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Identification of the forensically important sarcophagid flies *Boerttcherisca peregrina*, *Parasarcophaga albiceps* and *Parasarcophaga dux* (Diptera: Sarcophagidae) based on COII gene in China

Guo, Y.D., Cai, J.F.*, Li, X., Xiong, F., Su, R.N., Chen, F.L., Liu, Q.L., Wang, X.H., Chang, Y.F., Zhong, M., Wang, X. and Wen, J.F.
Department of Forensic Science, School of Basic Medical Sciences, Central South University, Changsha 410013, Hunan, China
* Corresponding author email address: cjf_jifeng@163.com
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Abstract. Accurate species identication is a crucial step in forensic entomology, as the insect collected on a corpse can provide useful information for estimation of postmortem interval (PMI). The utility of the forensically important Sarcophagidae (Diptera) for crime scene investigation has been severely restricted, as morphological identification is difficult, especially the identification of females and larvae. In this study, a method for using mitochondrial DNA (mtDNA) sequence data and phylogenetic analysis was performed to distinguish the three Chinese sarcophagid species: *Boerttcherisca peregrina* (Robineau-Desvoidy, 1830) *Parasarcophaga albiceps* (Meigen, 1826) and *Parasarcophaga dux* (Thompson, 1869) (Diptera: Sarcophagidae). DNA was extracted and analyzed by a 189 bp fragment of cytochrome coxidase subunit II (COII) gene. The monophyletic branches of the phylogenetic tree reveal that this marker is suitable for discrimination between these species, providing high support for separation on congeneric species. Therefore, the molecular method applied to the sarcophagid species identification is feasible.

INTRODUCTION

Forensic entomology involves the study of the interaction of insects and other anthropods with legal matters (Harvey *et al.*, 2003a). Especially in the estimation of postmortem interval (PMI), forensic entomology possesses a distinct advantage. In order to apply entomological evidence to the estimation of PMI or other related issues, it is essential to precisely identify the species of insects attracted to the remains (Kiyoshi *et al.*, 2005).

The Sarcophagidae or flesh flies (Diptera) comprise over 2,500 species in over 100 genera globally, with many species being carrion breeders and initial corpse colonizers (Fan & Pape, 1996). Many sarcophagid species have the potential to be used to estimate the PMI or death of place. Firstly, the larval stages of many species of Sarcophagidae are necrophagous and for this reason those species termed 'esh-ies' are signicant in forensic entomology, being second only to the Calliphoridae (Diptera) in terms of their usefulness (Pe'rez-Moreno *et al.*, 2006). Death scenes from which esh y larvae have been recovered vary from ones with relatively fresh remains to much older mummied corpses (Benecke, 1998). Secondly, the reproductive cycle of the sarcophagids makes them prospectively more reliable for PMI estimations compared with other initial dipteran colonizers (Kamal, 1958; Meiklejohn *et al.*,

2009). According to Introna *et al.* (1998), the postmortem interval (PMI) of charred remains was calculated based on third instar of *Bercaea africa*. Furthermore, in contrast to other forensically important flies, sarcophagids deposit live larvae rather than eggs, and these may be dropped in the vicinity of the corpse when an adult female cannot physically reach the body (Denno *et al.*, 1976). Sarcophagid flies, such as *B. peregrina*, *P. albiceps* and *P. dux* and others. are widely distributed in China (Xu *et al.*, 1996).

Species identification is a crucial step in forensic entomology. Accurate estimation of the PMI requires accurate identification of insects, as mis-identification may result in the application of incorrect development data (Harvey *et al.*, 2003b). Sarcophagids adults and larvae are easily recognized at family and generic level, as all sarcophagids share common characteristics (Pape, 1996; Byrd & Castner, 2001), but are morphologically remarkably similar subgenerically and inter-specically (Aspoas, 1991). For some sarcophagid species it is only the adult males that can be identified with certainty by taxonomic experts. Therefore, DNA-based method was used for forensic sarcophagid species identification (Wells *et al.*, 2001; Zehner *et al.*, 2001; Kiyoshi *et al.*, 2005, 2009; Harvey *et al.*, 2008; Meiklejohn *et al.*, 2009). To implement the use of sarcophagids for PMI estimation, a method for easy yet accurate species-level identification at any life stage is required (Meiklejohn *et al.*, 2009).

