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Molecular identification of forensically significant beetles (Coleoptera) in China based on COI gene

Identificación molecular de escarabajos (Coleoptera) forensicamente significativos en China basados en el gen COI

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Abstract: Precise identification of insect species plays an essential role in the accurate estimation of the postmortem interval (PMI), especially when information on the postmortem phenomenon is not available. Sarcosaphagous beetles infest and colonize human and animal remains in the late stage of decomposition, and their morphological similarity poses a great challenge for forensic entomologists, as an existing key may be incomplete or difficult for non-specialists to use. A method for easy and accurate species-level identification at any life stage is required. In this study, a 272-base pair region of the mitochondrial cytochrome oxidase I (COI) gene was used to explore its utility in the identification of forensically important beetles. Twenty-four specimens were collected from 14 locations in nine provinces of China. Phenogram analysis of the sequenced segments by the unweighted pairgroup method analysis (UPGMA) method showed that all specimens were properly assigned into six species with strong similarity, which indicates the possibility of separating congeneric species with the short COI fragment. These results will be instrumental for implementation of the Chinese database of forensically relevant beetles.

Key words: Forensic entomology. Sarcosaphagous beetles. Mitochondrial DNA.

Resumen: La identificación precisa de especies de insectos juega un papel esencial en la estimación del intervalo postmortem (IPM), especialmente cuando la información sobre el fenómeno postmortem no es disponible. Los escarabajos sarcosaprófagos infestan y colonizan los restos humanos y animales en la etapa tardía de descomposición y su similitud morfológica es un gran desafío para los entomólogos forenses ya que las claves taxonómicas existentes pueden ser incompletas o difíciles para los no especialistas. Se requiere un método fácil y preciso para la identificación a nivel de especie en cualquier estadio de vida. En este estudio se exploró la utilidad de una región del gen mitocondrial COI de 272 bases para identificar escarabajos de importancia forense. Se capturaron 24 ejemplares provenientes de 14 localidades de nueve provincias de China. El análisis de conglomerados de las secuencias por el método de ligamiento promedio no ponderado (UPGMA) mostró que todos los especímenes fueron apropiadamente asignados a seis especies con fuerte similitud lo que demuestra la posibilidad de separar especies congenéricas con este fragmento corto de COI. Estos resultados serán instrumentales en la implementación de una base de datos china de escarabajos importantes en el área forense.

Palabras clave: Entomología forense. Escarabajos sarcosaprófagos. ADN mitocondrial.

Introduction

The main purpose of forensic entomology is to provide information for the investigation of murders and suspicious deaths by determining the time, source, place and manner of death (Benecke 2008). The use of insects and other arthropods in forensic investigations has been increasingly gaining international recognition in the medicolegal discipline (Amendt 2004; Sukontason 2007). Some species of sarcosaphagous insects are attracted to a corpse within minutes of death, which is important for the estimation of postmortem interval (PMI) in cases of homicide, suicide or unexplained death and other forensic related issues (Catts 1992). The main advantage of the standard methods for the estimation of the early PMI is that arthropods, especially insects, can represent an accurate measure to determine time of death even in the late-stage decomposition of carcasses, when the classical forensic pathological methods fail (Byrd and Castner 2000). Additionally, the pattern of succession of insects is specific to the location and environmental conditions in which a carcass occurs. Because taxa can vary greatly with locality for precise estimation of the PMI, it is cruial to identify the forensically important insects that are specific to an area (Anderson 2000).

In forensic practice, the most common insects used for PMI are Diptera and Coleoptera (Lan *et al.* 2006). Previous studies focus mainly on blowflies. However, beetles can be also very informative (Wang *et al.* 2008). Diptera species usually appear early in the decomposition process (Peng *et al.* 2009), and Coleoptera tend to be associated with the later stage of the decomposition process, which is very important in terms of the dry bones of the body (Haskell *et al.* 1997). Some insects visit but do not colonize a carcass; rather, they exploit the carcass and developing maggots as food resources. These non-colonizing insects include predators and parasites of necrophagous species, such as beetles in the families Silphidae, Staphylinidae, and Histeridae, are useful in succession-based PMI estimations (Anderson 2000), and most of the beetles that are collected during succession studies fall into this category.

