Phylogenetic relationships of host insects of *Cordyceps sinensis* inferred from mitochondrial Cytochrome *b* sequences*

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Abstract This study used the sequence of the mitochondrial Cytochrome *b* (Cyt*b*) to estimate phylogenetic relationships among host Hepialidae insects of *Cordyceps sinensis*. Genome DNA of host insect was extracted from the dead larva head part of 18 cordyceps populations and 2 species of *Hepialus*, and the Cyt*b* fragment of host insect was amplified with PCR technique. The nucleotide sequence alignments and their homologous sequences of 24 species host Hepialidae insects of *Cordyceps sinensis* were obtained from GenBank and were used to construct phylogenetic trees based on neighbor-joining method. The results showed that genus *Bipectilus* diverged earlier than genus *Hepialus* and *Hepialiscus*. *Hepialus* host insects of *Cordyceps sinensis* have multitudinous species with different morphological characteristics and geographical distributions. The interspecific genetic differentiations are obvious in *Hepialus*. Thus, the genus *Hepialus* might be considered as polyphyletic origin. Cyt*b* sequences have abundant variations among the host insects of *Cordyceps sinensis* on specific and generic level. The divergence rate of Cyt*b* sequences among the species in *Hepialus* ranged from 0.23% to 9.24%, except that *Hepialus pratensis* and *Hepialus jinshaensis* have the same sequence. Cyt*b* sequence can be used for species identification of host insects of *Cordyceps sinensis*, but further confirmation in more host insect species is needed. To obtain the Cyt*b* sequence of host insect by amplifying DNA extracted from the head part of dead larva in cordyceps turns out to be an effective and accurate approach, which will be useful for studies on phylogeny and genetic structure of host insects of cordyceps populations, especially for analyzing relationships between *C. sinensis* and its host insects.

Keywords: mitochondrial Cytochrome *b*, *Cordyceps sinensis*, *Hepialus*, phylogeny.

Cordyceps is the complex of fungus *Cordyceps sinensis* (Berk.) Sacc. (Clavicipitaceae) parasitizing on the larva of Hepialidae[1,2]. It is only distributed in the prairie at an altitude of 3000—5100 meters in Qinghai-Tibet plateau of West China, mainly in Qinghai, Tibet, Yunnan and Sichuan. It is also well known in the traditional Chinese medicine for treating asthma, bronchial and lung inflammation, and kidney disease[1—3]. The morphology of cordyceps includes the upper fruiting body of fungus *C. sinensis* and lower larva of *Hepialus spp.*[2,3]. Previous researches[4—12] about *C. sinensis* and its correlative species confirmed that *Hirsutella sinensis* was the anamorph of *C. sinensis*[7—10], and about 68 species of host insects from 4 genera had been found and reported based on the knowledge of traditional morphological classification[11,12]. The host insects of *C. sinensis* are mainly from genus *Hepialus*, except for several species from genus *Hepialiscus*, *Forkalus* and *Bipectilus*. Presently, the studies on the host insects of *C. sinensis* are limited to the morphological analysis and biological characteristics[13—15], and geographical distribution of genus *Hepialus* insects in China[11]. Our understanding of the genetic relationships among the host insects of *C. sinensis* from different areas in China is rare and the limited knowledge significantly hindered effective utilization and conservation of the *C. sinensis* resources.

Mitochondrial DNA (mtDNA) has been widely used in insect molecular phylogeny because of its simple structure (no spacer and intron, no repetitive sequence), strict matrilinear inheritance, rare occurrence of reconstitution, rapid evolution as compared to nuclear DNA, and various rates of evolution among different genes. Cytochrome *b* (Cyt*b*) is in the mitochondrial membrane phospholipids bilayer and plays an importance role in the electron transport system of breath chain. Among the 13 mtDNA genes that en-

