Phylogenetic relationships of the Sarcophagidae (Diptera), using three mitochondrial loci (COI, COII, and ND4) and one nuclear locus (PER).

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Abstract

The family Sarcophagidae (Diptera) contains species of agricultural, forensic, and medical importance. However, relationships within the family are still poorly understood.
In order to provide greater insight into deeper phylogenetic nodes in these groups, we analyze sequence data for three mitochondrial genes [cytochrome oxidase I (COI), cytochrome oxidase II (COII), dehydrogenase subunit four (ND4)], and one nuclear gene, period (PER), from 43 species collected across the US. Together these loci comprise approximately 3800 bp of combined mtDNA and nDNA (COI 1929 bp, COII 637 bp, ND4 692 bp, PER 530 bp). Maximum Parsimony, Maximum Likelihood, and Bayesian Inference results are compared and contrasted amongst the various loci. We specifically compare our molecular-based results to both the classical, morphology-based taxonomic subdivisions and recent morphological phylogenetic studies.

Introduction

• Identification of species and relationships within groups is crucial in any molecular evolutionary study. It becomes central when we work on carrion flies which have great forensic importance - specifically, in the estimation of the postmortem interval (PMI).

• Our previous data set for phylogenetic analysis of the Sarcophagidae included only mtDNA (COI, COII and ND4). It provided good resolution for most nodes.

• The mtDNA gene tree might not be identical to the species tree, so it is necessary to obtain additional data from nuclear genes. Such new data might increase the support for the mtDNA tree or support a different phylogeny (e.g., Gibson et al., 2010).

• Existing sequence data for nuclear genes from Sarcophagidae are limited, but previous work exists on the circadian rhythm gene *period* (PER) in *Sarcophaga bullata* (Han, 2008). We used those data to design PCR primers, and here we report analyses that combine PER with our existing mtDNA data.

Objectives

1. Assess the impact of adding PER data to our mtDNA phylogeny
2. Test some novel phylogenetic hypotheses that were suggested by our mtDNA-only analysis

Materials and Methods

•We used one specimen from each of the 43 species of Sarcophagidae collected from different geographical locations across the continental U.S., plus two calliphorid outgroups.

• Specimens were preserved in 100%-EtOH at -80 C; one or two legs from each specimen were taken for DNA extraction by the Qiagen DNA extraction method (Qiagen's DNeasy kit, www.qiagen.com, Qiagen 2003-2010).

•Approximately 2.3kb of the mitochondrial cytochrome oxidase I and II (COI and COII) genes, 640bp of the ND4 gene and 530 bp of PER gene were amplified using PCR.

•The mtDNA was amplified by PCR in segments of ca. 1,000 bp, using our own PCR primers modified from standard, highly conserved dipteran primers (Simon et al., 1994 and others). Each segment was sequenced in both directions.

•Sequences were aligned using the following programs: BioEdit v.7.0.5 (Tom Hall 1999-2005 www.mbio.ncsu.edu/bioedit/bioedit.html), FinchTV v.1.4.0 (Geospiza 1997-2010, www.geospiza.com) and Mesquite v.2.73 and 2.74 (Wayne P. Maddison & David R. Maddison 1999-2010 http://mesquiteproject.org/mesquite/mesquite.html)

•Models and partitioning schemes were chosen based on Bayes' Factors, using the Stepping-Stone method (Kimura & Weiss, 1964) implemented in Phycas v. 1.2 (www.phycas.org). Bayesian inference was performed using MrBayes 3.1.2 (http://mrbayes.csit.fsu.edu/). MCMC analyses were run for 10^7 cycles, with trees sampled every 500 cycles. The first ¼ of the sampled trees were discarded as burn-in.

Results

Approximately 3kb of mtDNA data (2.3 kb from COI and COII, 640bp from ND4) were obtained for 43 sarcophagids plus 2 outgroups. Those data provide good resolution of most of the Sarcophagidae, with posterior probabilities > 95% (Fig.1).

We have, so far, obtained 530 bp of sequence data for PER for 22 species (shown in green in Fig. 1). In most cases, addition of PER maintains or improves support in the Bayesian Inference tree (Table 1).