Species identification of larval specimens of both Coleoptera and Diptera, however, requires a sophisticated technique

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to dissect under a stereomicroscope the larval mouthpart and also vast knowledge of the cephalopharyngeal skeleton morphology (Greenberg 2002). Therefore, accurate morphologic insect identification relies on detailed examination which can require expert entomologists and are extremely difficult for almost all forensic scientists within their routine work (Saigusa *et al.* 2006). Under these circumstances, DNA analysis appears promising to solve the species identification problem owing to the durability and stability of the DNA (Wallman and Donnellan 2001). It also can solve the problems of morphological identification with damaged specimens (Judith and Nicola 2008). This technique is based on the mitochondrial DNA (mtDNA) encoded cytochrome oxidase I gene (COI) (Wells and Sperling 2001). Partial sequences of this COI gene have been shown to have sufficient discrimination power (Stijn and Matthias 2009), which makes it suitable for forensic applications. The COI gene has been used for inferring phenogram analysis at various taxonomic levels of many animal groups (Avise 2000). COI is not only used widely for Diptera (Alessandrini *et al.* 2008; Harvey *et al.* 2003; Wells *et al.* 2007), but also helpful for Coleoptera identification (Paul *et al.* 2009; Dirk *et al.* 2007; Fang 2009).

Studies of carrion Coleoptera have been conducted in several regions of the western world to determine species composition by different sequences of the mtDNA gene (David *et al.* 2002; David *et al.* 2001; Arnoldi *et al.* 2007; Friedric *et al.* 2003). In East Asia, the partial sequences of mtDNA, including COI (Kim *et al.* 2000; Lee *et al.* 2003), COII (Suzuki *et al.* 2002, 2004) have also been determined and used to investigate the evolutionary and biogeographic relationships of some families of Coleoptera. However, there are few published data on the forensically important beetles in China. This paper reported 24 specimens from 14 districts of nine provinces in China within the last two years.

In this study, the genetic relationships of the COI gene between the species were visualised by phenogram analysis. Intra- and interspecific divergences were both valuable to form inferences about the relationships between the species. The comparison partly enabled us to study a Chinese geographical variability based on COI gene sequences between specimens of the same species of beetles. Our study has explored the utility of the independent COI sequences to identify the Coleoptera species. We hope our study can make a little contribution to the accumulation of genetic data for the database of sarcosaphagous beetles of China.

Materials and Methods

A fragment of the mitochondrial COI gene had been studied in 24 specimens of beetles obtained from 14 districts of nine provinces in China since 2009 (Fig. 1). The specimens are representatives of two suborders (Adephaga and Polyphaga), four families (Carabidae, Scarabaeoidea, Staphylinidae, and Silphidae), including six genera and six species: three *Harpalus herbivagus* (Say, 1823) specimens, two *Temnoplectron involucre* (Matthews, 1974) specimens, seven *Aleochara pacifica* (Casey, 1893) specimens, seven *Silpha carinata* (Herbst, 1783) specimens, two *Calosilpha bicolor* (Fairmaire, 1899) specimens, and three *Creophilus maxillosus* (Linnaeus, 1758) specimens. All the specimens were collected on rabbit cadavers by chopsticks and spoons for adults, and stored at room temperature by air drying. Specimens were referred by their generic names, number of specimens, vouchers and collecting locations (Table 1), sex was not recorded. All specimens were identified morphologically by expert entomologists through the use of relevant taxonomic keys (Lu and Wu 2003).

The thoracic muscles of each beetle were isolated for DNA extraction by the CTAB protocol used by Skevington *et al.* (2000). The head and abdomen of each specimen was retained to check its identity. DNA was resuspended in 50ml of 1×TE buffer [1×TE buffer, pH 8.0; 10mM Tris-HCl, 1mM EDTA, pH 8.0] and stored at 4°C.

All the COI sequences were aligned using the sequence alignment program DNASTAR (Megalign version 7.1.0). Conserved regions of the alignment were evaluated and marked. The most commonly occurring nucleotides at each position of the conserved sequence were selected and inputted in the primer design program Primer Premier 5.0. The primer-binding site should lie entirely within the conserved region. The general primer-design rules were considered to avoid false priming and primer-dimer formation in cross-family PCR. Genetic identification of these beetles was performed by amplifying a 272-bp fragment of the COI gene of mtDNA, using degenerate primers. Amplification of the fragment was performed by using the primers F: 5'-CAGATCGAAATTTAAATACTTC-3' and R: 5'-GTATCAACATCTATTCCTAC-3' (Guo *et al.* 2010; Liu *et al.* in press).