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code proteins, the configuration and function of Cyt*b* are best understood. Cyt*b* has moderate evolutionary rate, so it is very appropriate to reveal the phylogenetic relationship at the genera and species level[16—19], and has been used as the reliable marker to study molecular evolution, inheritance and phylogeny in insect[20—23]. Guryev et al.[22] inferred the phylogeny of genus *Chironomus* (Diptera) from DNA sequence of mitochondrial Cyt*b* and cytochrome oxidase I (COI). Cruz and Whiting[23] analyzed the genetic and phylogeographic structure of populations of *Pulex simulans* (Siphonaptera) in Peru based on gene sequences of Cyt*b* and COII. Chen et al.[24] estimated the phylogeny of 5 Chinese peculiar *Parnassius* butterflies using Cyt*b* sequence. Dai and Zheng[25] evaluated the phylogenetic relationships of 7 species of Pentatominae also based on the sequence of Cyt*b* gene. However, the understanding regarding the phylogenetic relationships of host insect of *C. sinensis* in China is insufficient. Chen et al.[26] firstly determined molecular evolutionary relationships of 5 species of *Hepialus* host insect of *C. sinensis* using Cyt*b* sequence. In this study, we analyzed the Cyt*b* sequence of host insects of *C. sinensis* from main distribution areas in China. Their molecular phylogenic trees were constructed to discuss phylogenetic relationships and geographical distribution pattern of host insects of *C. sinensis*. The results provided the genetic evidence to identify the producing area of cordyceps, and the basic information for further study on the relationships between *C. sinensis* and its host insects.

1 Materials and methods

1.1 Materials

Eighteen cordyceps populations were sampled in different areas that cover the distributing regions in China (Qinghai, Tibet, Yunnan and Sichuan) during May to July 2004. Meanwhile, *Hepialus yushuensis* Chu et Wang and *H. lagii* Yan were collected from Yushu County and Huangzhong County (Lagii mountain) in Qinghai Province to test whether Cyt*b* could be obtained from the genomic DNA of dead larva in cordyceps and whether the Cytb sequence obtained from larva body tissue is the same as that of the comprovincial *Hepialus spp*. The detailed locations of these samples are presented in Table 1 and Fig. 1. These specimens were identified by Mr. Xu Haifeng from Qinghai Academy of Science and Veterinary Medicine.

Table 1. Eighteen cordyceps populations used in this study

Population and code	Collection site	Altitude (m)	Longitude	Latitude
Maqin, MQ	Maqin county, Qinghai	4200	100°26′E	34°49′N
Yushu, YS	Yushu county, Qinghai	4500	96°97′E	33°03′N
Zaduo, ZD	Zaduo county, Qinghai	4300	95°03′E	32°92′N
Qilian, QL	Qilian county, Qinghai	2700	100°22′E	38°02′N
Huangzhong, HZ	Huangzhong county, Qinghai	2260	101°57′E	36°49′N
Gangcha, GC	Gangcha county, Qinghai	3200	100°17′E	37°32′N
Tianjun, TJ	Tianjun county, Qinghai	3200	99°03′E	37°28′N
Gonghe, GH	Gonghe county, Qinghai	3200	100°61′E	36°27′N
Xinghai, XH	Xinghai county, Qinghai	4300	99°99′E	35°06′N
Guinan, GN	Guinan county, Qinghai	3100	100°75′E	35°57′N
Henan, HN	Henan county, Qinghai	3600	101°62′E	34°75′N
Milin, ML	Milin county, Tibet	3700	94°08′E	29°11′N
Linzhi, LZ	Linzhi county, Tibet	3000	94°15′E	29°35′N
Dingqing, DQ	Dingqing county, Tibet	4300	95°38′E	31°25′N
Shiqu, SQ	Shiqu county, Sichuan	4200	98°06′E	33°01′N
Kangding, KD	Kangding county, Sichuan	4200	101°57′E	30°02′N
Shangrila, SG	Shangrila county, Yunnan	4500	98°72′E	27°78′N
Deqin, TQ	Deqin county, Yunnan	3559	98°56′E	28°29′N

