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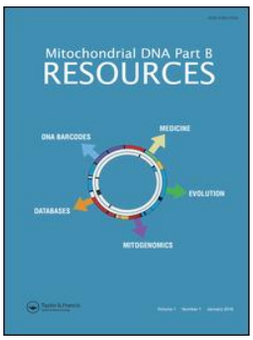

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MITOGENOME ANNOUNCEMENT

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Complete mitochondrial genomes of three species of fresh flies of forensic entomology interest from the genus *Sarcophaga* (Sarcophagidae) from Portugal and Brazil

Carmo Anderson Oliveira[a,b], Susanne Faccin[a], Patrícia J. Thyssen[c], Deodália Dias[b,d], Evanguedes Kalapothakis[a] and Maria Teresa Rebelo[b,d]

[a]Laboratório de Biotecnologia e Marcadores Moleculares, Universidade Federal de Minas Gerais, UFMG, Brazil; [b]CESAM – Centro de Estudos do Ambiente e do Mar Faculdade de Ciências da Universidade de Lisboa, Portugal; [c]Departmento de Biologia Animal, Universidade Estadual de Campinas, UNICAMP, Brazil; [d]Departamento de Biologia Animal Faculdade de Ciências da Universidade de Lisboa, Portugal

ABSTRACT

The Sarcophagidae family of fresh flies bears strong importance in the context of forensic entomology due to their application in the estimation of the Post Mortem Interval (PMI). *Sarcophaga* is the major genus in the Sarcophagidae family and includes cosmopolitan species, which are distributed worldwide. In this communication, we present the analysis of the complete mitochondrial genome (mtDNA) of two species from Portugal – *S. melanura* and *S. dux* – and one from Brazil – *S. ruficornis*. The mtDNA of these species range from 14,882 bp to 15,190 bp and have 22 tRNA genes, 13 protein-coding genes (PCG), and two rRNAs distributed along both strands. Our data include the first record of complete *Sarcophaga* mtDNA sequences from species collected in Portugal and in Brazil. These genomes represent an advance in the understanding about this group, expand the database, and can be used for the development of new markers for species identification.

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Forensic entomology; Sarcophaga; NGS; complete mtDNA; flesh flies

Sarcophagidae is one of the most important families in forensic entomology due to its widespread distribution and diversity of species (Pape 1996). The genus *Sarcophaga* is considered the most diverse, with 133 subgenera and 790 species described (Pape 1996). Species belonging to this genus are frequently used and represent an important source of information in forensic investigations (Moura et al. 1997; Carvalho et al. 2000; Oliveira et al., 2011; Rolo et al., 2013). The correct identification of species from the *Sarcophaga* genus is usually made using the male genitalia. However, in contrast with other genus, the females of some *Sarcophaga* species can be identified by their external morphology (Zhang et al. 2014). Nevertheless, morphological identification still presents its limitations, which hinder the identification of larval stages and dandified specimens. We show novel complete mtDNA sequences of flesh fly species of forensic importance. Our data expand the knowledge on the molecular database for the identification of these species.

In this work, we collected *Sarcophaga* specimens from two sites in Portugal and one in Brazil. In Portugal, *S dux* (38°35′02" N 9°10′30" W), and *S melanura* (38°42′52" N 9°11′45" W). *Sarcophaga ruficornis* was collected in Brazil (22°51′12" S 46°19′18" W). Each specimen was identified following the taxonomic key (Buenaventura and Pape 2015) and preserved in 70% ethanol. The mtDNA was extracted from the thorax, legs, and wings of the flies (Françoso et al. 2016), and the head and abdomen of *S. ruficornis* (Voucher 14) were deposited in the database of UNICAMP, whereas *S. dux* (Voucher 2) and *S melanura* (Voucher GE) were registered in the database of the Universidade de Lisboa. The mtDNA library was generated using the Nextera XT kit according to the manufacturer's instructions and sequenced using a paired-end strategy 2×250 on the MiSeq platform (Illumina, CA). The genomes were constructed by mapping the reads against the *Sarcophagidae* mitogenomes available on the NCBI database, followed by *de novo* assembly of the mapped reads using the CLC Genomics Workbench. The mitogenomes were annotated using the MITOS WebServer (Bernt et al. 2013) and manually verified using the NCBI database.

