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Identification of forensically important beetles (Coleoptera: Histeridae) in China based on 16S rRNA and Cyt b

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Abstract. Exact identification of an insect sample is usually the first essential step in a forensic entomological analysis. However, the morphological similarity of beetles in the level of species usually poses a challenge for forensic scientists within their routine work. As a supplementary to traditional morphological method, molecular genetics identification turns out to be simple and time-saving. A molecular identification method involving a 288-bp segment of the 16S ribosomal RNA (16S rRNA) gene and a 334-bp segment of the cytochrome b (Cyt b) gene from 23 histerid beetles specimens, collected from 7 locations in 6 Chinese provinces, was evaluated. The 16S rRNA and Cyt b genes are sequenced to examine the ability of the region, resolve species identities and enrich the local databases. The monophyletic branches of the phylogenetic tree showed the potential of the markers in identifying beetles within families. Combined analysis is a more accurate approach for species identication than independent analysis.

INTRODUCTION

Necrophilous beetles, which cannot be replaced by flies, play a pivotal role not only in estimating minimum postmortem interval time (m-PMI) of dry skeletal remains in the later stages of decomposition, but also to determine the destruction, and posture changes of carcasses. Forensic entomology is the study of insects (or even other arthropods such as mites and ticks) that forms part of the evidence in legal cases (Hall, 1990) . It is related with the fields of medical entomology, taxonomy, and forensic pathology (Catts & Haskell, 1997), and is used mainly to estimate the time of death or minimum postmortem interval (m-PMI) based on the developmental rates and the successional ecology of specific insects that feed on carcasses (Wolff *et al.*, 2001).

Hundreds of arthropod species are attracted by corpses, primarily flies (Diptera), beetles (Coleoptera), and their larvae, and also mites, isopods, opiliones and nematodes (Benecke, 2001). Insects found on the body provide an important source of information (Amendt *et al.*, 2004). Numerous ecological studies have investigated the abundance, species diversity, succession, and ecological interactions of insects on decaying cadavers (Schilthuizen *et al.*, 2011). Most of the developmental studies of carrion-related insects have been focused on maggots (fly larvae), but beetles' larvae development have been largely neglected (Midgley & Villet, 2009; Guo *et al.*, 2012), despite the fact that they are taxonomically and ecologically more diverse than Diptera (Schilthuizen *et al.*, 2011). The reasons for the relative rarity of Coleoptera compared to

many other necrobiont insect communities in forensic entomological practice is probably due to their taxonomic inaccessibility (Krikken & Huijbregts, 2001). Thus, potential Coleoptera identification that could yield forensic information complementary to that obtained from Diptera are uttermost important in criminal investigations (Schilthuizen *et al.*, 2011). Forensically important beetles include Staphylinidae, Scarabaeidae, Carabidae, Histeridae, Silphidae, and Dermestidae (Goff & Catts, 1990).

Beetles develop more slowly than flies, and, therefore, offer an opportunity to estimate m-PMI from developmental data after maggots have left the bodies (Kulshrestha & Satpathy, 2001). Among Coleoptera, Silphidae were the first attracted group, followed by Staphylinidae and Histeridae (Peng *et al.*, 2009; Tang *et al.*, 2012;). All three families were reported as predators that feed on fly larvae and frequently found when fly larvae were abundant (Reed, 1958; Wolff *et al.*, 2001; Kocárek, 2003). Estimation of m-PMI based on beetle development are reputedly less precise (days or weeks) than those based on maggot development (hours or days) (Catts, 1992), but in the condition where maggots are no longer present on a carcass, the beetle larvae could be the only available source for the determination of time of colonization (Midgley & Villet, 2009). However, the rate of decomposition is also affected by a wide range of variables associated with the corpse itself and the surrounding abiotic environment (Amendt *et al.*, 2004), such as geographical region, habitat, season, and climatological and microclimatic conditions, (Anderson, 2001). Coleoptera have been recognized to provide significant entomological evidence in the forensic field, particularly its association with dry human skeletal remains in the later stages of decomposition (Kulshrestha & Satpathy, 2001). In cases where mummification occurs, beetles frequently contribute to the decay of a human body, even inside human houses (Schroeder *et al.*, 2002). Histeridae beetles have been proved useful during forensic investigations, especially in the estimation of time of death (Ortloff *et al.*, 2012). It is reported that Histeridae, after Staphylinidae, is the second family having the most number of species on carcasses, and they are found mainly during active and advanced decomposition stages (Ozdemir & Sert, 2009).