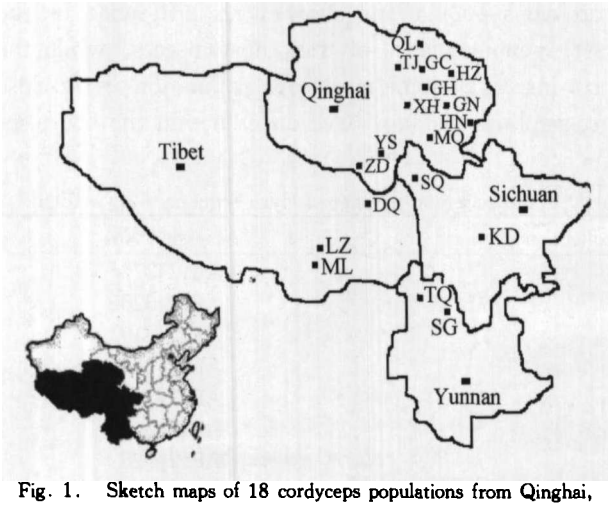

Fig. 1. Sketch maps of 18 cordyceps populations from Qinghai, Tibet, Sichuan and Yunnan, China. Population codes refer to Table 1.

1.2 DNA extraction

In the preliminary experiments, 10 cordyceps individuals of Yushu population (YS) and Gonghe population (GH) were selected to extract DNA and amplify Cyt*b* sequences of host insects, and no difference in the Cyt*b* sequence was detected within population. Thus, whole genomic DNA was extracted from 10 mg head-part of dead larva in one single cordyceps individual in each population with CTAB method[27]. The whole DNA is a mixture of *C. sinensis* genomic DNA and host insect genomic DNA. The quality of extracted DNA was detected by electrophoresis in a 0.7% agarose gel. One moth was also selected from populations of *H. yushuensis* and *H. lagii*, respectively, and their genomic DNA was extracted with the same method.

1.3 PCR amplification and DNA sequencing

The Cyt*b* gene was amplified with the above DNA by the following primers CB1: 5′-TATGTACTACCATGAGGACAAATATC-3′ and CB2: 5′-ATTACACCTC CTAATTTATTAGGAAT-3′[28]. The reaction mixture (50 μL) for PCR consisted of 10 mmol/L Tris-HCL (pH 8.0), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 0.2 μmol/L primer, 2U *Ex Taq* DNA polymerase (TaKaRa), and 50 ng template DNA. PCR was run for 40 cycles after preheating for 5 min at 94℃. Each cycle consisted of a 94℃ denaturation for 45 s, an annealing for 60 s at 46℃, and a 72℃ extension for 60 s in a Mastercycler Gradient PCR (Eppendorf, Germany). At the end of the 40 cycles, a final extension period was appended for 10 min at 72℃, then soaked at 10℃. Amplified products were detected by electrophoresis in 1.5% agarose gels, and purified and sequenced (GeneCore Biotechnologies, Shanghai, China).

1.4 Phylogenetic analysis

The nucleotide sequences were aligned using the Clustal X multiple alignments program[29] with minor adjustments by visual inspection. The sequence distances were calculated with MEGA3.1[30] using Kimura's two-parameter model with pairwise deletion to estimate their divergence. Molecular phylogenetic tree was constructed by neighbor-joining (NJ) analysis for host insects of 18 cordyceps populations and *H. yushuensis* and *H. lagii*. The homologous sequences of host insects of *C. sinensis* from GenBank were also combined with the total 20 Cyt*b* gene fragment sequences detected in this study for phylogenetic analysis. *Bombyx mori* from GenBank was used as the outgroup (Table 2). Bootstrap values for the interior nodes in the NJ tree were performed with 1000 replicates.

2 Results

2.1 Sequence variation analysis

The sequences containing 433 bp of partial Cyt*b* gene were obtained from *H. yushuensis*, *H. lagii* and the host insects of 18 cordyceps populations. In the fragment of 433 bp, no insertion or deletion of base pairs was evident. Of the 433 sites in the initial alignment there were 63 variable sites, and the divergence rate was 14.5%. It indicates that Cyt*b* sequences have abundant variations among the host insects of each cordyceps population from different producing areas. The average A + T content (75.8%) was obviously higher than that of G + C (24.2%). Cyt*b* sequences of *H. yushuensis* and *H. lagii* were the same as the Cyt*b* sequences that were amplified from the cordyceps collected from the same place (*H. yushuensis* vs YS and *H. lagii* vs HZ), respectively, and their genetic distance is 0 (Table 3). Sequence alignment also revealed that the sequence of *H. yushuensis* was 100% identical with the host insect of cordyceps populations collected from the other four sites, Maqin (MQ), Henan (HN) and Zaduo (ZD) populations of Qinghai Province and Shiqu population (SQ) of Sichuan Province. Meanwhile, *H. lagii* showed the same Cyt*b* fragment sequence as its comprovincial Huangzhongcordyceps population (HZ)