The use of sarcophagids as PMI estimators is restricted due to inadequate documentation of their thermobiological histories, especially for the Chinese species. Molecular identification can be carried out on any lifecycle stage without further rearing and on dead, preserved or live samples. Molecular identification can be regarded as an alternative method proposed to eliminate the issues surrounding correct species-level identification based solely on taxonomy. Mitochondrial DNA (mtDNA has been widely used for analyses of metazoan phylogenetic relationships at various taxonomic levelsbecause of the lack of intermolecular recombination, its maternal inheritance, the relatively rapid evolutionary rate, and the large numbers of copies (Machida *et al.*, 2004). Sequence of the mitochondrial region encoding the cytochrome oxidase II (COII) gene is particularly useful in evolution studies, population genetics and systematics due to the relatively high degree of variation in the region molecular systematic study (Ying *et al.*, 2007).

Genetic species identification is crucial in matching the sequence of the evidence item to an authenticated reference DNA sequence (Altschul *et al.*, 1976). However, molecular data available with respect to the sarcophagid flies are very limited in China. To this day, few genetical species identication studies on Sarcophagidae in China have been published yet. In this study, we investigated the ability of the COII region to accurately identify adult sarcophagid specimens from 13 districts of 9 provinces in China. At the same time, molecular data was accumulated for the future implementation of Chinese Sarcophagidae as a tool in forensic investigations.

MATERIALS AND METHODS

Specimens

Twenty two dried adult specimens of Sarcophagidae (Diptera), including three species (*B. peregrina*, *P. albiceps*, *P. dux*), were obtained from 13 districts of 9 provinces in China during the year 2007 to 2009. Another two dried adult specimens of *Musca domestica* (Diptera: Muscidae) were obtained from Xi'an (Shannxi) and Yongzhou (Hunan) in the year 2009. All samples were trapped using animal (rabbit, dog or pig) corpse-baited traps, stored at room temperature by air drying, and were identified to subfamily level using traditional morphological characters by entomologist. Specimens used in this study

are listed in Table 1, the distributions of collection locations were are also showed in Fig. 1.

The southernmost collection location was Wanning located in Hainan province, SA (18°802, 110°392), the northernmost and westernmost location was Urumqi in Sinkiang municipality, SA (43°462, 87°362).

DNA extraction, polymerase chain reaction (PCR) methods and nucleotide sequencing

The thoracic muscle of each fly was isolated for DNA extraction by a CTAB protocol described by Skevington *et al.* (2000). The head and abdomen of each specimen was retained to check its identity. DNA was resuspended in 50ul of 1xTE buffer [1xTE buffer, pH 8.0; 10mM Tris–HCl, 1mM EDTA, pH 8.0] and stored at 4°C.

All of the COII sequences were aligned using the sequence alignment programme 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 programme 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. A portion of 189bp fragment of the mitochondrial COII gene was amplified and sequenced by using forward primer (5'-ATTAGATGTTGATAATCG-3') and reverse primers (5'-ACAAATTTC-TGAACATTG-3').

The PCR reaction volume was 25μl, containing 1-5μl (20-40ng) of template DNA, 12.5μl 2xGoTaq® Green Master Mix (4μl dNTP (1mmol/ml), 1.0u Taq polymerase, 2.5μl 10xbuffer (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 Thermo Cycler (Perkin-Elmer 9600) and programmed with the following parameters: initial step at 94°C (3 min), continued for 30 cycles of 94°C (30s) and 50°C (30s for mt-rDNA annealing) and 72°C (30s). An elongation