The PCR reaction volume was 25ml, containing 1-5ml (20-40ng) of template DNA, 12.5ml 2×GoTaq® Green Master Mix (4ml dNTP (1mM/ml), 1.0ml Taq polymerase, 2.5ml 10×buffer (Mg^{2+}1.5mmol/l)), 0.25-2.5ml each primer (10mM), Nuclease-Free Water added to a total volume of 25ml. PCR amplifications were performed in a Thermo Cycler (Perkin-Elmer 9600) and programmed with the following parameters: initial step at 94°C (3min), continued for 30 cycles of 94°C (30s) and 50°C (30s for mt-rDNA annealing) and 72°C (30s).

The PCR products were purified with QiaQuick columns cycle. Then sequencing was performed on both forward and reverse strands through using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Bio-

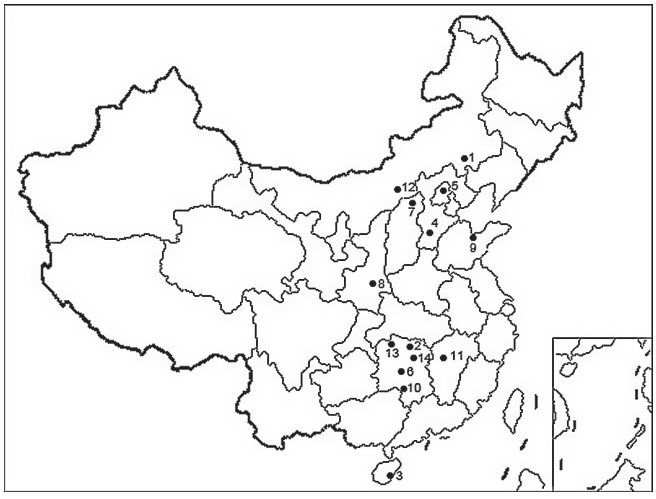

Figure 1. Map showing collecting locations of sarcosaphagous beetles in China. The locality numbers are as follows: 1. Chifeng, 2. Xiangyin, 3. Wanning, 4. Shijiazhuang, 5. Beijing, 6. Hengshan, 7. Datong, 8. Xi'an, 9. Weifang, 10. Yongzhou, 11. Yichun, 12. Hohhot, 13. Zhangjiajie, 14. Changsha.

systems). Removal of excess dye-deoxyterminator primers and buffer were executed by DYE-EX spinoclumns (Qiagen). Sequence chromatograms were edited and discrepancies between forward and reverse sequences resolved using Sequence Navigator (v1.01, Applied Biosystems). Since the sequences were protein coding and did not contain any indels, all resulting sequences in this study were aligned using Clustal W (http://www.ddbj.nig.ac.Jp/E-mail/clastal-e.html). Their accession numbers were listed in Table 1.

Table 1. Individuals included in the study with location and accession number.

No.	Species	Location (Latitude (°), Longitude (°))	Accession no.
1	*H.herbivagus* (Say, 1823)	Chifeng, Inner Mongolia (42.26, 118.89)	GU270028
2		Xiangyin, Hunan (28.68, 112.87)	GU270027
3		Wanning, Hainan (18.80, 110.39)	GU270026
4	*T. involucre* (Matthews, 1974)	Beijing (39.92, 116.46)	GU270025
5		Hengshan, Hunan (27.25, 112.86)	GU270024
6	*A. pacifica* (Casey, 1893)	Datong, Shanxi (40.12, 113.30)	GU270002
7		Xi'an, Shannxi (34.23, 108.91)	GU270000
8		Xi'an, Shannxi (34.23, 108.91)	GU270001
9		Shijiazhuang, Hebei (38.04, 114.51)	GU270005
10		Weifang, Shandong (36.62, 119.10)	GU270003
11		Yongzhou, Hunan (26.22, 111.63)	GU270006
12		Yichun, Jiangxi (27.81, 114.38)	GU270004
13	*S. carinata* (Herbst, 1783)	Hohhot, Inner Mongolia (40.82, 111.65)	GU269994
14		Hohhot, Inner Mongolia (40.82, 111.65)	GU269998
15		Xi'an, Shannxi) (34.23, 108.91)	GU269997
16		Shijiazhuang, Hebei (38.04, 114.51)	GU269999
17		Shijiazhuang, Hebei (38.04, 114.51)	FJ763719
18		Shijiazhuang, Hebei (38.04, 114.51)	GU269995
19		Zhangjiajie, Hunan (29.08, 110.29)	GU269996
20	*Ca. bicolor* (Fairmaire, 1899)	Xi'an, Shannxi (34.23, 108.91)	GU270017
21		Changsha, Hunan (28.23, 112.94)	GQ118427
22	*C. maxillosus* (Linnaeus, 1758)	Shijiazhuang, Hebei (38.04, 114.51)	GQ118409
23		Shijiazhuang, Hebei (38.04, 114.51)	GQ118419
24		Datong, Shanxi (40.12, 113.30)	FJ763716