and farside Xinghai cordyceps population (XH), but one nucleotide site showed difference from its near neighbour Guinan cordyceps population (GN). These results demonstrated that the fungi-host relation can be complex in cordyceps, since some distantly isolated cordyceps populations showed no difference in the Cyt*b* gene sequence of their host insects, while the host insects of some cordyceps populations with close geographical distance have difference in the Cyt*b* sequence.

Table 2. Main host insects of *C. sinensis*, their distributing area[11,12] and accession number of Cyt*b* sequences in GenBank

Species	Distributing area or collection site	Accession No.
Hepialus yushuensis	Qinghai (Yushu), Tibet	AF124322
H. menyuanicus	Qinghai (Menyuan)	AF124323
H. oblifurcus	Qinghai, Sichuan	AF124319
H. baqingensis	Tibet (Baqing)	AF124304
H. jialangensis	Tibet (Meili Snow Mountain)	AF124318
H. zaliensis	Tibet (Zhali Snow Mountain)	AF124327
H. dongyuensis	Tibet, Yunnan	AF124328
H. damxungensi	Tibet (Dangxiong)	AF124313
H. armoricanus	Sichuan, Yunnan	AF124303
H. kangdingroides	Sichuan (Kangding)	AF124301
H. litangensis	Sichuan (Litang), Tibet	AF124302
H. pratensis	Yunnan (Baima Snow Mountain)	AF124308
H. jinshaensis	Yunnan (Western shore of Jinshajiang River)	AF124307
H. ferrugineus	Yunnan (Baima Snow Mountain)	AF124320
H. baimaensis	Yunnan (Baima Snow Mountain)	AF124314
H. albipictus	Yunnan (Renzhi Snow Mountain)	AF124310
H. renzhiensis	Yunnan (Renzhi Snow Mountain)	AF124315
H. callinivalis	Yunnan (Meili Snow Mountain)	AF124309
H. anomopterus	Yunnan (Northwest slope of Laojun Mountain)	AF124325
H. jianchuanensis	Yunnan (Stock farm, Laojun Mountain)	AF124311
H. yunnanensis	Yunnan (Northwest slope of Laojun Mountain)	AF124324
H. yulongensis	Yunnan (Yulong Snow Mountain)	AF124316
H. luquensis	Gansu (Luqu), Qinghai	AF124312
Hepialiscus sylvinus	Sichuan (Kangding)	AF124306
Bipectilus yunnanensis	Yunnan	AF124305
Bombyx mori		AF149768

Table 3. The pairwise sequence distances among the host insects of 18 cordyceps populations and 2 species of *Hepialus spp.* using Kimura's two-parameter model

