PROOF COVER SHEET

Author(s): Huidan Liao, Xiang Yang, Zhilong Li, Yanjun Ding, and Yadong Guo

Article title: The complete mitochondria genome of *Parasarcophaga albiceps* (Diptera: Sarcophagidae)

Article no: IMDN_A_1106507

Enclosures: 1) Query sheet
2) Article proofs

Dear Author,
Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the ''Submit All Corrections'' button.

Please review the table of contributors below and confirm that the first and last names are structured correctly and that the authors are listed in the correct order of contribution.

Contrib. No.	Prefix	Given name(s)	Surname	Suffix
1		Huidan	Liao	
2		Xiang	Yang	
3		Zhilong	Li	
4		Yanjun	Ding	
5		Yadong	Guo	

AUTHOR QUERIES

Q1: Please check whether the author names (first name followed by last name) and affiliations are correct as presented in the proofs.

Q2: Please provide complete details for (Meigen, 1826) in the reference list or delete the citation in the text.

Q3: Please check and confirm the unit ''ul'' changed to ''**µ**l'' in the sentence is correct.

Q4: As per journal style, funding statement has been moved to declaration of interest section. Please check.

Q5: Please update volume number and page range for Fu (2014), Guo (2014a), Shi (2014), Yan (2014), Zhang (2015), Zhang (2014), Zhong (2014), if now available.

Q6: Please clarify whether the reference referred to as Guo (2014) in the text should be (2014a) or (2014b).

Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, Early Online: 1–3
© 2015 Taylor & Francis. DOI: 10.3109/19401736.2015.1106507

Taylor & Francis
Taylor & Francis Group

MITOGENOME ANNOUNCEMENT

The complete mitochondria genome of *Parasarcophaga albiceps* (Diptera: Sarcophagidae)

Huidan Liao[1], Xiang Yang[2], Zhilong Li[3], Yanjun Ding[1], and Yadong Guo[1]

[1]*Department of Forensic Science, School of Basic Medical Sciences, Central South University, Changsha, Hunan, China,* [2]*Xiangya Medical College, Central South University, Changsha, Hunan, China, and* [3]*Department of Forensic Science, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, Sichuan, China*

Abstract

Parasarcophaga albiceps is one of the forensically important fly species which belongs to the family Sarcophagidae. In this study, we report the complete mitochondrial genome of *P. albiceps* to provide a supplemental data for species identification. The 14 935 bp-long mitogenome is composed of 13 protein-encoding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a non-coding AT-rich region. The permutation of the genes is in conformity with that observed in the ancestral arthropod. The overall base compositions of A, G, C and T are 39.24%, 9.70%, 14.44%, and 36.62%, respectively. Phylogenetic analysis shows the composition of the *P. albiceps* mitochondrial genome, which is very similar to that of another eight species of Sarcophagidae. The monophyletic branches of the phylogenetic tree reveal that complete mitochondrial genome 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. The complete mitochondrial genome of *P. albiceps* is supposed to make contributions to enriching the dipteran mitochondrial genomes and provide a potential tool for species identification.

Keywords

Mitochondria genome, *Parasarcophaga albiceps*, phylogenetic analysis, species identification

History

Received 22 September 2015
Revised 28 September 2015
Accepted 7 October 2015
Published online ▮▮▮

PROOF ONLY

Sarcophagid flies are the common type of insects found to be colonizer in the early stage of corpse decomposition (Jordaens et al., 2013). *Parasarcophaga albiceps* (Meigen, 1826) is one of the Sarcophagid species with a widely distribution in China (Guo et al., 2010). Unequivocal species identification of *P. albiceps* is a crucial step in the estimation of postmortem interval (PMI) (Guo et al., 2011). Considered that *P. albiceps* has the potential to be useful in forensic investigations, the complete mitochondrial genome sequence and phylogenetic analysis of *P. albiceps* is reported for species identification (GenBank accession no. KT444443).