Data analyses were conducted in MEGA4 (Tamura *et al*. 2007). Similarities were calculated by the simple matching method, and a phenogram was constructed using the unweighted pairgroup method analysis (UPGMA) as reported in Sneath and Sokal (1973).

Results

Alignment of sequences. A 272-bp fragment of the mitochondrial COI gene was successfully sequenced for all the specimens, and the alignment of all specimens considered in this study lacked any insertion or deletion and revealed 77 variable positions (59 at the codon third position, 15 at the first position, and 3 at the second position) on 272-bp analysed.

A total of six species were sequenced over COI regions. The morphological identification and original locations of all the 24 specimens are displayed in Table 1.

Nucleotide patterns of substitution. In Table 2, each entry showed the probability of substitution from one base (row) to another base (column) instantaneously. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The average base composition was 36.5% thymine (T), 13.3% cytosine (C), 32.4% adenine (A) and 17.8% guanine (G). The transition/transversion (ts: tv) rate ratios were k_1 = 0.716 (purines) and k_2 = 3.632 (pyrimidines). The overall transition/transversion bias was R = 0.637, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$.

Phenogram construction. All individual sequences for a given species (as identified using morphological characters) clustered closely together with 100% similarities respectively, indicating the strong basis for species distinction in the six main clades (Fig. 2). Some species were grouped together with high similarity values but to be separated into several small branches intraspecifically like *A. pacifica*, *S. carinata*, *H. herbivagus* and *C. maxillosus*. *A. pacifica* and *H. herbivagus* formed a single grouping with low value at 53%, but separated into two distinct species groups both with 100% similarity respectively. In addition to *A. pacifica* and *H. herbivagus*, together with *S.carinata* and *T. involucre,* also formed a single group with a lower similarity of 77%. Two specimens of *H. herbivagus* from Chifeng (GU270028) and Wanning (GU270026) also formed a small clade at a good similarity at 95%. On the contrary, two specimens of *A. pacifica* from Xi'an (GU270000 and GU270001) clustered at a low similarity at 56%, as well as two specimens of *C. maxillosus* from Shijiazhuang (GQ118409) and Datong (FJ763716) at 74%, which showed their difference based on intraspecific variation. Within species *S. carinata*, three specimens from Shijiangzhuang (GU269999, FJ763719 and GU269995) and one specimen from Hohhot (GU269994) were grouped well at 96%, as they were all obtained from northern China; one specimen of the same species from Zhangjiajie (GU269996) clustered with the former four specimens at a low similarity (56%).