Insect species need to be accurately identified in order to obtain the precise developmental information for use in forensic investigations (Ames *et al.*, 2006). Species-diagnostic anatomical characters are not known for many immature stages of forensically important insects, and the existing key may be incomplete or difficult for non-specialists to appreciate (Wells & Stevens, 2008). The advent of DNA-based identification techniques for use in forensic entomology began in 1994 (Sperling *et al.*, 1994). The use of DNA techniques could provide a suitable alternative approach but also give information on population substructures (Hillis & Moritz, 1990). This identification can be carried out on any life stages without further rearing and can be applied on dead, preserved or live samples (Ames *et al.*, 2006). DNA techniques are also relatively insensitive to the preservation methods used for specimens or age of samples (Paabo *et al.*, 1988; Caterino & Tishechkin, 2006).

Mitochondrial DNA (mtDNA) exhibits several properties that make it a valuable tool in the study of population genetics, phylogenetics, molecular evolution, and even conservation genetics, due to its relatively simple genetic structure, maternal mode of inheritance (in most cases), and high rate of evolution (Kim *et al.*, 2009). Validated molecular markers are needed for the determination of population structure and gene flow occurrence among subpopulations (Dotson & Beard, 2001). In previous studies in China, focus were mainly on genes such as CO I and CO II (Guo *et al.*, 2010); 16S rRNA and Cyt b were usually examined less, especially Cyt b of beetles. Meanwhile, the risks of relying on a single locus are well illustrated by several studies (Stevens *et al.*, 2002; Wells *et al.*, 2007; Wells & Stevens, 2008). A few authors have included more than one locus in a single analysis (Guo *et al.*, 2012). 16S rRNA is a molecular marker

existing in mitochondria and has a high conserved region. It is different from COI that 16S rRNA was widely used to analyses the relationship of edge species and the higher categories of different insects (Long & Pang, 2003). The Cyt b locus has been used extensively in taxonomic and forensic studies(Linacre & Tobe, 2011), and sequence analysis of the Cyt b gene proved to be a very sensitive and powerful technique for forensic species identification (Parson *et al.*, 2000). In this study, independent and combined analysis of the partial sequences of the 16S rRNA gene and the Cyt b gene of mtDNA are used to investigate the phylogenetic relationships of beetles in China.

The present study emphasizes Histeridae beetles in forensic entomology particularly with reference to remains recovered in the later stages of decomposition. The main purposes of this study were: (1) to evaluate the potential value of 16S rRNA gene and Cyt b gene for basic biological studies of necrophilous beetles, (2) to evaluate the function of combined analysis of necrophilous beetles, (3) to provide a molecular basis for the species delimitation in the species, (4) to be instrumental for implementation of the Chinese necrophilous beetles.

MATERIALS AND METHODS

Specimens

We sequenced the nucleotide sequences of the 16S rRNA and Cyt b regions of 20 specimens, comprising 10 of *Merohiser jekeli* (Marseul, 1857), five of *Saprinus tenuistrius* (Marseul, 1855), three of *Saprinus subvirescens* (Menetries, 1832). Another two more beetles, namely *Saprinus*, cannot be identified to species level by taxonomist, due to the size of the beetles and morphological imperfection. All samples were caught by using animal carcass-baited traps. Nine-month-old pigs (35-50 kilograms) were placed on grassland or under trees. Beetles were caught by forceps and put into gas chamber (ethyl acetate). They were stored at room temperature using specimen box (25ºC) with dry air at an humidity level of 50 percent. All samples were identified by traditional morphological characters (Cai, 2011). Two *Dermestes maculates* (De Geer, 1774) were introduced as outgroup to ascertain their relationship to Histeridae lineages. Specimens used in this study are listed in Table 1.

DNA extraction

In order to avoid possible contamination of DNA from ingested protein and gut parasites of maggots, the thoracic muscle of each beetle was isolated for DNA extraction by a CTAB protocol that was used by Skevington & Yeates (2000). DNA was resuspended in 50ul of 1×TE buffer [1×TE buffer, pH 8.0; 10mM Tris–HCl, 1mM EDTA, pH 8.0] and stored at 4°C.