The complete mtDNA of *S. ruficornis* (MH879755) was 14,940 bp long and presented 24.3% of CG. *S. dux* (MH879759) mtDNA was 14,882 bp long and showed 23.8% of CG. *S. melanura* (MH879758) presented the longest mtDNA, with 15,190 bp and 24.4% of CG. The annotation of these genomes revealed 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes, and a noncoding control region (D-loop) located between the 12S rRNA and $tRNA^{Ile}$. The PCG sequences commonly start with an ATT, ATA, or ATG codon (12 PCGs), but COX1 starts with CAA. Five PCGs have the T–stop codon completed to TAA by posttranscriptional polyadenylation (Ojala et al. 1981). The light strand codifies eight of the 22 tRNAs, five PCGs and the two rRNAs, while the

CONTACT Evanguedes Kalapothakis kalapothakis@gmail.com Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil

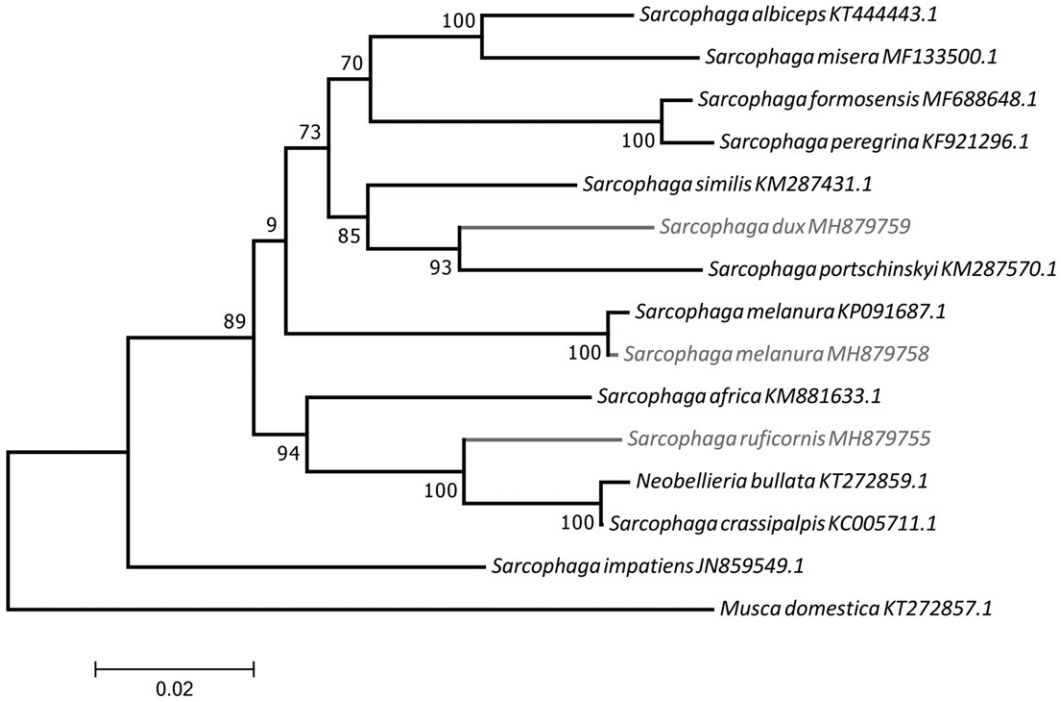

Figure 1. Molecular phylogenetic inferences of *Sarcophaga* mitogenomes. The phylogenetic analyses were made using the MEGA 7 software (Kumar et al.2016) using the maximum likelihood model with the Nearest-Neighbour-Interchange heuristic method. The D-loop region was excluded from this analysis due to its high degree of variability (Gonder et al.,2007). The tree showed a well-structured separation of the novel species. *S. melanura* (collected in Lisbon, Portugal) showed few differences in comparison with the species from NCBI collected in China (KP091687.1). *S. dux* (Aroeira, Portugal) is more related to *S. portschinskyi* (China). On the other hand, *S. ruficornis* (Brazil) clustered with the branch including *S. crassipalpis* and *N. bullata* (both from the United States). The novel mtDNA published herein are highlighted in grey. The mitogenomes for comparison were obtained from the NCBI database: *Musca domestica* (KT272857.1) – used as outgroup, *Sarcophaga impatiens* (JN859549.1), *Sarcophaga melanura* (KP091687.1), *Neobellieria bullata* (KT272859.1), *Sarcophaga crassipalpis* (KC005711.1), *Sarcophaga peregrina* (KF921296.1), *Sarcophaga formosensis* (MF688648.1), *Sarcophaga africa* (KM881633.1), *Sarcophaga portschinskyi* (KM287570.1), *Sarcophaga similis* (KM287431.1), *Sarcophaga albiceps* (KT444443.1), *Sarcophaga misera* (MF133500.1).

remaining genes are encoded on the heavy strand. The phylogenetic analysis and comparison with others *Sarcophaga* species are showed in Figure 1.

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Disclosure statement

The authors report no potential conflict of interest.

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ORCID

Carmo Anderson Oliveira http://orcid.org/0000-0003-4646-513X
Susanne Faccin http://orcid.org/0000-0003-2145-0396
Patrícia J. Thyssen http://orcid.org/0000-0001-7343-2419
Deodália Dias http://orcid.org/0000-0002-8771-6727
Evanguedes Kalapothakis http://orcid.org/0000-0002-8326-249X
Maria Teresa Rebelo http://orcid.org/0000-0002-2724-2195

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