We find strong support for several previously proposed relationships:
Monophyly of the subfamilies Sarcophaginae and Miltogramminae
Monophyly of all genera for which we include multiple species, including *Sarcophaga*
A sister-group relationship between *Oxysarcodexia* and *Ravinia*

We also find support for some novel relationships:
A clade comprising *Blaesoxipha*, *Fletcherimyia*, and *Mecynocorpus* (PP = 1.0)
A clade comprising *Boettcheria*, *Tripanurga*, and *Tricharaea* (PP = 0.99)
A sister-group relationship between *Sarcophaga* and *Peckia* (PP = 0.84)

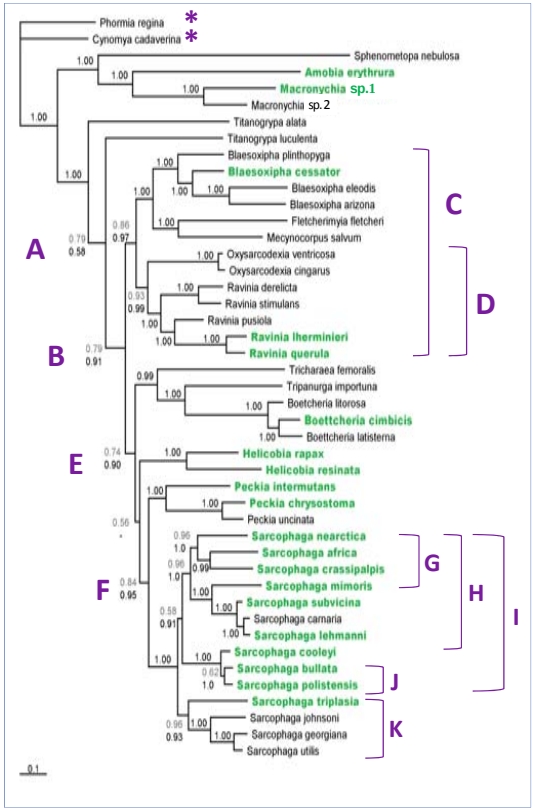

Figure 1. The current COI+COII+ND4+PER tree constructed for 45 taxa based on Bayesian estimation of phylogeny. (** = Calliphoridae. Values in *light-grey* are the Posterior Probabilities received for mtDNA-tree only, while *full dark* values are the Posterior Probabilities for the combined mtDNA+PER tree. The species *in green* are the ones for which we have data from PER gene. Clades' letters *in purple* correspond to those in Table 1.)

Results (continued)

Clades		Posterior probabilities	
		COI+COII+ND4	COI+COII+ND4+ PER
A	Paraphyly of *Titanogrypa*	0.79	0.58
B	*Titanogrypa* + all sister groups	0.79	0.91
C	*Blaesoxipha* + *Ravinia*	0.86	0.97
D	*Oxysarcodexia* + *Ravinia*	0.93	0.99
E	From *Boettcheria* to *Sarcophaga*	0.74	0.90
F	*Peckia* + *Sarcophaga*	0.84	0.95
G	*Sarcophaga africa* + *S. crassipalpis* + *S. nearctica* (= group 1)	0.96	1.0
H	Group1 + Group2 (*S. mimoris* through *S. carnaria*)	0.96	1.0
I	Group1 + Group2 + *S.bullata*'s group	0.58	0.91
J	*S. bullata* + *S. polistensis*	0.62	1.0
K	*S. triplasia* through *S. georgiana*	0.96	0.93

Table 1. The changes in the posterior probabilities among groups after adding PER sequences to the mtDNA-tree.

Conclusions

• Mitochondrial data provides good resolution for most nodes in our sampling of the Sarcophagidae.

• Adding data from the nuclear gene PER increases support for most nodes.

• A thorough understanding of sarcophagid phylogeny will require sampling additional species as well as additional nuclear loci.

Future Work

• Explore additional nuclear loci.

• Sample >1 individual per species – to allow use of coalescent-based species tree methods.

• Increase taxonomic coverage.

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Acknowledgments

This project was supported by:
•Grant No. 2005-DA-BX-K102 (National Institute of Justice, Office of Justice Programs, US Department of Justice). The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect the views of the Department of Justice.
•The University Research Council's Undergraduate Student Research Fellowship program (University of Cincinnati).
•Department of Biological Sciences, University of Cincinnati.