PCR conditions

A portion of 288-bp fragment of the mitochondrial 16S rRNA gene was amplified and sequenced by using forward primer (5'-CGCTGTTATCCCTAAGGTAA-3') and reverse primer (5'-CTGGTATGAAAGGTTT GACG-3'), detail of the primers were described in (Li *et al.*, 2010). For cytochrome b (Cyt b) gene, a region of 334-bp of the Cyt b gene was amplified and sequenced by using forward primers (5'-CCATCCAACATCTC AGCATGATGAAA-3') and reverse primers (5'-CCCCTCAGAATG ATATTTGTCCTCA-3'). The PCR reaction volume for each locus was 25µl, containing 1-5µl (20-40ng) of template DNA, 6µl dNTP (1mmol/ml), 12.5µl 2×GoTaq® Green Master Mix (applied by Promerga, Madison, WI, USA, containing 4µl dNTP (1mmol/ml), 1.0u Taq polymerase, 2.5µl 10×buffer (Mg2+1.5mmol/l)), 0.25-2.5µl each primer (10µM), Nuclease-Free Water added to a total volume of 25 µl. PCR amplifications were performed in a thermocycler (Perkin-Elmer9600), with initiative denaturing for 3 minutes at 94º, followed by 38 cycles of 94º for 30 seconds, 50.8º for 30 seconds and 72º for 30 seconds.

Sequencing

PCR products were purified with QiaQuick PCR Purification Kit (Qiagen, Germany). Columns cycle sequencing was performed on both forward and reverse strands using

Table 1. Locality and reference data of specimens newly sequenced for this study

No.	Species	Collection locality	Accession No.		Date
			16S rRNA	Cyt b	
1	*Merohiser jekeli* (Marseul, 1857)	Qiqihar, Heilongjiang [123°33E, 47°522 N]	JF794567	JF794621	2010
2		Chifeng, Inner Mongolia [118.88E, 42.28N]	JF794568	JF794622	2010
3		Qiqihar, Heilongjiang [123°33E, 47°522N]	JF794569	JF794623	2010
4		Suihua, Heilongjiang [124°132E, 45°32N]	JF794570	JF794624	2010
5		Suihua, Heilongjiang [124°132 E, 45°32N]	JF794571	JF794625	2010
6		Datong, Shanxi [113.29E, 40.08N]	JF794572	JF794626	2010
7		Chifeng, Inner Mongolia [118.88E, 42.28N]	JF794575	JF794629	2010
8		Chifeng, Inner Mongolia [118.88E, 42.28N]	JF794576	JF794630	2010
9		Xiangyin, Hunan [112.88E,28.71N]	JF794573	JF794627	2010
10		Datong, Shanxi [113.29E, 40.08N]	JF794574	JF794628	2010
11	*Saprinus tenuistrius* (Marseul, 1855)	Fuzhou, Fujian [118°082E,25°152N]	JF794577	JF794631	2010
12		Fuzhou, Fujian [118°082E,25°152N]	JF794578	JF794632	2010
13		Chifeng, Inner Mongolia [118.88E, 42.28N]	JF794579	JF794633	2010
14		Qiqihar, Heilongjiang [123°33E, 47°522N]	JF794580	JF794640	2010
15		Qiqihar, Heilongjiang [123°33E, 47°522N]	JF794581	JF794641	2010
16	*Saprinus subvirescens* (Menetries, 1832)	Fuzhou, Fujian [118°082E,25°152N]	JF794582	JF794634	2010
17		Fuzhou, Fujian [118°082E,25°152N]	JF794583	JF794635	2010
18		Fuzhou, Fujian [118°082 E,25°152N]	JF794584	JF794636	2010
19	*Saprinus*	Qiqihar, Heilongjiang [123°33E, 47°522N]	JF794586	JF794638	2010
20		Chifeng, Inner Mongolia [118.88E, 42.28N]	JF794587	JF794639	2010
21	*Dermestes maculates* (De Geer, 1774)	Datong, Shanxi [113.29E, 40.08N]	JF794607	JF794643	2010
22		Tianjin [117.21E,39.16N]	JF794608	JF794642	2010

ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit by ABI PRISM 3730 (Applied Biosystems, Foster City, USA). And the sequencing agent was BigDye terminator v3.1. Sequence chromatograms were edited and discrepancies between forward and reverse sequences resolved using Sequence Navigator (v1.01, Applied Biosystems, Foster City, USA).

