were not shown). *Cordyceps sinensis* from 8 localities (Nyaramu, DEGE, MAQU, YUSHU, Qingshashan, Kangding, LUHUO, BINGHU) grouped together in the ITS1-ITS2, 18S and 28S rDNA analyses. In ITS1-ITS2 analysis, they separated into two subgroups; one (subgroup 1) with 6 localities (Nyaramu-1, -2, 5 Kangding isolates, Litang, Rusyasya, Shade, Yeniushan) and the second (subgroup 2) with 6 localities (DEGE, MAQU, Qingshashan, YUSHU, LUHUO, BINGHU), with the DNA GenBank data available for 3 *Cordyceps sinensis* and 2 *Hirsutella sinensis* Liu et al. sequences (Table 2). Thus, the 28S and 18S rDNA sequences are considered to be more conservative and less informative for the analyses of intraspecific differentiation in *C. sinensis*.

One cultural strain SHANGHAI provided by Prof. Y. Pan, Edible fungi Institute, Shanghai Academy of Agricultural Science, Shanghai, China was grouped with *C. sinensis* AF291749, *Tolypocladium cylindrosporum* W. Gams AJ 303055 and *T. inflatum* W. Gams U35308 in ITS-ITS2 and 28S rDNA analysis. This result suggests that *C. sinensis* AF291749 and the teleomorph of isolate SHANGHAI might be a same species but different from *C. sinensis*, because *Tolypocladium* is an anamorphic species, which is related to other *Cordyceps* species (Hodge et al., 1996). Another possibility is that the isolate SHANGHAI, *C. sinensis* AF291749 and *Tolypocladium* spcecies might be a contaminant or associated fungi of *C. sinensis* because many contaminant anamorphic fungi are associated with *Cordyceps* species.

Discussion

Many collections of *C. sinensis* deposited at the herbarium are often immature or lack the ascostromatal parts. The commercial commodities at traditional Chinese medicinal shops or markets have the same tendencies. Therefore, scientific studies concerning this fungus have frequently been confronted with taxonomical problems. In this study, we investigated the habitat of the fungus and collected fresh materials from typical growing regions in China. Then we reexamined the accurate ascostromatal morphology of the fungus. The results of comparison of ITS1-ITS2 rDNA sequences indicated that the variations among different localities were rather small, although clear differences were recognized in their pharmaceutical activity (data not shown). The molecular phylogenetic evidence showed that the morphologi-

Table 2. ITS rDNA data obtained from DNA GenBank.

Species	Accession No.	Note
Cordyceps sinensis	AF291749	MPNU 8002
Cordyceps sinensis	AJ243775	China:Tibet, sclerotium
Cordyceps sinensis	AJ243776	China:Tibet, ascospore
Cordyceps sinensis	AJ243778	China:Tibet, stroma
Cordyceps sinensis	AJ245559	China:Tibet, LiT9704
Hirsutella sinensis	AJ243979	China:Guangdong, Zhw01
Hirsutella sinensis	AJ243980	China:Beijing, HMAS 55469
Cordyceps militaris	AJ243774	not described
Cordyceps militaris	AJ242923	not described
Cordyceps heteropoda	AB027373	not described
Tolypocladium cylindrosporum	AJ303055	CBS 719.70
Tolypocladium inflatum	U35308	not described