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

NO.	Species	Accession NO.	Locality and coordinates	Date
1	*B. peregrina* (R-D,1830)	HM016746	Urumqi, Xinjiang [87:36E, 43:46N]	2009.7
2		HM016747	Changsha, Hunan [112:59E 28:12N]	2009.8
3		HM016748	Linyi, Shangdong [118:35E 35:05N]	2007.8
4		HM016749	Shijiazhuang, Hebei [114:26E, 38:03N]	2009.8
5		HM016750	Urumqi, Xinjiang [87:36E, 43:46N]	2009.8
6		HM016751	Lanzhou, Gansu [103:83E, 36:06N]	2007.8
7		HM016752	Jishou, Hunan [109:43E 28:18N]	2008.8
8		HM016753	Changsha, Hunan [112:59E 28:12N]	2009.8
9		GU270079	Shijiazhuang, Hebei [114:26E, 38:03N]	2007.8
10		GU270087	Xiangxiang, Hunan [112:5 E 27:75N]	2008.8
11		HM016754	Nanning, Guangxi[108:21E 22:47N]	2007.8
12	*P. albiceps* (Meigen, 1826)	HM016755	Shijiazhuang, Hebei [114:26E, 38:03N]	2009.8
13		HM016756	Hohhot, Inner Mongolia [111:38E, 40:48N]	2009.8
14		HM016757	Wanning, Hainan [110:39E, 18:80N]	2007.8
15		HM016758	Hohhot, Inner Mongolia [111:38E, 40:48N]	2009.8
16		GU270084	Datong, Shanxi [113:13E 40:07N]	2009.7
17		HM016759	Zhangjiajie, Hunan [114:51E 38:04N]	2009.8
18	*P. dux* (Thompson, 1869)	HM016760	Wanning, Hainan [110:39E, 18:80N]	2009.8
19		HM016761	Yongzhou, Hunan [111:61E, 26:42N]	2009.8
20		HM016762	Changsha, Hunan [112:59E 28:12N]	2007.8
21		HM016763	Yongzhou, Hunan [111:61E, 26:42N]	2008.8
22		HM016764	Wanning, Hainan [110:39E, 18:80N]	2007.8
23	*M. domestica* (Linnaeus, 1758)	GU269967	Xi'an, Shannxi[108:91E, 34:23 N]	2009.8
24		GU269968	Yongzhou, Hunan [111:61E, 26:42N]	2009.8

Figure 1. From north to south, 14 sample collecting locations shown in a Chinese map
1. Urumqi; 2. Changsha; 3. Linyi; 4. Shijiazhuang; 5. Lanzhou; 6. Jishou; 7. Xiangxiang; 8. Nanning; 9. Hohhot; 10. Wanning; 11. Datong; 12. Zhangjiajie; 13. Yongzhou; 14. Xi'an

of PCR products by 72°C for 5min completed the reaction.

PCR products were purified with QiaQuick PCR Purification Kit (Qiagen). Columns cycle sequencing was performed on both forward and reverse strands using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit by ABI PRISM 3730 (Applied Biosystems). 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).

The resulting sequences were compared with the Diptera sequences in the NCBI web site by Blastn function to identify species. The sequences have been deposited in GenBank by Sequin (http://www.ncbi.nlm.nih.Gov/equin/index.htmland the accession numbers are listed in Table 1. As the sequences were protein coding and did not contain any insertions or deletions, all resultant sequences in this study were aligned using ClustalW (http://www.ddbj.nig.ac.Jp/E-mail/clastalw-e.html).

Sequencing analysis

A total of 189 aligned sites for the 22 fragments of the mitochondrial COII sequences were included in the analyses. We tested whether the sequences were of mitochondrial origin or represented paralogous sequences resident in the nucleus using MEGA4 (Tamura *et al.*, 2007). The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al.*, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (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). Neighbor-joining analysis was performed using the Tamura-Nei model of substitution and bootstrapping (n=500) conducted using MEGA4.

RESULTS

Alignment of COII sequences

The COII region was straightforward to both amplify and sequence. A 189-bp fragment of the COII gene was sequenced from 22 specimens: 11 *B. peregrina*, 6 *P. albiceps*, and 5 *P. dux*. The alignment of all specimens lacked any insertion or deletion and revealed 143 variable positions on 189 analysed (Figure 1). All COII sequences were aligned through the program DNASTAR (Megalign version 7.1.0), before the final adjustments were made by eyes. The same bases were marked dark, while the different ones were marked light.