Population[a] or host insect	MQ	YS	KD	SG	ZD	SQ	HN	DQ	TQ	HZ	GN	XH	ML	LZ	TJ	GC	GH	QL	*H. yushuensis*
MQ	—																		
YS	0.000	—																	
KD	0.019	0.019	—																
SG	0.009	0.009	0.019	—															
ZD	0.000	0.000	0.019	0.009	—														
SQ	0.000	0.000	0.019	0.009	0.000	—													
HN	0.000	0.000	0.019	0.009	0.000	0.000	—												
DQ	0.002	0.002	0.016	0.012	0.002	0.002	0.002	—											
TQ	0.009	0.009	0.023	0.009	0.009	0.009	0.009	0.012	—										
HZ	0.012	0.012	0.026	0.012	0.012	0.012	0.012	0.014	0.016	—									
GN	0.009	0.009	0.023	0.009	0.009	0.009	0.009	0.012	0.014	0.002	—								
XH	0.012	0.012	0.026	0.012	0.012	0.012	0.012	0.014	0.016	0.000	0.002	—							
ML	0.092	0.092	0.094	0.094	0.092	0.092	0.092	0.089	0.089	0.102	0.100	0.102	—						
LZ	0.094	0.094	0.096	0.094	0.094	0.094	0.094	0.091	0.089	0.097	0.094	0.097	0.066	—					
TJ	0.076	0.076	0.083	0.081	0.076	0.076	0.076	0.073	0.076	0.089	0.086	0.089	0.078	0.078	—				
GC	0.073	0.073	0.081	0.078	0.073	0.073	0.073	0.071	0.073	0.086	0.084	0.086	0.076	0.076	0.002	—			
GH	0.073	0.073	0.081	0.078	0.073	0.073	0.073	0.071	0.073	0.086	0.084	0.086	0.076	0.076	0.002	0.000	—		
QL	0.073	0.073	0.081	0.078	0.073	0.073	0.073	0.071	0.073	0.086	0.084	0.086	0.076	0.076	0.002	0.000	0.000	—	
H. yushuensis	0.000	0.000	0.019	0.009	0.000	0.000	0.000	0.002	0.009	0.012	0.009	0.012	0.092	0.094	0.076	0.073	0.073	0.073	—
H. lagii	0.012	0.012	0.026	0.012	0.012	0.012	0.012	0.014	0.016	0.000	0.002	0.000	0.102	0.097	0.089	0.086	0.086	0.086	0.012

a) Population codes refer to Table 1

2.2 Genetic relationships of host insects of cordyceps populations from different producing areas

The 433 bp nucleotide sequences of Cyt*b* gene of *H. yushuensis*, *H. lagii* and host insects of 18 cordyceps populations were used for the phylogenetic analysis. Their molecular phylogenetic tree generated by neighbor-joining (NJ) method using MEGA3.1 is shown in Fig. 2. In the NJ tree, the 18 cordyceps populations could be obviously divided into 3 clusters. The first cluster contained the 4 populations of Gonghe (GH), Gangcha (GC), Tianjun (TJ) and Qilian (QL) distributed around the Qinghai Lake. The genetic relationships of their host insects were very close, with the genetic distances ranging from 0 to 0.002 (Table 3). The Linzhi (LZ) and Milin (ML) populations from Tibet were included in the second cluster, and the farthest genetic distance between their host insects was 0.066. Other 12 populations from mid-south of Qinghai Province, Shangri-la population (SG) of Yunnan Province and Kangding population (KD) of Sichuan Province were encompassed in the third cluster. The genetic distance ranged from 0 to 0.026 in this cluster, and the genetic relationships of host insects from some populations were very close. The bootstrap values with 1000 replicates were 100%, 95% and 100% for the 3 interior nodes, respectively.