The resulting sequences were compared with the Coleoptera sequences in the NCBI web site by Blast function to identify species. The sequences have been deposited in GenBank by Sequin (http://www.ncbi,nlm.nih.Gov/equin/index.html) and the accession numbers are listed in Table 1.

Sequence analysis

Sequences were visualized using Chromas v1.43 (http://trishul.sci.gu.edu.au/~conor/Chrom-as.html), and alignments and editing conducted using ClustalW (http://www.ddbj.nig.ac.Jp/E-mail/clastal-e.html). There were 288 positions for 16S rRNA in the final dataset and a total of 334 positions for Cyt b. Phylogenetic analyses were conducted in MEGA 4.0 (Tamura *et al.*, 2007). The evolutionary history was inferred using the UPGMA method (Sneath & Sokal, 1973). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

RESULTS

A total of 22 individuals from four taxa were sequenced and 1 outgroup taxa (*D. maculates*) were aligned by 288-bp 16S rRNA gene and 334-bp Cyt b gene. In order to gain estimates of phylogeny, analyses of the data sets individually and in combination (i.e., Cyt b, 16S, Cyt b+16S) were carried out (Nixon & Carpenter, 1996).

Cytochrome b gene partial sequence analysis

A 334-bp region of the Cyt b gene contained 140 variable characters including 129 that were parsimony-informative. No insertions or deletions were located over this region. Our data showed a predictably high AT richness (65.5%) compared to C (17.5%) and G (17.0%) nucleotide frequencies in the variable informative characters. It is also reported that the AT richness could accelerate amino acid variations (Hugall *et al.*, 1997). There are 273 identical pairs, 29 transitional pairs (ts) and 31 transversional pairs (tv), while R (ts/tv) is 0.9. The frequency of base substitute for different sites is showed in Table 2.

The UPGMA tree displays the relationships between species by using the distance method of analysis (Fig. 2). Three distinct clusters were formed based on the sequence data. The percentage of replicate

Table 2. The frequency of base substitute for different sites of Cyt b and 16S rRNA sequence

	ii	ts	tv	R	TT	TC	TA	TG	CC	CA	CG	AA	AG	GG	Total
Cyt b	273	29	31	0.9	100	20	18	1	42	10	3	81	9	50	334.0
16S rRNA	206	29	53	0.5	85	10	29	13	16	8	3	68	19	37	288.0

identical pairs (ii), transitional pairs (ts), transversional pairs (tv), while R = (ts/tv)/

