

Short Communication

Sequence Change and Phylogenetic Signal in Muscoid COII DNA Sequences

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The complete DNA sequence of the mtDNA cytochrome oxidase II gene from house fly, *Musca domestica*, face fly, *Musca autumnalis*, stable fly, *Stomoxys calcitrans*, horn fly, *Haematobia irritans*, and black garbage fly, *Hydrotaea aenescens*, are reported. The nucleotide sequence codes for a 229 amino acid peptide. The COII sequence is A + T rich (74.1%), with up to 12.3% nucleotide and 8.4% amino acid divergence among the five taxa. Of the 688 nucleotides encoding for the gene, 135 nucleotide sites (19.6%) are variable, and 55 (8.0%) are phylogenetically informative. A phylogenetic analysis using three calliphorids as the outgroup taxa, indicates that the two haematophagus species, horn fly and stable fly, form a sister group.

Keywords: Muscidae; Diptera; mtDNA; Cytochrome Oxidase II

Mitochondrial genes have been studied increasingly because of the ease of recovering genetic information that may be useful for investigating molecular and organismal evolution. The predominance of maternal inheritance, lack of extensive recombination, and accelerated rates of nucleotide substitution are features that have favored the use of mtDNA as an informative evolutionary marker.

Most studies of dipteran species are related to the occurrence of flies of medical and economic importance. The Dipteran family Muscidae has several important subfamilies including the Stomoxyinae, Muscinae, and Phaoniinae. Flies of the subfamily Stomoxyinae have a slender, strongly sclerotized proboscis and feed on the blood of warm-blooded vertebrates. There are three genera in the subfamily, *Haematobia* Lepeletier and Serville,

Lyperosiops Townsend, and *Stomoxys* Geoffroy. The most important species in North America are the horn fly, *Haematobia irritans* (L.), a pest of cattle, and the stable fly, *Stomoxys calcitrans* (L.), a pest of humans and livestock. Flies of the subfamily Muscinae are ubiquitous. Species of the genus *Musca* Linnaeus, notably the house fly, *Musca domestica* L. are important as pests and disseminators of disease. The other species of *Musca*, *M. autumnalis* De Geer, is an important pest of livestock in the United States since its introduction in 1952. The black dump fly, *Hydrotaea aenescens* (Wiedemann) is a member of the subfamily Phaoniinae. The larvae are commonly found in association with decaying vegetable and animal matter, and the black dump fly has been used extensively for biology control of house flies (Turner *et al.*, 1992).

In this work, we describe nucleotide and amino acid variation among 5 economically important muscid species representing 3 subfamilies for the mtDNA COII gene. These results should allow the identification of species-specific genetic markers, and analysis of phylogenetic information for understanding dipteran evolution.

DNA sequencing of the amplicon revealed that it averaged 780 bp in size. The 3' portion of the COI gene along with the tRNA leucine were removed from the DNA sequence leaving 688 bp of the COII gene (Fig. 1). The average base frequencies were A = 0.33, C = 0.13, G = 0.13, and T = 0.41. Pairwise Tajima-Nei distances (Tajima and Nei, 1984) among the muscid taxa ranged from 8 to 12% (Table I). Genetic divergence between *Musca domestica* and

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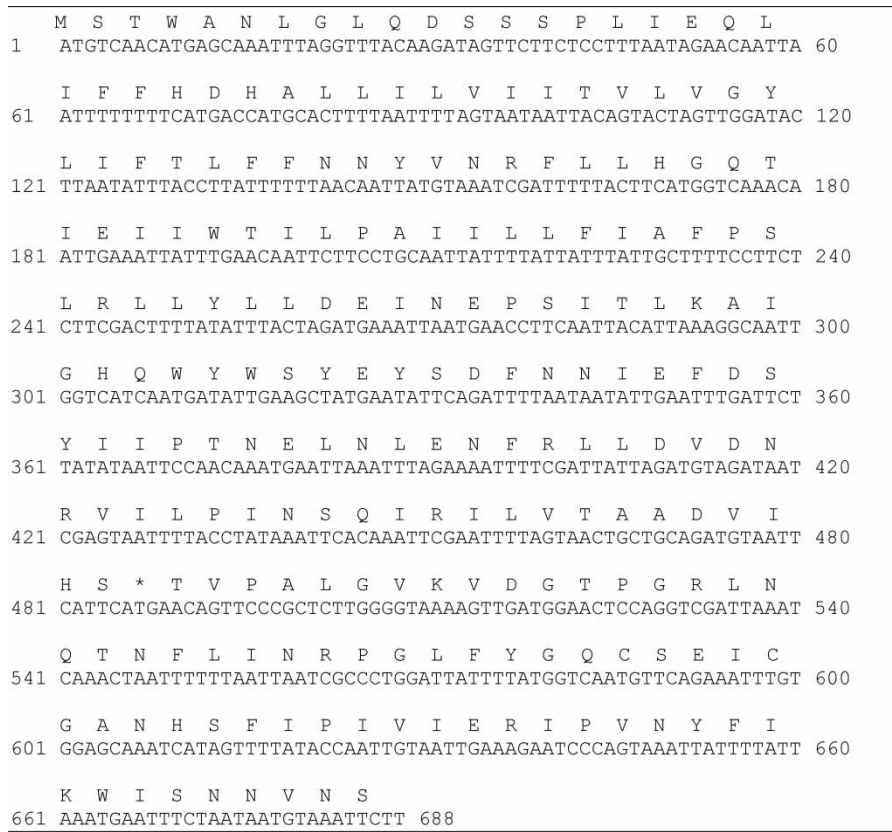