Phylogenetic analysis

A total of 189 aligned sites for the COII sequences were included in the analyses. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches, which provides an indication of the percentage support for a grouping by randomly resampling the data.

Two *M. domestica* (Linnaeus, 1758) samples belonging to the family Muscidae were used as outgroup for phylogenetic analyses (Table 1). These two specimens clustered together with a supporting bootstrap of 100%, and they were clearly separated from the family Sarcophagidae in NJ tree (Fig. 2). Several distinct congeneric clusters were formed based on the sequence data. The high bootstrap values (100%) provide an indication of good percentage support for the grouping nodes of *P. dux*. Within the *B. peregrina* species, two different clades were formed (two

	A	C	T	A	T	G	C	T	T	T	G	T	T	T	A	T	T	T	A	T
	45	54	60	63	72	79	81	84	87	88	94	102	111	121	123	141	144	147	156	165
B.peregrina(Urumqi)HM016746	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Changsha)HM016747	A	T	C	A	T	A	C	T	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Linyi)HM016748	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Shijiazhuang)HM016749	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Urumqi)HM016750	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Lanzhou)HM016751	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Jishou)HM016752	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Changsha)HM016753	A	T	C	A	T	A	C	T	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Shijiazhuang)GU270079	A	C	T	T	A	A	C	C	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Xiangxiang)GU270087	A	T	C	A	T	A	C	T	T	C	G	T	T	T	A	T	C	T	A	T
B.peregrina(Nanning)HM016754	A	T	C	A	T	A	C	T	T	C	G	T	T	T	A	T	C	T	A	T
P.albiceps(Shijiazhuang)HM016755	C	T	T	A	T	G	T	T	T	T	A	G	T	T	A	A	T	C	T	C
P.albiceps(Hohhot)HM016756	C	T	T	A	T	G	T	T	T	T	A	G	T	T	A	A	T	C	T	C
P.albiceps(Wanning)HM016757	C	T	T	A	T	G	T	T	T	T	A	G	T	T	A	A	T	C	T	C
P.albiceps(Hohhot)HM016758	C	T	T	A	T	G	T	T	T	T	A	G	T	T	A	A	T	C	T	C
P.albiceps(Datong)GU270084	C	T	T	A	T	G	T	T	T	T	G	T	T	T	A	A	T	T	C	T
P.albiceps(Zhangjiajie)HM016759	C	T	T	A	T	G	T	T	T	T	G	T	T	T	A	A	T	T	C	T
P.dux(Wanning)HM016760	A	C	T	A	T	G	A	T	C	T	G	T	A	C	T	A	T	C	A	T
P.dux(Yongzhou)HM016761	A	C	T	A	T	G	A	T	C	T	G	T	A	C	T	A	T	C	A	T
P.dux(Changsha)HM016762	A	C	T	A	T	G	A	T	C	T	G	T	A	C	T	A	T	C	A	T
P.dux(Yongzhou)HM016763	A	C	T	A	T	G	A	T	C	T	G	T	A	C	T	A	T	C	A	T
P.dux(Wanning)HM016764	A	C	T	A	T	G	A	T	C	T	G	T	A	C	T	A	T	C	A	T

Figure 2. Variable positions in the 189 bp COII gene fragment alignment of *B. peregrina*, *P. albiceps*, and *P. dux* obtained from different regions of China. The numbers of horizontal axis represent the order of the bases, and only the bases that exist variance were shown

specimen from Changsha, one from Nanning and one from Xiangxiang formed clade 2 and the other specimens clustered as clade 1, clade 1 had a supporting value of 99% and clade 2 had a supporting value of 96%) sharing a supporting values of 95%. Two *P. albiceps* samples were clustered as a clade with the supporting value of 72%, while the other *P. albiceps* specimens formed a clade with the supporting values of 98%. These two clades of *P. albiceps* share a weak supporting value of 63%.