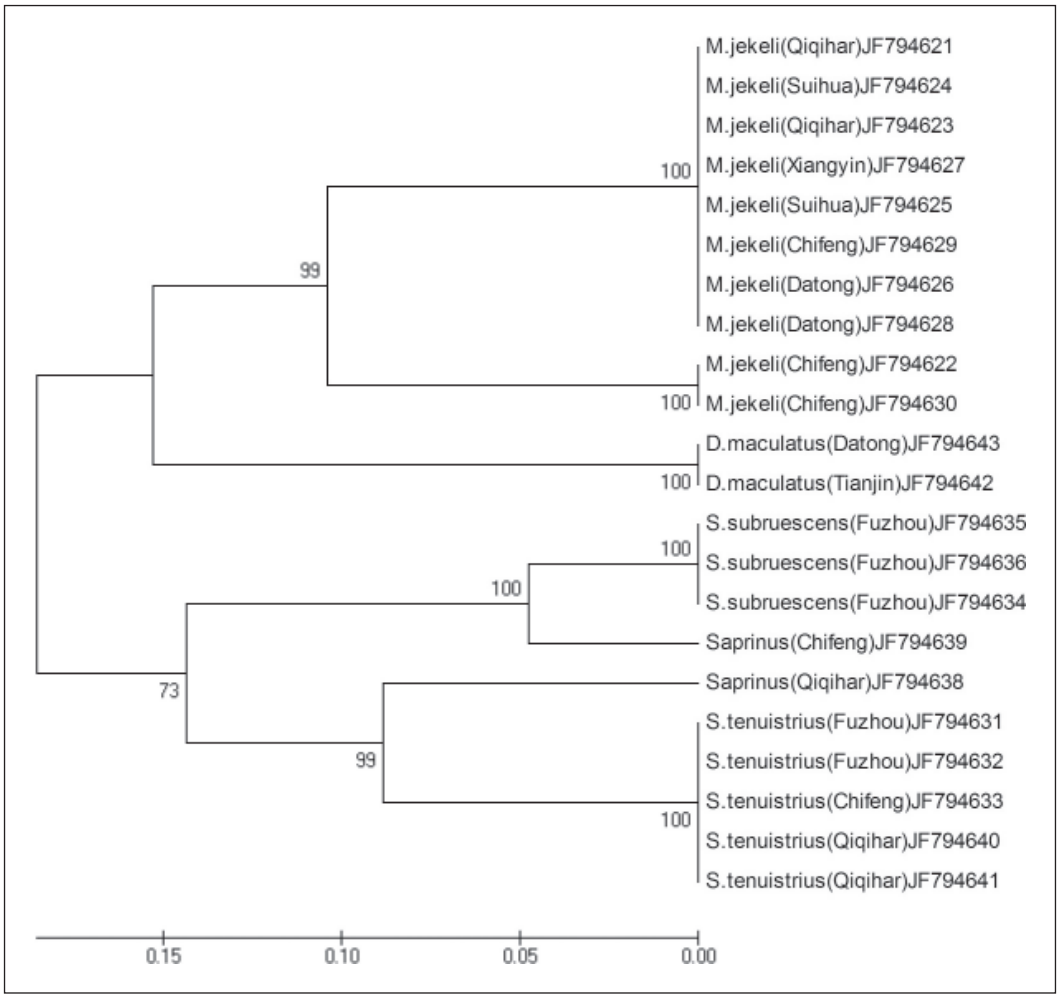

Figure 1. Evolutionary relationships of necrophilous Histeridae beetles based on Cyt b UPGMA tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The bar indicates 0.5 substitutions per site

trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches, which provides an indication of the percentage support for a grouping by randomly resampling the data. At the level of species, every species was well-resolved as monophyletic groups with the bootstrap value 99%. Two *Saprinus* specimens were clustered with the species of *S. tenuistrius* and *S. subruescens* respectively sharing a bootstrap value of 99% and 100%. Unexpectedly, the *D. maculates* outgroup was not clearly separated from the Histeridae in the UPGMA tree.

16S rRNA gene partial sequence analysis

The length of the studied 16S rRNA fragment was 288-bp, and it also had a high AT content (71.6%), compared to C (9.4%) and G (19.0%) on average. All 173 variable positions were informative sites. There is 206 identical pairs, 29 transitional pairs (ts) and 53 transversional pairs (tv), while R (ts/tv) is 0.5. The frequency of base substitute for different sites is showed in Table 2.

The UPGMA tree displays the relationships between species by using the distance method of analysis (Fig. 3). Three distinct clusters were formed based on the sequence data. The percentage of replicate