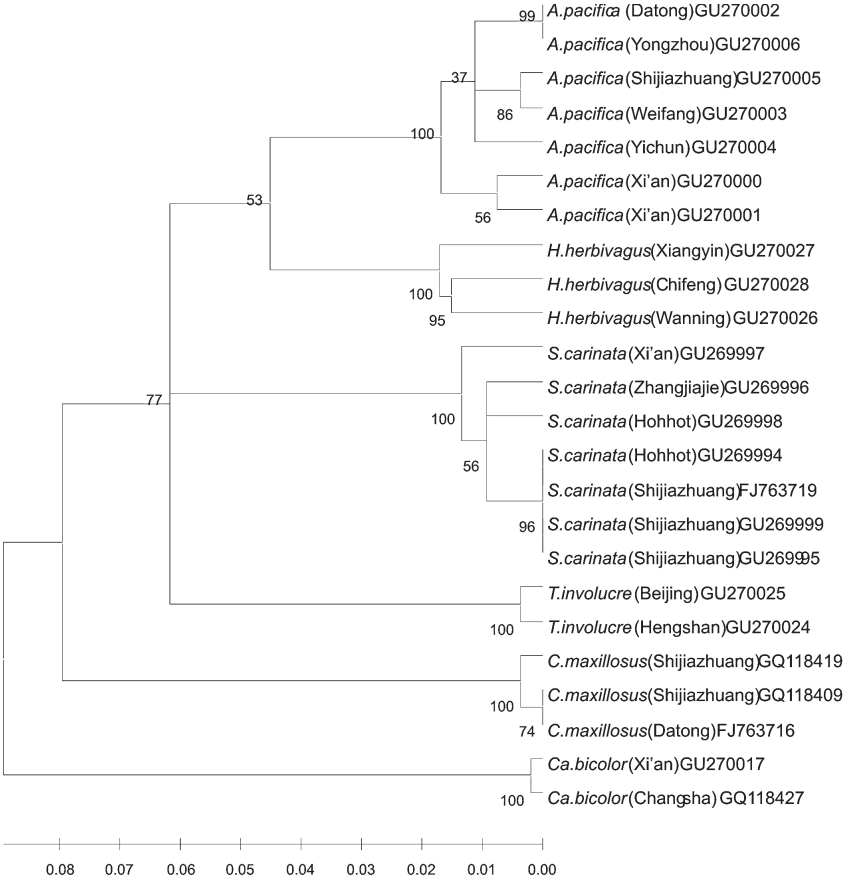

Figure 2. Phenogram from UPGMA clustering of correlation coefficients among 24 operating taxonomic units of Coleoptera in China.

Table 2. Maximum composite likelihood estimate of the pattern of nucleotide substitution.

	A	T	C	G
A	-	*8.76*	*3.19*	**3.05**
T	*7.77*	-	**11.59**	*4.26*
C	*7.77*	**31.82**	-	*4.26*
G	**5.57**	*8.76*	*3.19*	-

Intraspecific and interspecific variation. The average of base substitutions per site for all specimens was 0.109 (Table 3). The minimum intraspecific divergence mean value was found in three specimens of *C.maxillosus* from two northern cites (Shijiazhuang and Datong) of China, which was at 0.2% (Table 4). The maximum intraspecific divergence mean value was found in three specimens of *H. herbivagus* from Xiangyin (south inland), Chifeng (north inland) and Wanning (south coast) that was at 2.7%. Levels of interspecific variation varied from 9.4% to 20.1%.The highest interspecific variation was between *T. involucre* and *Ca.bicolor* (Table 5). Furthermore, in comparison with *Ca.bicolor,* the interspecific variations of the other species were all very high, all beyond 16.4%. The lowest interspecific variation was between the two species pairings of *H. herbivagus* and *A. pacifica*. There was also a low divergence between *A. pacifica* and *S. carinata* at 9.9%.

Discussion

To the best of our knowledge, there were few studies using the COI sequences to identify forensically important beetle species in China. The major purpose of our study was to confirm that published molecular identification methods can also be applied on beetles from China. Sometimes the identification of sarcophagous insects including the species from the Coleoptera can be puzzling because of the similar morphological markers, and it can be difficult or even impossible to identify the immature stages of many species. From the correlation coefficients for each clade and the level of nucleotide divergence between groups, the 272-bp region of COI was shown as a potential marker for identification of sarcosaphagous beetles. And the results indicated that the used technique is as effective as morphological method in identification of Coleoptera species, while, in order to acquire correct identification, the morphologic method needs expertise in specialized taxonomy (Leclercq and Lecomte, 1978), yet the technology using mtDNA is easier to perform and saves time. Moreover, the limited amount of the insect tissue in this study made the possibility for further morphological study and genetic analyses.

The COI gene of all the specimens exhibited a high proportion of AT nucleotides, which was in agreement with previous findings made in Coleoptera and other insects (Crozier and Crozier 1993; Lunt *et al.* 1996; Simon *et al.* 1994; Daniel 1999). The AT richness increased the amount of potential transversions and led to a low proportion within the ts: tv ratio, which was also supported by Su *et al.* (2004) who used a 1059-bp fragment of COI gene of the carabid ground beetle (Carabidae).