Interspecific and intraspecific variation
All results were based on the pairwise analysis of the 22 sequences. Analyses were conducted using the Maximum Composite Likelihood method in MEGA 4.0 (Tamura *et al.*, 2004, 2007). Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Pairwise divergence between species was calculated and was showed on in Table 2. And variation among all individuals of the species was calculated. The number of base substitutions per site from analysis between sequences was shown. The overall average of all specimens was 0.05.

In Table 2, levels of interspecific variation varied from 0% to 9%. The maximum and minimum levels of divergence between the *B. peregrina* and *P. albiceps* were 9% and 4%, that of *B. peregrina* and *P. dux* were both 7%, and that of *P. albiceps* and *P. dux* were 7% and 5%. The mean value of interspecific variation between each species was as follows: the values between *B. peregrina* and other two species were both 7%, and between *P. albiceps* and *P. dux* was 6%.

The data matrix (Table 3) displays the maximum, minimum and mean intraspecific divergence of uncorrected percentage sequence. And the Table 2 showed the detail of the intraspecific divergence of each species. The mean intraspecific variation of *B. peregrina* and *P. albiceps* were both 1%, and the maximum values were both 3%. However, there is no significant intraspecific variation within the *P. dux* samples. Both the mean and maximum intraspecific variation of *P. dux* was 0%.

DISCUSSION

Although our primary purpose was to accumulate genetic data for the future study of forensically important sarcophagid flies, these data provide an opportunity to evaluate the potential value of COII

Table 2. Pairwise distance matrix of *B. peregrina*, *P. albiceps*, and *P. dux*189bp COII sequences

B. peregrina (Urumqi) HM016746	[1]	–																					
B. peregrina (Changsha) HM016747	[2]	0.03	–																				
B. peregrina (Linyi) HM016748	[3]	0.00	0.03	–																			
B. peregrina (Shijiazhuang) HM016749	[4]	0.00	0.03	0.00	–																		
B. peregrina (Urumqi) HM016750	[5]	0.00	0.03	0.00	0.00	–																	
B. peregrina (Lanzhou) HM016751	[6]	0.00	0.03	0.00	0.00	0.00	–																
B. peregrina (Jishou) HM016752	[7]	0.00	0.03	0.00	0.00	0.00	0.00	–															
B. peregrina (Changsha) HM016753	[8]	0.03	0.00	0.03	0.03	0.03	0.03	0.03	–														
B. peregrina (Shijiazhuang) GU270079	[9]	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03	–													
B. peregrina (Xiangxiang) GU270087	[10]	0.03	0.00	0.03	0.03	0.03	0.03	0.03	0.00	0.03	–												
B. peregrina (Nanning) HM016754	[11]	0.03	0.00	0.03	0.03	0.03	0.03	0.03	0.00	0.03	0.00	–											
P. albiceps (Shijiazhuang) HM016755	[12]	0.09	0.07	0.09	0.09	0.09	0.09	0.09	0.07	0.09	0.09	0.07	–										
P. albiceps (Hohhot) HM016756	[13]	0.09	0.07	0.09	0.09	0.09	0.09	0.09	0.07	0.09	0.09	0.07	0.00	–									
P. albiceps (Wanning) HM016757	[14]	0.09	0.07	0.09	0.09	0.09	0.09	0.09	0.07	0.09	0.09	0.07	0.00	0.00	–								
P. albiceps (Hohhot) HM016758	[15]	0.09	0.07	0.09	0.09	0.09	0.09	0.09	0.07	0.09	0.09	0.07	0.00	0.00	0.00	–							
P. albiceps (Datong) GU270084	[16]	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.04	0.06	0.04	0.04	0.03	0.03	0.03	0.03	–						
P. albiceps (Zhangjiajie) HM016759	[17]	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.04	0.06	0.04	0.04	0.03	0.03	0.03	0.03	0.00	–					
P. dux (Wanning) HM016760	[18]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.05	0.05	–				
P. dux (Yongzhou) HM016761	[19]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.05	0.00	–			
P. dux (Changsha) HM016762	[20]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.05	0.00	0.00	–		
P. dux (Yongzhou) HM016763	[21]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.05	0.00	0.00	0.00	–	
P. dux (Wanning) HM016764	[22]	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.05	0.00	0.00	0.00	0.00	–