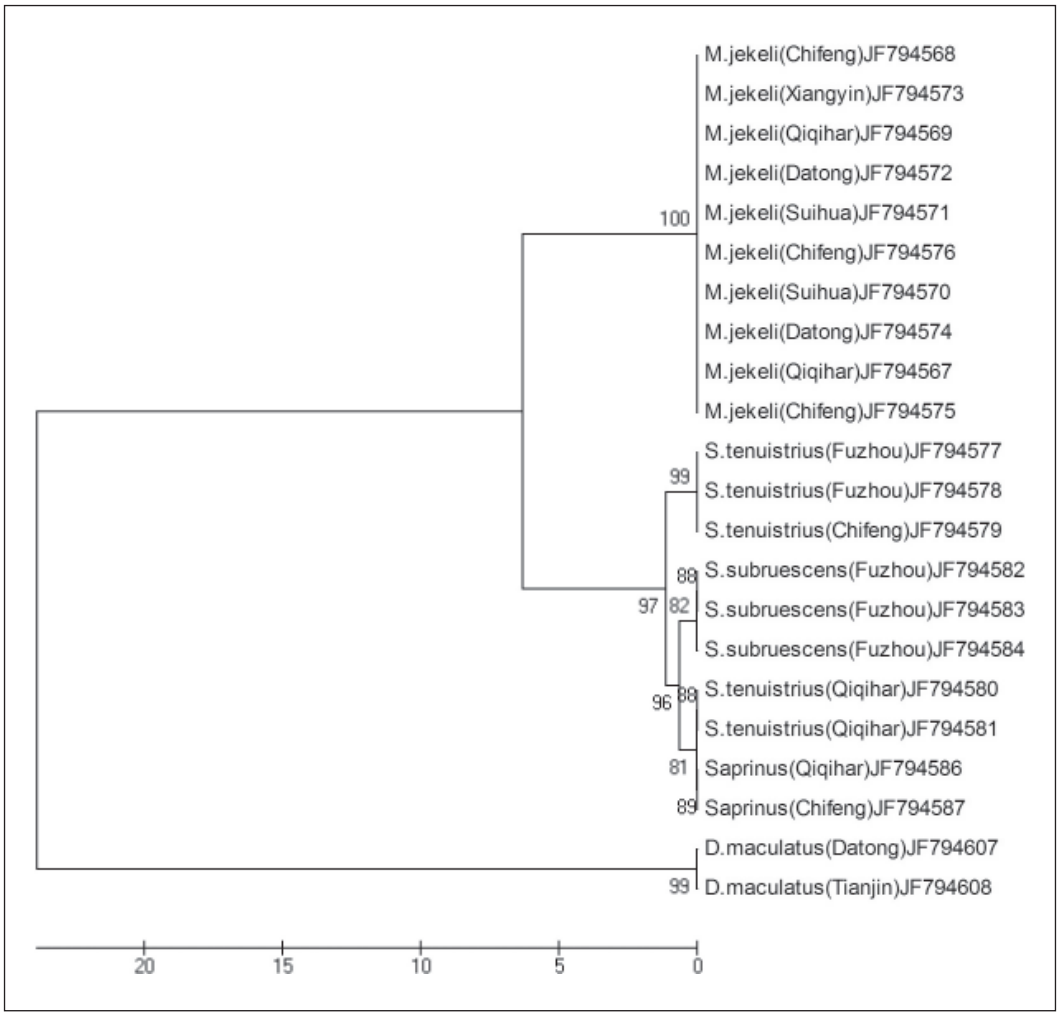

Figure 2. Evolutionary relationships of necrophilous Histeridae beetlesbased on 16S rRNA UPGMA tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The bar indicates 0.5 substitutions per site

trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches, which provides an indication of the percentage support for a grouping by randomly resampling the data. At the genus level, *Merohiser* was well-separated from *Saprinus* with high bootstrap values of each genus. At the species level, each species was resolved as monophyletic groups except *S. tenuistrius* (formed two branches), *M. jekeli* (100%), *S. subruescens*(82%). Two *Saprinus* specimens and two of *S. tenuistrius* clustered with a bootstrap value of 81%, and both of them were probably related to the lower branch of *S. tenuistrius* supported by 96% bootstrap value.

Molecular combined analysis

The UPGMA tree constructed by Cyt b and 16S rRNA partial sequences (Fig. 4) showed clear combination information of the two loci. Specimens of each species clustered together and are present in different clades obtained. Two *Saprinus* specimens presented the same distribution as in the Cyt b tree, clustering with *S. tenuistrius* and *S. subruescens* respectively sharing a bootstrap value of 83% and 82%.