FIGURE 1 The nucleotide sequence and predicted amino acid sequence of *Stomoxys calcitrans*. Alcohol preserved specimens were allowed to dry on filter paper, and DNA was extracted from individual thoraces using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris-EDTA and stored at -20°C. Polymerase chain reaction (PCR) was conducted using the primers TL2-J-3037 (5'-ATGGCAGATTAGTGCAATGG-3') designed by Liu and Beckenbach (1992) and described by Simon *et al.* (1994) and Miura *et al.* (1998), and primer TK-N-3785 (5'-GTTTAAGAGACCAGTACTTG-3') from Simon *et al.* (1994). These primers amplify a 3' portion of the mtDNA COI gene, tRNA-Leu, and the entire COII gene. PCR reactions were conducted using 1 µl of the extracted DNA (Szalanski *et al.*, 2000a,b), with a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. PCR product was resolved on 1% agarose gels per Taylor *et al.* (1996). Amplified DNA from individual flies was purified, and concentrated using Microcon-PCR Filter Units (Millipore, Bedford, MA). Samples were sent to The University of Arkansas DNA Sequencing Facility (Fayetteville, AR) for direct sequencing on both strands using an ABI Prism 377 DNA sequencer. GenBank accession numbers for the flies subjected to DNA sequencing in this study are AY184815 to AY184819.

M. autumnalis was 0.8% which falls in the levels of genetic divergence observed among species of the globeflower fly, *Chiastocheta* spp. for COI and COII (Després *et al.*, 2002). The nucleotide sequence codes for a 229 amino acid peptide (Fig. 1). Up to 19 of the amino acids were variable, with amino acid divergence ranging from 2.1 to 8.4% (Table I). The aligned DNA data matrix, including the outgroup taxa (available at TreeBASE, <http://www.treebase.org>, study accession number SN1281) resulted in a total

of 688 characters. Of these characters, 180 (26%) were variable and 105 (15%) were phylogenetically informative. Maximum likelihood analysis (Fig. 2), of the aligned Muscidae species and the outgroup taxon resulted in a consensus tree with several distinct branches. The horn fly and stable fly formed a sister group, as did the two two *Musca* species.

In this study, we have successfully amplified and sequenced the mtDNA COII gene from five muscid fly species. The COII gene was 688 bp pair in length, codes for a 229 amino acid protein and was 74% AT rich. The COII gene length of 688bp is shared with *Cochliomyia hominivorax* and *Drosophila yakuba* (Lessinger *et al.*, 2000). The A + T content of COII gene from the muscid flies, was similar to other Diptera including *Cochliomyia hominivorax* (Diptera: Calliphoridae), *D. yakuba* (Diptera: Drosophilidae), and *Chiastocheta* spp. (Diptera: Anthomyiidae) which are 72.5, 73.9, and 73.6% AT rich, respectively (Lessinger *et al.*, 2000; Després *et al.*, 2002). The protein size of 229 amino acids for the muscid flies

TABLE I Nucleotide substitution rates (below the diagonal) and amino acid substitution rates (above the diagonal) among 5 muscid species

Species	1	2	3	4	5
1 <i>M. autumnalis</i>	-	0.02	0.05	0.06	0.08
2 <i>M. domestica</i>	0.08	-	0.04	0.05	0.07
3 <i>H. irritans</i>	0.10	0.09	-	0.04	0.06
4 <i>S. calcitrans</i>	0.12	0.10	0.08	-	0.05
5 <i>H. aeneszens</i>	0.12	0.10	0.09	0.11	-