Table 3. Maximum, minimum and mean intraspecific variation of *B. peregrina*, *P. albiceps*, and *P. dux* expressed as a percentage of the total of 189base pairs of COII data

Species	Numbers	Max (%)	Min (%)	Mean (%)
S. peregrina	11	3	0	1
S. albiceps	6	3	0	1
S. dux	5	0	0	0

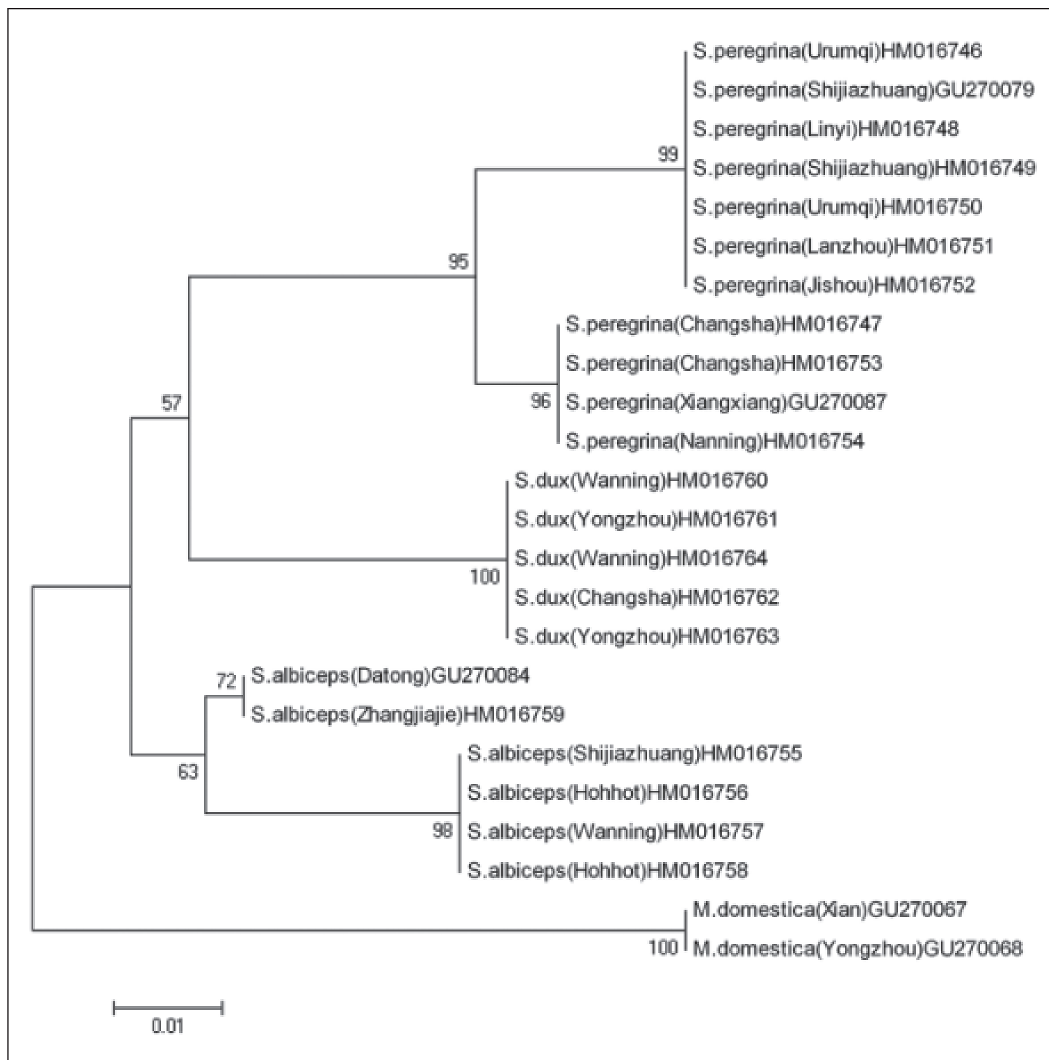

Figure 3. Single most parsimonious phylogram of *B. peregrina*, *P. albiceps*, and *P. dux* (heuristic search with 500 random step-wise additions) based on a 189 bp region of the COII gene. Numbers on branches indicate the support value. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The bar indicates 0.01 substitutions per site