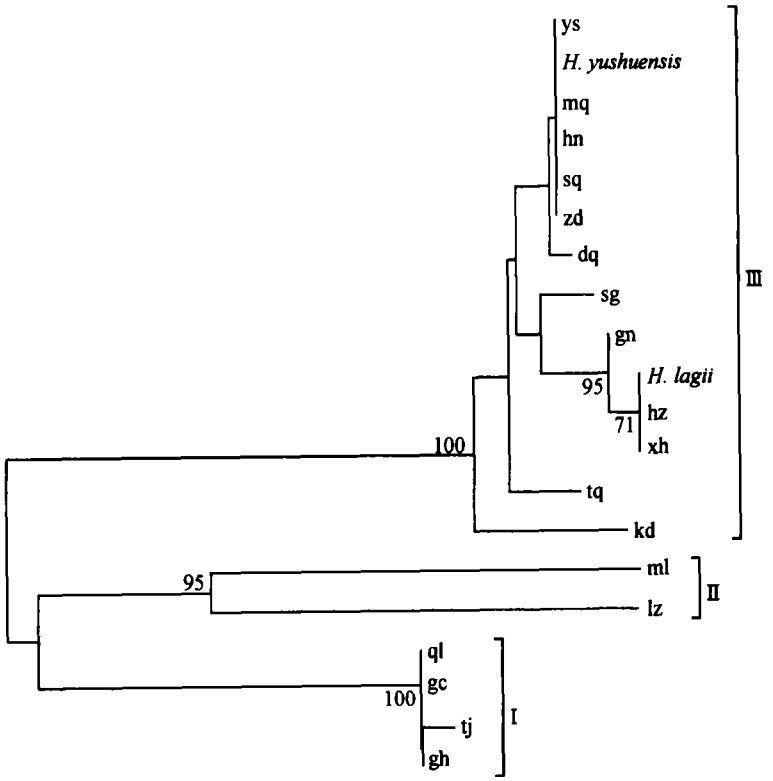

Fig. 2. The NJ phylogenic tree of host insects of 18 cordyceps populations and 2 species of *Hepialus spp*. based on Cyt *b* gene sequence. Numbers at nodes represent bootstrap values (%) with 1000 replicates. Population codes refer to Table 1.

2.3 Phylogenetic analysis of host inset of *C. sinensis*

The homologous sequences of 22 species of genus *Hepialus*, *Hepialiscus sylvinus*, *Bipectilus yunnanensis* and *Bombyx mori* from GenBank were combined with the total 20 Cyt*b* gene fragment sequences of 433 bp detected in this study for constructing the NJ phylogenic tree (Fig. 3). In the NJ tree, the insects of genus *Hepialus* as major host of *C. sinensis* were completely separated from the minor host of genus of *Hepialiscus* and *Bipectilus* with the bootstrap value of almost 100%. Among the 3 genera, *Hepialiscus* showed a close relationship to *Hepialus*, and *Bipectilus* was relatively distant to *Hepialus* and *Hepialiscus*. Within the genus *Hepialus*, all host insects of *C. sinensis* and those of the 18 cordyceps populations could also be divided into 3 distinct clades with high bootstrap values in the NJ tree (Fig. 3). Among the 18 cordyceps populations, the host insects of 4 cordyceps populations distributed around the Qinghai Lake and 4 species of *Hepialus spp*. formed the first clade; the LZ and ML populations from Tibet were independently clustered in the second clade;

and the other 12 cordyceps populations were encompassed in the third clade with 20 species of *Hepialus spp*. The phylogenetic analysis, combined with the geographic location of cordyceps populations and *Hepialus spp*. habitats, indicates the host insects of *C. sinensis* distribution is obviously regional-specific. Major cordyceps populations and *Hepialus spp*. with close sampling sites or habitats usually showed a close genetic relationship. For example, all *Hepialus spp*. clustered together in the third clade can be subdivided into 4 regional groups of south Qinghai-north Tibet, central Qinghai, Yunnan-east Tibet, and Sichuan. However, a few geographically separated cordyceps populations and *Hepialus spp*. also showed close genetic relationships. For example, in the first clade, the host insects of cordyceps populations distributed around the Qinghai Lake showed a close relationship with *H. yulongensis* and *H. jianchuanensis* which are only distributed in Yunnan Province.