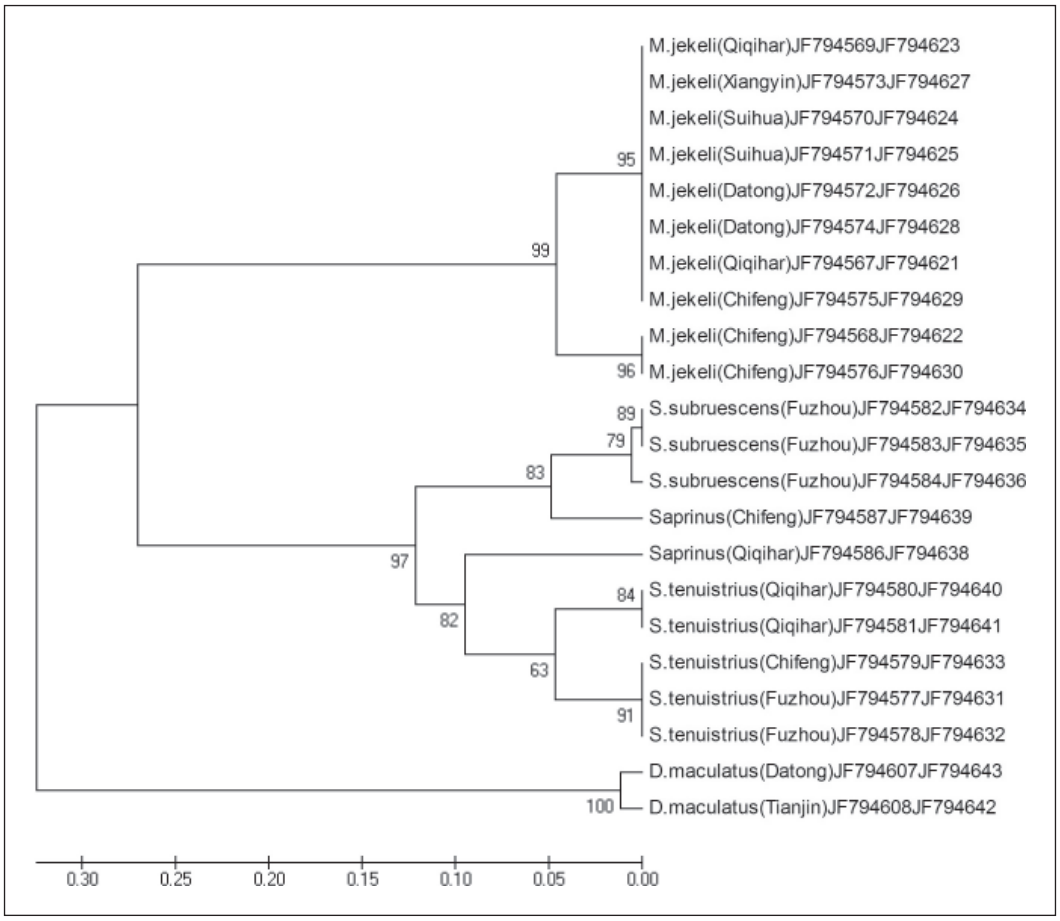

Figure 3. Evolutionary relationships of necrophilous Histeridae beetles based on Cyt b and 16S rRNA combined analysis by UPGMA tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The bar indicates 0.5 substitutions per site

Divergence analysis

The overall mean distance of all specimens was 0.228 (Cyt b), 4.327 (16S rRNA) and 4.065 (combined). Genetic distances between species were large (Cyt b= 0.106-0.387, 16S rRNA= 0.00-8.053, combined= 0.213-7.499), whereas within species they were small. Table 3 and Table 4 showed the details of the inter-specific divergence of each species. Species gain the lowest inter-specific divergence indicating that the two species almost had no major change in the inter-specific variation. A subset of these insects was analyzed to infer intra-specific genetic variation (Table 5). Since intra-specific variation will mostly be smaller than inter-specific variation, unambiguous identification at the species level may be possible (Amendt *et al.*, 2004).

DISCUSSIONS

Four different species of necrophilous beetles (Histeridae) were successfully sequenced and ultimately diagnosed and delimited based on the phylogenetic tree, in accordance with their morphological characters. The two unknown species from *Saprinus* were discriminated by different phylogenetic tree. A high bootstrap value of all specimens indicated that the DNA-based beetle

Table 3. Estimates of average evolutionary divergence over sequence pairs between groups based on Cyt b sequences

	M. jekeli	*S. tenuistrius*	*S. subruescens*	*Saprinus* (Qiqihar)	*Saprinus* (Chifeng)
M. jekeli		0.303	0.377	0.255	0.387
S. tenuistrius	8.053		0.266	0.165	0.281
S. subruescens	7.205	1.421		0.289	0.106
Saprinus (Qiqihar)	7.567	1.380	0.844		0.289
Saprinus (Chifeng)	7.567	1.380	0.844	0.00	

Table 4. Estimates of average evolutionary divergence over sequence pairs between groups of combined analysis based on 16S rRNA

	M. jekeli	*S. tenuistrius*	*S. subruescens*	*Saprinus* (Qiqihar)	*Saprinus* (Chifeng)
M. jekeli					
S. tenuistrius	6.966				
S. subruescens	7.138	2.639			
Saprinus (Qiqihar)	6.332	1.478	2.459		
Saprinus (Chifeng)	7.499	2.468	0.213	1.542	

Table 5. Estimates of average evolutioary divergence over sequence pairs within groups