Specimens of *P. albiceps* were collected in August 2015 in Changsha, China (28°9′N; 112°54′ E). The *P. albiceps* specimen has been assigned a unique code. The complete mitogenome was amplified in eight fragments and the circular dsDNA was sequenced by primer walking within each long PCR product (Nelson et al., 2012a). The PCR reaction volume was 20 µl, containing 0.5 µl of DNA, 0.5 µl of each primer, 1.0 µl (1.25 U) of TaKaRa MightyAmp Taq polymerase (Takara Co., Dalian, China), 10 µl of MightyAmp PCR Buffer, and 7.5 µl of sterilized distilled water. Amplifications were programmed with the following parameters: initial denaturation at 94 °C for 2 min; 35 cycles of 98 °C for 10 s, 50 °C for 30 s, and 68 °C for 2.5 min, and a final elongation at 68 °C for 10 min. DNA fragments were sequenced on both strands by the commercial service (Transduction Bio Co. Ltd., Wuhan, China). The obtained sequences have been deposited in GenBank by Sequin (http://www.ncbi.nlm.nih.gov/Sequin/index.html). The remains with unique codes will be preserved in our lab permanently.

The total 14 935 bp genome of *P. albiceps* was composed of 22 transfer RNA genes, 13 protein-coding genes, 2 ribosomal RNA genes, and a control region that can usually be observed in arthropod (Boore et al., 2005). The mitochondrial genome size, the order, and orientation of the genes are identical to that of the inferred ancestral arthropod genome (Clary & Wolstenholme, 1985). Similar to *Parasarcophaga similis* (Yan et al., 2014) and *Sarcophaga africa* (Fu et al., 2014), the 125 bp putative control region of *P. albiceps* was located between *12S rRNA* and *tRNA-Ile*. Based on the translations using the invertebrate mitochondrial code, 12 of the 13 protein-coding genes were identified with ATN as start codon coding for M except COI coding for S, which is different from the standard code (Nelson et al., 2012a; Weigl et al., 2010) but the same to the former result (Guo et al., 2014 ; Yan et al., 2014; Zhong et al., 2014).

We conducted a phylogenetic analysis using the complete mitochondria gene sequences from nine species of Sarcophagidae, along with two from family Calliphoridae as outgroup species. The 11 mt genomes sequence data from GenBank which are *P. albiceps*, *P. similis*, *S. africa*, *P. crassipalpis*, *H. melanura*, *S. peregrina*, *P. portschinskyi*, *S. impatiens,* and *R. pernix* of Sarcophagidae and two *L. cuprina* from family Calliphoridae (except *P. albiceps* the other genomes originally published in Fu et al., 2014; Guo et al., 2014 ; Nelson et al., 2012a,b; Ramakodi et al., 2015; Shi et al., 2014; Yan et al., 2014; Zhang et al.,2014; Zhong et al., 2014). Each of the 11 mt genes was aligned separately using Vector NTI 9.0. (Lu & Moriyama, 2004). Individually aligned gene datasets were translated into amino acid

Correspondence: Yadong Guo, Department of Forensic Science, School of Basic Medical Sciences, Central South University, Changsha 410013, Hunan, China. Tel: +86 731 82355414. Fax: +86 731 82355414. E-mail: gdy82@126.com