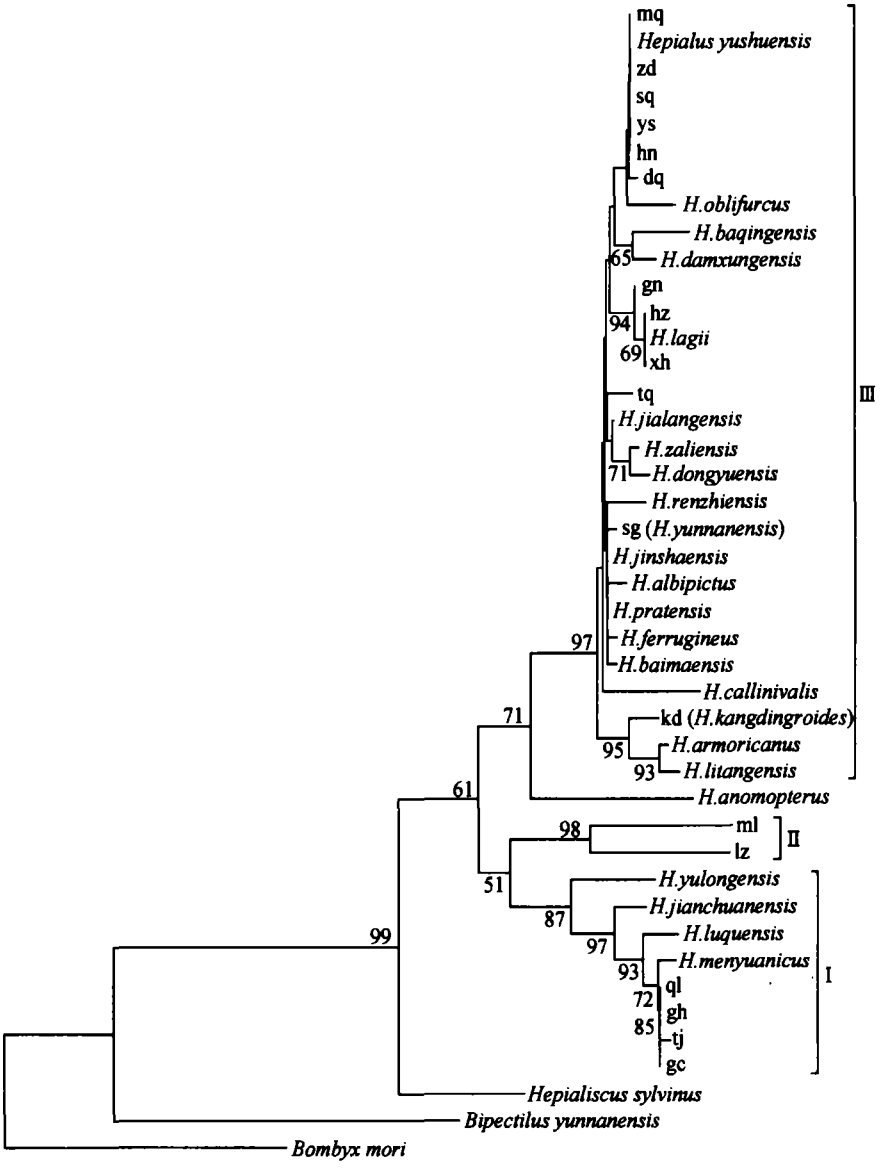

Fig. 3. The NJ phylogenic tree of host insects of 18 cordyceps populations and 26 species of host Hepialidae insects based on Cyt *b* gene sequence. Numbers at nodes represent bootstrap values (%) with 1000 replicates. Population codes refer to Table 1.

3 Discussion

In this study, the genomic DNA with good quality could be extracted from both *Hepialus spp*. and the head part of dead larva in cordyceps. The specific Cyt*b* fragments of host insects could also be successfully amplified with the genomic DNA. The identical Cyt*b* sequence was detected from the cordyceps and the host *Hepialus spp*. which were both collected from a same place. Moreover, the Cyt*b* sequence am-

plified from KD and SG cordyceps populations was the same as that of *H. kangdingroides* and *H. yunnanensis* from GenBank, respectively (Fig.3). These results indicate that it is an effective and accurate approach to obtain the Cyt*b* sequence of host insect by amplifying the extracted DNA from the head part of dead larva in cordyceps. The mixed DNA of *C. sinensis* and its host insect, which was extracted from dead larva section of cordyceps, can be used for specific amplification of multifarious gene sequences of host insect to obtain gene sequences for genetic diversity analysis and species identification of host insects of cordyceps populations from different producing areas. The approach makes it possible to evaluate the genetic diversity of *C. sinensis* and its host insects simultaneously at the population level, and to illustrate the relationships between *C. sinensis* and its host insects.

The 433 bp Cyt*b* gene sequence of host insects of *C. sinensis* was obtained in this study with the universal primer pair of CB1 and CB2[28]. The sequence length is the same as that of *Parnassius* butterflies[24] and Pentatominae insects[25]. The distinct genetic variations in the 433 bp sequence of Cyt*b* gene were detected among the host insects of 18 cordyceps populations. The divergence rate (14.5%) of the Cyt*b* sequence is a little higher than that of 10.7% among 5 species of *Hepialus spp.*[26]. The difference of the two values might be related to the geographic distributing extent of sampling, and they are almost at the same level. Commonly, the content of A + T in Cyt*b* gene of insect is higher than that of G + C. The Cyt*b* sequence of host insects of *C. sinensis* also shows the characteristic of rich A + T, the same as other insects in Lepidoptera, Hymenoptera and Hemiptera[24,25,31,32].