	Mean			Maximum			Minimum		
	16s	Cyt b	combined	16s	Cyt b	combined	16s	Cyt b	combined
M. jekeli	0.001	0.060	0.044	0.003	1.69	0.126	0.000	0.000	0.000
S. tenuistrius	1.171	0.000	0.077	1.952	0.000	0.128	0.000	0.000	0.000
S. subruescens	0.018	0.000	0.008	0.027	0.000	0.012	0.000	0.000	0.000
Saprinus (Qiqihar)	\	\	\	\	\	\	\	\	\
Saprinus (Chifeng)	\	\	\	\	\	\	\	\	\

discrimination of Cyt b and 16S rRNA genes were valid even with short markers, while the morphologic method needs expertise in specialized taxonomy in order to provide correct identification (Leclercq & Lecomte, 1978). Also, mtDNA technology is easier to perform and can save more time. The well-combined information of the two fragments of mtDNA suggests that combined analysis is a useful tool at the level of population genetic structure. Pairwise distances can be used as a confirmation of validity to support both the morphological and the molecular-based analyses conducted.

In our study, *Saprinus* discrimination resulted differently in two phylogenetic trees of Cyt b and 16S rRNA fragments. The combined analysis, including information from 16S rRNA fragment, supported the result of Cyt b. Combined analysis is a more accurate approach than independent analysis for species identication. Based on the phylogenetic tree, *Saprinus* (Chifeng) could be identified as *S. subruescens* and *Saprinus* (Qiqihar) could be *S. tenuistrius*. The observation was also noticed by other authors that the combination of data sets with varying rates of evolution (e.g. mitochondrial and

nuclear genes) yielded results that best accounted for the variation present in results from individual analyses (Flook *et al.*, 1999; Cryan *et al.*, 2001). Meanwhile, it is reported that the inclusion of additional loci in a multi-locus framework helps to minimize the risk of inaccurate species identification, and to increase the proportion of successful species identifications relative to single-locus data sets. (Roe *et al.*, 2010).

Short markers discrimination may be of great convenience and efficiency, but its performance stability is questionable. In this research, the *D. maculates* outgroup was not clearly separated from the Histeridae in the Cyt b UPGMA tree, albeit the two samples clustered together. It might be explained that the discriminating ability of short Cyt b gene at the family and genus level is relatively poor. Thus, accuracy factor needs to be taken into account when using shorter markers.

Monophyly of each species is supported by UPGMA tree. However, two different branches were formed sharing low supporting values for *S. tenuistrius* in combined analysis. The genetic distance of group 1 from group 2, depending on strong discrimination ability of 16S rRNA, suggests that the two groups could represent two different sub-species. In different regions of mtDNA, there were differences in evolution rates, and in the abilities of decoding such mutations. Fernández *et al.* (2005) analyzed the intra-specific genetic variation among *Triatoma dimidiate* (Latreille, 1811) from seven Costa Rican populations and compared the variability of 16S rRNA and Cyt b genes. It was reported that the variability of 16S rRNA was 2.39% and that of Cyt b was 10.17%, which revealed high evolutionary rates of Cyt b genes. In our study, there were 173 variable sites in 288-bp 16S rRNA fragments and 140 variable sites in 334-bp Cyt b fragments. As a result, evolutionary rate of 16S rRNA is relatively high, while that of Cyt b gene is conservative. Therefore, evolutionary rates of different mtDNA genes vary in different species.

Forensic entomology is known as a locality-specific science and molecular studies are generally directed to the specific fauna found in a region (Wallman & Donnellan, 2001; Harvey *et al.*, 2003a; 2003b). Specimens from new localities may not exactly match published DNA sequences, raising questions regarding acceptable levels of variation for distinction (Harvey *et al.*, 2008). In addition, the longitude and latitude span of China is large that the climate condition and geographical environment of different provinces may be greatly diversified. Also, it is possible for a forensic scientist to rely on others' data for identification purposes provided that he or she knows the local fauna well enough to narrow down the choices of species presented in that particular areas (Wells & Sperling, 2001). Furthermore, it is recommended that systematic studies related to beetle infestation on human remains should be conducted by forensic entomologists (Kulshrestha & Satpathy, 2001) and setting up local database is strongly suggested. Since forensic important beetles have been less studied compared with dipterans, this database of necrophilous beetles will be of great significance.

This paper is the first combined DNA-barcoding study of histerid beetles in China. Besides, we found combined analysis is a more accurate approach for species identication than independent analysis. The 16S rRNA and Cyt b gene could be useful in discriminate among the four beetle species in our study. Evolutionary rate of 16S rRNA is relatively high, while Cyt b gene is conservative. Future work could include the extension of the sample size, and the analysis of additional species of beetles from other parts of China.

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