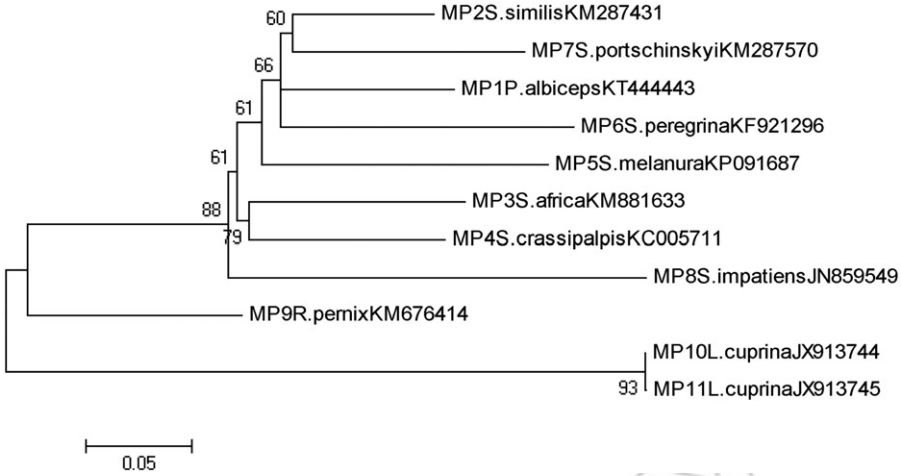

Figure 1. Neighbor-joining (NJ) tree of maximum composite likelihood method for the complete mitochondria gene sequences from 9 species of Sarcophagidae. Voucher ID, city, morphological species identification, and accession number are given in specimen label. Numbers on branches indicate the support value. Two *lucilia cuprina* from family Calliphoridae are included as the outgroup. Evolutionary distance divergence scale bar is 0.05.

sequences using MEGA 5 (Tamura et al., 2011). The evolutionary history was inferred using the neighbor-joining method (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the analyzed taxa (Felsenstein, 1985). Branches corresponding to partitions reproduced in <50% bootstrap replicates were collapsed. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (500 replicates), are shown next to the branches. The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004). Codon positions included were first + second + third noncoding.

The phylogeny of sarcophagine flies based on the complete mitochondria gene sequences was separated into different genetic clades (Figure 1). As outgroup, the two Calliphorid samples were clustered together and clearly separated from the sarcophagid mitotypes. The interspecific variation for the nine Sarcophagidae that is being studied ranged from 15.2 to 38.3%. Compared with the previous study findings (Guo et al., 2014; Zhang et al., 2015), the interspecific variation were obvious higher by the complete mitochondria gene analysis, which provided us the superior of using of the complete mitochondria gene as an identification tool for Sarcophagidae species to other genetic markers.

The utilization of sarcophagid species in the estimation of PMI has been severely restricted as most of these species are morphologically highly similar (Alessandrini et al., 2008) and *P. albiceps* is supposed to be one of the species of greatest forensic importance throughout the world (Zhang et al., 2015). In addition, no suitable key for the identification of the immature stages of sarcophagid flies exists (Wells et al., 2001). Molecular identification is viewed as a supplemental means to provide reliable species-specific identification regardless of insect life-cycle stages (Guo et al., 2012; Wells & Stevens, 2008). It has been well demonstrated that mitochondrial DNA (mtDNA) sequences could be successfully employed to distinguish some species of sarcophagid flies (Guo et al., 2011; Wells et al., 2001). This study provides the entire mitochondrial genome of *P. albiceps*, which is probably valuable to the database improvement of diptera and provide a potential tool or gene market selection for the forensic species identification.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This study was supported by the National Natural Science Foundation of China (No. 81302615) and the Central South University Student Innovation Test Plan (201510533329).

References

Alessandrini F, Mazzanti M, Onofri V, Turchi C, Tagliabracci A. (2008). Mt DNA analysis for genetic identification of forensically important insects. Foren Scie Int: Genet Suppl Ser 1:584–5.

Boore JL, Macey JR, Medina M. (2005). Sequencing and comparing whole mitochondrial genomes of animals. Methods Enzymol 395: 311–48.

Clary DO, Wolstenholme DR. (1985). The mitochondrial DNA molecular of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code. J Mol Evol 22:252–71.

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–91.

Fu X, Che K, Zhu Z, Liu J, Guo Y. (2014). The complete mitochondria genome of *Sarcophaga africa* (Diptera: Sarcophagidae). Mitochondrial DNA 24:1–2. [Epub ahead of print]. doi: 10.3109/19401736.2014.982582.

Guo J, Xie K, Che K, Hu Z, Guo Y. (2014a). The complete mitochondria genome of *Ravinia pernix* (Diptera: Sarcophagidae). Mitochondrial DNA 24:1–2. [Epub ahead of print]. doi: 10.3109/19401736.2014.982560.

Guo Y, Cai J, Chang Y, Li X, Liu Q, Wang X, Wang X, et al. (2011). Identification of forensically important Sarcophagid Flies (Diptera: Sarcophagidae) in China, based on *COI* and 16S rDNA gene sequences. J Foren Sci 56:1534–40.