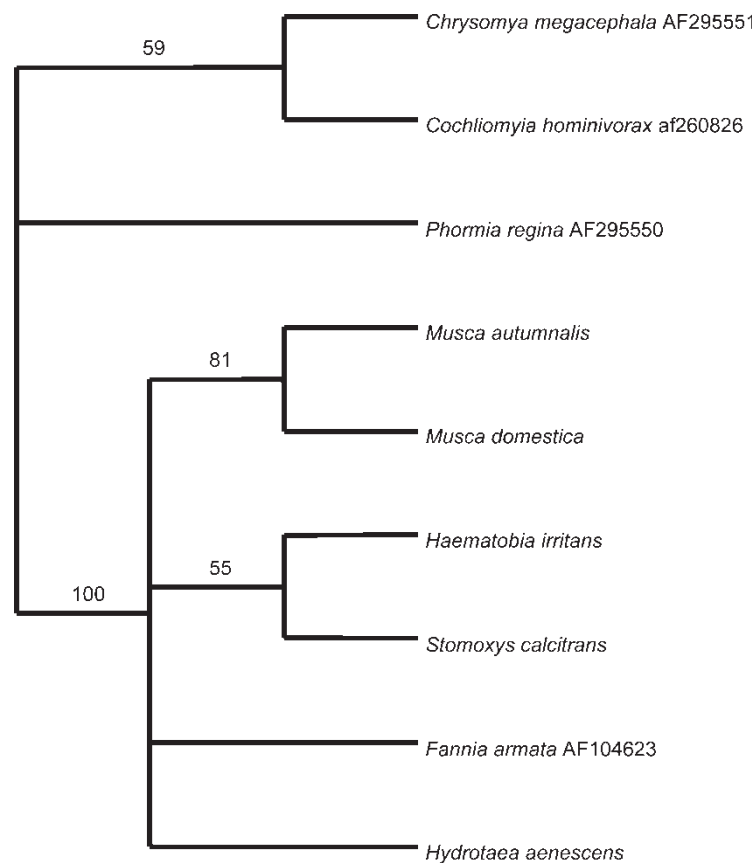


FIGURE 2 A phylogenetic tree for six muscid species based on DNA sequence analysis of the mtDNA COII gene, derived from maximum likelihood analysis ($-\ln L = 2448.72110$). Bootstrap values are provided along with GenBank accession numbers from sequences obtained from GenBank. The distance matrix option of PAUP* 4.0b8 (Swofford, 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura, 1980) of sequence evolution. The three calliphorid species, *Chrysomya megacephala*, *Cochliomyia hominivorax*, and *Phormia regina* were used as the outgroup. DNA sequences were aligned using the PILEUP program in GCG (Genetics Computer Group, Madison, WI) and adjusted manually. Maximum likelihood analysis on the alignments were conducted using PAUP* 4.0b8 (Swofford, 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein, 1985). For maximum likelihood analysis (Yang, 1994), the default likelihood parameter settings were used (HKY85 6-parameter model of nucleotide substitution, empirical base frequencies, and transition/transversion ratio set to 2:1). These parameters were used to carry out a branch and bound search using PAUP*.

was similar to *C. hominivorax* (228 a.a.) and *D. yakuba* (227 a.a.) (Lessinger *et al.*, 2000).

Results of the present study were congruent with those derived from morphological classification (Stone *et al.*, 1965) by supporting the classification of horn fly and stable fly in the subfamily Stomoxyinae. Our study confirmed the phylogenetic relationship between the two haematophagus species, stable fly and horn fly. Despite their importance as pest species, the phylogenetic relationships among Muscids using molecular markers is poorly understood. Bernasconi *et al.* (2000) evaluated a 1100bp region of the entire mtDNA COI and a 5' portion of the COII gene for a phylogenetic analysis of the Muscoidea, using only two muscids, *M. domestica* and *Fannia armata*. Vossbrinck and Friedman (1989) used partial 28S rRNA sequences for a phylogeny of cyclorrhaphous Diptera including *Stomoxys calcitrans*, *Musca domestica*, *Glossina simulans* and *Fannia scalaris*. The only supported relationship among these taxa was

S. calcitrans and *M. domestica* forming a sister group. Our study found the studied muscid taxa to be monophyletic relative to *Cochliomyia hominivorax*. Because of the limited number of taxa used, we cannot confirm the classification of the other subfamilies used in this study.

This study provides a baseline for the phylogenetic relationships of the economically important family. The COII marker contains adequate information for phylogenetic assessment of the five muscids studied and should prove useful for understanding the relationships of other muscids, and could provide the basis for species specific molecular diagnostic markers.

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