for basic biological studies of these sarcophagid flies. Although some samples were clustered as a clade with weak supporting value, the three species groups of Sarcophagidae were ultimately diagnosed and delimited. From the bootstrap support for each group and the level of nucleotide divergence between groups, it is thus evident that these gene sequences have potential for identification of sarcophagid flies.

Phylogenetic analysis was performed to examine the ability of the region to resolve species identities and taxonomic relationships between species (Harvey, 2003b). The monophyletic separation and support for the congeneric groupings and sub-familial arrangements in the phylogenetic tree (Fig. 2) basically agreed with the classical morphological-based phylogeny and again affirmed the ability of the 189 bp COII region in interspecific distinction. The three species groups of Sarcophagidae were separated and most of the bootstrap values were over 95%. However, one *P. albiceps* sample from Datong (GU270084) and one from Zhangjiajie (HM016759) were clustered as a clade with weak supporting value. Low levels of variation between some species indicate that sarcophagid flies from more locations should be studied in the future.

Table 2 and Table 3 show the intraspecific variation analysis. The pairwise divergence from COII revealed interspecific and intraspecific divergences that could be useful for species identification. The maximum mean intraspecific variability for all specimens was 5%, while the minimum interspecific variability was 6%, this difference between the thresholds levels indicates that is possible to distinguish between *B. peregrina*, *P. albiceps*, and *P. dux* in China. According to the strong bootstrap support for *P. dux* samples, both the mean and maximum intraspecific variation of *P. dux* was 0%, which makes partial sequences of this COII gene sequence in particular suitable for distinguishing *P. dux* from other sarcophagid species.

Species identication of Sarcophagidae by molecular methods has the advantage of speed and simplicity, because the stage, preservation and sex of the samples can be ignored. For exploring better species identification, different single mtDNA fragment should be sequenced and studied. Some entomologists recommended combined analysis of different mtDNA fragments for Diptera species identication (Amendt *et al.*, 2004). The combined analysis of COI and COII fragments is a more accurate approach for Diptera species identication than single mtDNA fragment analysis (Wallman, *et al.*, 2001; Alessandrini *et al.*, 2007). However, before the combined analysis was carried out, the superiority and limitation of single fragment of mtDNA should be researched. To the best of our knowledge, there are no previous reports on the COII sequences of *B. peregrina*, *P. albiceps*, and *P. dux* from China. The COII sequences of three species analyzed in this study were different from each other, indicating that species identication by COII sequences was possible. However, the sarcophagid family is a part of a larger taxonomic group, and seven species are considered to be forensiclly important in China, including *B. peregrina*, *P. albiceps*, and *P. dux* (Min, 1999). Further investigation of the sarcophagid species found on the remains and their molecular biological features are required in order to accumulate forensic entomological evidence.

Although entomology is used in a variety of forensic cases in some countries, its potential has not yet been realized in China. One of the limiting factors is that the longitude and latitude span of China is so large that the climate condition and geographical environment of different provinces exhibits great diversity. Consequently, those forensically-important species that have a widespread distribution could potentially have marked phenotypic differences between populations – and this could compromise their identification by molecular methods. Consequently, local databases are strongly recommended.

This study indicated that the partial COII region has sufficient discrimination power for Sarcophagidae identification. Future work with more Sarcophagidae family species from different provinces of China could indicate the examination of more variable mitochondrial genes, and then improve the molecular method for identification of forensically important sarcophagid species. The distribution and succession of sarcophagid flies from other parts of China should be studied and local database set up are strongly recommended in China.

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