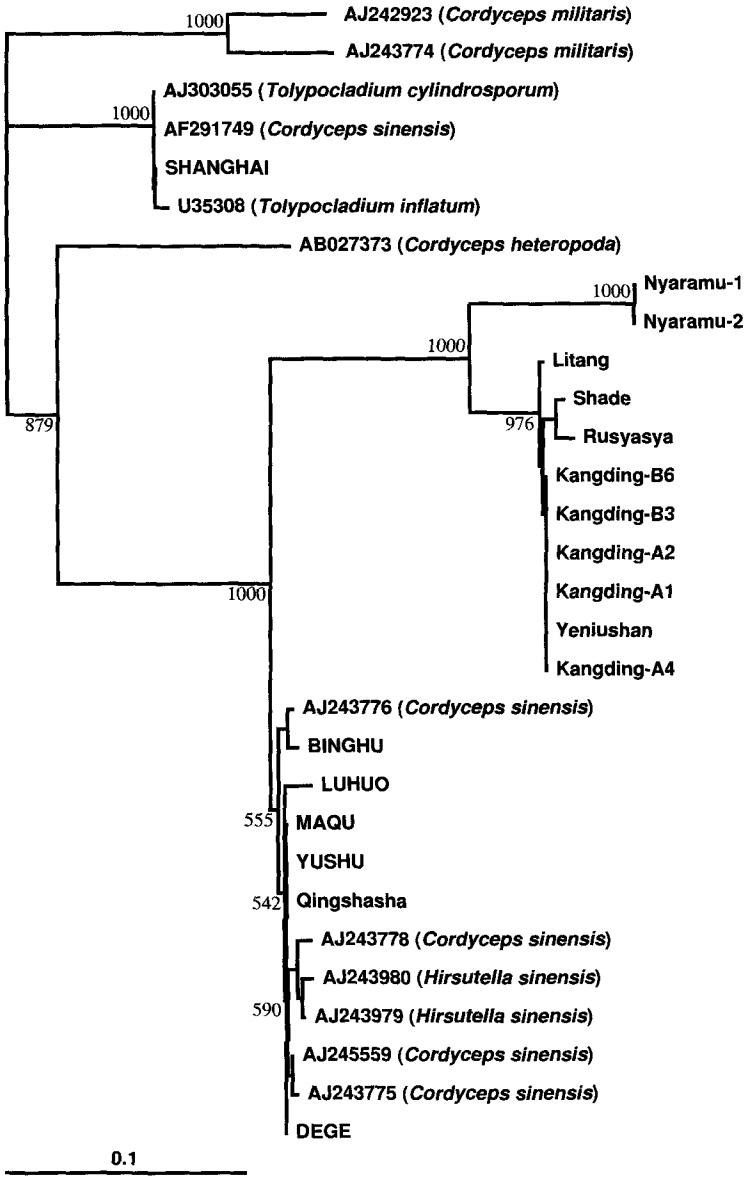

Fig. 7. Phylogram resulting from the neighbor-joining analysis of ITS1, 2 and 5.8S regions of rDNA of *Cordyceps sinensis*, and other related fungi. The values shown at nodes are the confidence levels from 1,000 replicate bootstrap samplings. Bar=distance corresponding to 10 base changes per 100 nucleotide positions.

cally similar fungi distributed in alpine regions of China on bat moths might be a single species with variations. In our analyses of 17 collections and cultures along with GenBank data, *C. sinensis* was divided into 2 groups. One group is composed of materials from Himalayan north slope to southwest parts of Sichuan Province. The other group is from a more northern part of Sichuan Province to Qinghai and Gansu provinces, although both groups were not strictly represented by the geographical divergence. Local variation in the fungus was suggested by ITS sequence analyses (Liu et al., 2001) and by comparisons with RAPD markers (Chen et al., 1999; Zhang et al., 1999). However, consideration of genetic diversity and its relation to geographic distribution should await future analyses with more specimens.

The existence of small variations in morphology of the fungus has been recognized. Thus two species have been reported as similar but different species on bat moth; *C. gansuensis* Zhang et al. from Gansu Province (Zhang et al., 1989), and *C. crassispora* Zang et al. from the north part of Yunnan Province (Zang et al., 1990). These were proposed as new species on the basis of minor morphological differences although they are both parasitic on bat moth larvae (Ying and Zang, 1994). The species description and figures given by Chaudhuri (1931) based on the east Tibetan collections are also different from other reports (e.g., Kobayasi, 1941). However, the morphological variations in *C. sinensis* have been analyzed and the morphology of fruiting parts are varied depending on their maturation stages (Liang et

al., 1995). Moreover, we noticed that many commercial samples had been collected in early stage of ascostromatal development (Figs. 3–5), and sometimes we recognized the deformed individuals assignable as different species during our expeditions. We concur with Liang et al. (1995) because only small differences were found in DNA sequences (Fig. 7). Our results strongly suggest that the *Cordyceps* species on bat moth larvae, including *C. gansuensis* and *C. crassispora* , might be a single species assignable as *C. sinensis*. Unfortunately, we failed to amplify the DNA from the specimens from Bei-Ma Shan; this possibility was supported by the recent report concerning anamorph-teleomorph connections (Liu et al., 2001).

As for the anamorphic cultures, our rDNA sequence data for all materials assignable as *C. sinensis* were consisted with the data deposited at GenBank identified as *Hirsutella sinensis*. Thus, the anamorph of the fungus would be *Hirsutella* stage. Growth of cultured strains of *C. sinensis* on SGA at 18–20°C were rather slow and only reached some 5 mm in diam after 30 d. The growth at 25°C was poor and no growth was observed at 30°C. These cultural characteristics will be summarized in a future publication. One of our tentative conclusions is that the isolation of the fungus must avoid contamination by associated fungi.

The next goal is the identification of the host insect. Our collections made at the Himalayan north slope were on *Thitarodes* species. In the literatures, the host insect larvae of *C. sinensis* was described as *Hepialus* species. It is very interesting that the fungus is able to use plural genera or species as the host insect.

Finally, we need to identify suitable DNA regions for comparison of intraspecific variations in order to relate pharmaceutical effects found in specimens/isolates or commercial commodities of the fungus collected at different locations.

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