The Coleoptera is significant in forensic studies. The main families among them are Staphylinidae, Scarabaeidae, Carabidae, Histeridae, Silphidae and Dermestidae (Goff and Catts 1990). In this study, specimens were all obtained from four families: Staphylinidae, Scarabaeidae, Carabidae

Table 3. Pairwise distance matrix of 272-bp COI sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
[1]																								
[2]	0.034																							
[3]	0.015	0.034																						
[4]	0.124	0.119	0.115																					
[5]	0.124	0.119	0.115	0.007																				
[6]	0.119	0.111	0.102	0.128	0.128																			
[7]	0.090	0.081	0.073	0.111	0.111	0.038																		
[8]	0.086	0.077	0.069	0.102	0.102	0.038	0.019																	
[9]	0.098	0.090	0.082	0.107	0.107	0.019	0.019	0.019																
[10]	0.102	0.094	0.086	0.111	0.111	0.015	0.022	0.022	0.004															
[11]	0.119	0.111	0.102	0.128	0.128	0.000	0.038	0.038	0.019	0.015														
[12]	0.102	0.094	0.086	0.102	0.102	0.026	0.030	0.030	0.022	0.019	0.026													
[13]	0.119	0.119	0.111	0.124	0.124	0.115	0.090	0.106	0.102	0.098	0.115	0.086												
[14]	0.102	0.111	0.094	0.115	0.115	0.102	0.073	0.090	0.090	0.086	0.102	0.073	0.015											
[15]	0.119	0.120	0.120	0.133	0.133	0.115	0.081	0.090	0.094	0.098	0.115	0.086	0.022	0.038										
[16]	0.119	0.119	0.111	0.124	0.124	0.115	0.090	0.106	0.102	0.098	0.115	0.086	0.000	0.015	0.022									
[17]	0.119	0.119	0.111	0.124	0.124	0.115	0.090	0.106	0.102	0.098	0.115	0.086	0.000	0.015	0.022	0.000								
[18]	0.119	0.119	0.111	0.124	0.124	0.115	0.090	0.106	0.102	0.098	0.115	0.086	0.000	0.015	0.022	0.000	0.000							
[19]	0.128	0.128	0.119	0.141	0.141	0.115	0.090	0.106	0.102	0.098	0.115	0.086	0.015	0.030	0.030	0.015	0.015	0.015						
[20]	0.174	0.188	0.169	0.203	0.203	0.165	0.165	0.160	0.160	0.156	0.165	0.165	0.165	0.178	0.179	0.165	0.165	0.165	0.174					
[21]	0.169	0.183	0.165	0.198	0.198	0.170	0.169	0.165	0.165	0.161	0.170	0.160	0.160	0.174	0.174	0.160	0.160	0.160	0.169	0.004				
[22]	0.133	0.132	0.151	0.133	0.142	0.155	0.146	0.137	0.142	0.146	0.155	0.133	0.146	0.165	0.138	0.146	0.146	0.146	0.165	0.174	0.170			
[23]	0.137	0.137	0.146	0.129	0.138	0.151	0.142	0.133	0.137	0.142	0.151	0.128	0.151	0.169	0.142	0.151	0.151	0.151	0.169	0.170	0.165	0.004		
[24]	0.133	0.132	0.151	0.133	0.142	0.155	0.146	0.137	0.142	0.146	0.155	0.133	0.146	0.165	0.138	0.146	0.146	0.146	0.165	0.174	0.170	0.000	0.004	

The numbers in this table is corresponding with Table 1.

[1] *H. herbivagus* (GU270028); [2] *H. herbivagus* (GU270027); [3] *H. herbivagus* (GU270026); [4] *T. involucre* (GU270025); [5] *T. involucre* (GU270024); [6] *A. pacifica* (GU270002); [7] *A.pacifica* (GU270000); [8] *A. pacifica* (GU270001); [9] *A. pacifica* (GU270005); [10] *A. pacifica* (GU270003); [11] *A. pacifica* (GU270006); [12] *A. pacifica* (GU270004); [13] *S. carinata* (GU269994); [14] *S. carinata* (GU269998); [15] *S. carinata* (GU269997); [16] *S. carinata* (GU269999); [17] *S. carinata* (FJ763719); [18] *S. carinata* (GU269995); [19] *S. carinata* (GU269996); [20] *Ca. bicolor* (GU270017); [21] *Ca. bicolor* (GQ118427); [22] *C. maxillosus* (GQ118409); [23] *C. maxillosus* (GQ118419); [24] *C. maxillosus* (FJ763716).