Guo YD, Cai JF, Li X, Xiong F, Su RN, Chen FL, Liu QL, et al. (2010). Identification of the forensically important sarcophagid flies *Boerttcherisca peregrina, Parasarcophaga albiceps* and *Parasarcophaga dux* (Diptera: Sarcophagidae) based on COII gene in China. Trop Biomed 27:451–60.

Guo YD, Cai JF, Meng FM, Chang YF, Gu Y, Lan LM, Liang L, Wen JF. (2012). Identification of forensically important flesh flies based on a shorter fragment of the cytochrome oxidase subunit I gene in China. Med Veterin Entomol 26:307–13.

Guo Y, Zha L, Yan W, Li P, Cai J, Wu L. (2014b). Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) in China based on COI and period gene. Int J Legal Med 128:221–8.

Jordaens K, Sonet G, Richet R, Dupont E, Braet Y, Desmyter S. (2013). Identification of forensically important Sarcophaga species (Diptera: Sarcophagidae) using the mitochondrial COI gene. Int J Legal Med 127:491–504.

Lu G, Moriyama EN. (2004). Vector NTI, a balanced all-in-one sequence analysis suite. Brief Bioinf 5:378–88.

Nelson LA, Cameron SL, Yeates DK. (2012a). The complete mitochondrial genome of the flesh fly, *Sarcophaga impatiens* Walker (Diptera: Sarcophagidae). Mitochondrial DNA 23:42–3.

Nelson LA, Lambkin CL, Batterham P, Wallman JF, Dowton M, Whiting MF, Yeates DK, Cameron SL. (2012b). Beyond barcoding: A mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). Gene 511:131–42.

Ramakodi MP, Singh B, Wells JD, Guerrero F, Ray DA. (2015). A 454 sequencing approach to dipteran mitochondrial genome research. Genomics 105:53–60.

Saitou N, Nei. M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–25.

PROOF ONLY

Shi J, Guo Y, Zhang C, Zhou Y, Yan J, Liao H, Lagabaiyila Z. (2014). The complete mitochondrial genome of the flesh fly, *Parasarcophaga portschinskyi* (Diptera: Sarcophagidae). Mitochondrial DNA 16:1–2. [Epub ahead of print]. doi:10.3109/19401736.2014.971282.

Tamura K, Dudley J, Nei M, Kumar. S. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol E 28: 2731–9.

Tamura K, Nei M, Kumar. S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101:11030–5.

Weigl S, Testini G, Parisi A, Dantas-torres F, Traversa D, Colwell DD, Otranto D. (2010). The mitochondrial genome of the common cattle grub, *Hypoderma lineatum*. Med Veterin Entomol 24:329–35.

Wells JD, Pape T, Sperling FA. (2001). DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). J Forens Sci 46:1098–102.

Wells JD, Stevens JR. (2008). Application of DNA-based methods in forensic entomology. Annu Rev Entomol 53:103–20.

Yan J, Liao H, Zhu Z, Xie K, Guo Y, Cai J. (2014). The complete mitochondria genome of *Parasarcophaga similis* (Diptera: Sarcophagidae). Mitochondrial DNA 11:1–2. [Epub ahead of print]. doi:10.3109/19401736.2014.958708.

Zhang C, Fu X, Xie K, Yan W, Guo Y. (2015). MtDNA analysis for genetic identification of forensically important Sarcophagid Flies (Diptera: Sarcophagidae) in China. J Med Entomol. [Epub ahead of print]. doi: 10.1093/jme/tjv131.

Zhang C, Fu X, Zhu Z, Xie K, Guo Y. (2014). The complete mitochondrial genome sequence of *Helicophagella melanura* (Diptera: Sarcophagidae). Mitochondrial DNA 4:1–2. [Epub ahead of print]. doi:10.3109/19401736.2014.987258.

Zhong M, Wang X, Liu Q. (2014). The complete mitochondrial genome of the flesh fly, *Boettcherisca peregrine* (Diptera: Sarcophagidae). Mitochondrial DNA. [Epub ahead of print]. doi:10.3109/19401736.2013.873925.

PROOF ONLY