According to the traditional morphological classification, approximately 68 species from 4 genera, mainly from genus *Hepialus* had been found and reported as the host insects of *C. sinensis*[11,12]. Cyt*b* with the moderate evolutionary rate has been used as an effective molecular marker for analyzing the generic and interspecific relationships[16–19]. The variation of Cyt*b* sequences ranging from 0.9% to 19.4% has been detected among 11 species of Pentatominae[25]. Our research shows that Cyt*b* sequences of host insects have no variation within cordyceps population (data not shown), and supposes no variation in this sequence within the species of its host insect. A variation ranging from 0.23% to 9.24% was detected among the 22 species out of 24 species of *Hepialus spp.* with known mitochondrial Cyt*b* sequence, except 2 species of *Hepialus pratensis* and *Hepialus jinshaensis* which have the same sequence. Various species of *Hepialus spp.* with each own Cyt*b* sequence indicate evident interspecific genetic diversity. It is concluded from the present study that the host insects of *C. sinensis* should belong to different species if their Cyt*b* sequence is different, and the host insects of *C. sinensis* with the same Cyt*b* sequence might be considered as the same species but do not exclude the possibility that they are different species. Hereby, Cyt*b* sequence should be used for species identification of host insects of *C. sinensis*, but further confirmation is needed in more species of host insects.

The host insects of *C. sinensis* are usually studied using morphological and biological characteristics[11–15], but their phylogenic relationships are not clear. The phylogenetic relationship of host insects of *C. sinensis* based on the Cyt*b* sequence suggests that genus *Bipectilus* diverged earlier than genus *Hepialus* and *Hepialiscus*. Most species of genus *Hepialus*, from the majority of producing areas in China except the area around Qinghai Lake, are considered as a monophyletic union from a common ancestor with bootstrap value of 97%. Genus *Hepialus* also contains other clades with relatively distant genetic relationships, conjecturing that it might be polyphyletic origin. All host insects of *C. sinensis* are distributed with distinct regional characteristic. The distributing region of most *Hepialus spp.* is narrow, but several species like *Hepialus yushuensis* are distributed widely. Generally, close genetic relationship is revealed between the *Hepialus spp.* with near distributing areas. However the host insects of two cordyceps populations (LZ and ML) with near geographical location from Tibet show extremely distant genetic relationship and form an independent clade with bootstrap value of 98% from other host insects, indicating it might be related to the special environment of Linzhi and Milin areas. Because of the special landform, forest vegetation, microclimate etc. of the area locating in Namjagbarwa mountain, the insect species distributed in this area are very different from those of other places in China and other countries, and the similarity coefficients among the Lepidoptera insects even from the four counties of this area are al-

so very low[33]. It should be further studied why the relationships are so close between the host insects of the four cordyceps populations around the Qinghai Lake, and *H. yulongensis* and *H. jianchuanensis* from Yunnan Province. Yang et al.[11] reported that each kind of *Hepialus spp.* is distributed with a special pattern and geographical location, and different species are usually known among different mountain ranges, even on different sides and at different altitudes of the same mountain. Our study based on the Cyt*b* sequence analysis of host insects of *C. sinensis* populations and some *Hepialus spp.* supports that various morphologic characters and special geographical distributing patterns of *Hepialus spp.* are attributed to the active interspecific divergence of genus *Hepialus*, which is determined by the complex geographical and environmental factors in the Qinghai-Tibet plateau. However, the genetic relationships among most species of *Hepialus spp.* are quite close. Results of this study also show that the highest species abundance of *Hepialus spp.* and extremely large interspecific divergence are found in Yunnan area, which can be considered as the diversity center of *Hepialus spp.*, especially some *Hepialus spp.* from distant, isolated areas also show close genetic relationships to the species of Yunnan. This study analyzed the Cyt*b* gene sequence of host insects from several cordyceps populations. More populations and gene sequences will be further used to analyze phylogeny and genetic structure of *Hepialus spp.* and the relationship between *C. sinensis* and its host insects.

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