Table 4. Mean intraspecific divergence expressed as a percentage of the total of 272 base pairs of COI data.

Species	Numbers of specimens	Mean (%)
H. herbivagus	3	2.7
T. involucre	2	0.7
A. pacifica	7	2.3
S. carinata	7	1.5
Ca. bicolor	2	0.4
C. maxillosus	3	0.2

and Silphidae, and the families Silphidae and Staphylinidae are the first visitors of sarcosaphagous beetles to the human and animal remains (Ozdemir and Sert 2009). According to the study by Elena *et al*. (2005), a longer fragment (759-bp) of the mitochondrial COI that was sequenced to distinguish 119 specimens of the same tribe Harpalini was shown as a credible tool to distinguish the taxonomy of beetles at the tribe level. Similar results were also found in the study of the tribe Cychrini using 1059-bp fragment of the COI gene (Su *et al*. 2004). Therefore, the identification of Chinese specimens at the tribe level should be discussed in future studies. Phenogram analysis using UPGMA tree was performed to examine the ability of the region to resolve species identities and taxonomic relationships between species of six genera owing to every species forming its own group with very high similarity respectively, and this illustrated the potential of COI sequence for use at the species level. Regional variability is one kind of the evidence to infer the geographical origin of forensically important insects species. In addition, it can also determine the scene of crime (SOC). As mentioned before in results, three specimens of *S. carinata* from Shijiazhuang and one specimen of *S. carinata* from Hohhot formed a highly supportive clade but one specimen of *S. carinata* from Zhangjiajie (a mountainous region in southern China) grouped with a relatively low support with the former four specimens (flatlands in northern China) showed that the intraspecific variation was possibly influenced by geographical and climatic differences, which is in agreement with previous estimations made in mitochondrial genomes of two luminous beetles (Li *et al*. 2007). These above might be because many beetles are hindwingless and the elytra are fused at suture, so that they cannot fly like most species of Diptera, thus revealing considerable geographically linked phenogram diversification (Su *et al*. 2004). However, one specimen of *T. involucre* from Hengshan (a mountainous region in southern China) and another specimen of the same species from Beijing (a flatland in northern China) were grouped together at full similarity value, as well as the specimens of the same species *Ca. bicolor* from two different cites (a northern city Xi'an and a southern city Changsha), which indicated these species were hardly influenced by geographical reasons or prompted more samples are needed from this two locations to explain this phenomenon. Similar findings were also found in two specimens of *H. herbivagus* from Chifeng (an inland city in northern China) and Wanning (a coastal city in southern China) discussed above, the maximum mean intraspecific variability for all specimens was 2.7%, while the minimum interspecific variability was 9.4%, this difference between the threshold levels enabled differentiation to be observed between forensically important Coleoptera species in China. In the interspecific variation analysis, compared wih *Ca. bicolor,* the interspecific variations of the other species were all very high, all beyond 16.4%. Additionally, the lowest value was between *H. herbivagus* and *A. pacifica* which belong to two different suborders Adephaga and Polyphaga. These results above are different from the classical classification of beetles, which indicated large samples should be supplied in future study.

A COI and COII, or with other gene regions like 16S rDNA, combined analysis would be carried out to explore Chinese forensically correlative beetles identification in future study. A multidisciplinary approach and the study of new taxa are needed to establish solid monophyletic lineages that eventually will lead to a more natural classification of Coleoptera.

Conclusion

Our results stress the utility of using DNA-based method for purposes of molecular identification of species. The 272-bp fragment of the mitochondrial COI gene in this study displayed that, besides the morphological method of identification, this region has potential as a discriminatory tool in identification of Coleoptera species. To some unexplained problems in this paper, further analysis is required to reveal the cause of this discrepency. We should do new studies to obtain more specimens of different species of Coleoptera in a wider area of China, and then improve the molecular method for identification of forensically important beetles.

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Table 5. Pairwise divergence between species expressed as a percentage of 272 base pairs of COI data.

Species	*H. herbivagus*	*T. involucre*	*A. pacifica*	*S. carinata*	*Ca. bicolor*	*C. maxillosus*
H. herbivagus						
T. involucre	0.119					
A. pacifica	0.094	0.113				
S. carinata	0.116	0.126	0.099			
Ca. bicolor	0.175	0.201	0.164	0.168		
C. maxillosus	0.139	0.136	0.143	0.152	0.171	

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