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Short Communication

Molecular phylogeny of the *Drosophila tripunctata* and closely related species groups (Diptera: Drosophilidae)

Luciane Mendes Hatadani^a, James O. McInerney^b, Hermes Fonseca de Medeiros^a, Ana Carolina Martins Junqueira^c, Ana Maria de Azeredo-Espin^{a,c}, Louis Bernard Klaczko^{a,*}

^a Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), P.O. Box 6109, Campinas, SP 13083-970, Brazil

^b Bioinformatics Laboratory, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland

^c Laboratório de Genética Animal, Centro de Biologia Molecular e Engenharia Genética e Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), P.O. Box 6010, Campinas, SP 13083-875, Brazil

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1. Introduction

The Drosophila tripunctata species group is presently the second largest Neotropical group of Drosophila (surpassed by the D. repleta group), comprising 78 species according to the Taxodros database of May 2008 (Bächli, 2008). Frota-Pessoa (1954) subdivided the group into four subgroups (I–IV) based on morphological characters. The D. tripunctata group is almost endemic to the Neotropics (Throckmorton, 1975), where its species are abundant, particularly in forest areas, and a dominant component of the drosophilid fauna (Ashburner et al., 2005; Klaczko, 2006).

The *tripunctata* radiation was created by Throckmorton (1975) and included the *tripunctata* group, as well as other groups (*calloptera, cardini, guarani, macroptera, pallidipenis, rubrifrons* and *sticta*). According to this author, a radiation which he called *immigrans-Hirtodrosophila* originated in the Paleotropics, where it initially diversified and from where it sent two separate lineages to the Neotropics: *tripunctata* (composing the *tripunctata* radiation) and *Hirtodrosophila*. Throckmorton (1975) also suggested that the *tripunctata* group itself should not be considered a monophyletic group. This statement is in agreement with cytological observations (Kastritsis et al., 1970) and recent molecular studies (Yotoko et al., 2003; Robe et al., 2005). The monophyly of the *tripunctata*

E-mail address: lbk@unicamp.br (L.B. Klaczko).

ABSTRACT

We suggest a new phylogenetic hypothesis for the *tripunctata* radiation based on sequences of mitochondrial genes. Phylogenetic trees were reconstructed by parsimony, maximum likelihood and Bayesian methods. We performed tests for hypotheses of monophyly for taxonomic groups and other specific hypotheses. Results reject the monophyly for the *tripunctata* group whereas monophyly is not rejected for the *tripunctata* radiation and other specific groups within the radiation. Although most of the basal nodes were unresolved we were able to identify four clusters within the *tripunctata* radiation. These results suggest the collection of additional data before a proper taxonomic revision could be proposed. © 2009 Elsevier Inc. All rights reserved.

> radiation as a whole has also been questioned (Remsen and O'Grady, 2002; Robe et al., 2005; Yotoko et al., 2003). In general, phylogenetic relationships among species belonging to the *tripunctata* radiation have been poorly studied, which has been pointed out by Markow and O'Grady (2006). Even though it seems clear that the *tripunctata* group is not monophyletic, the monophyly of the *tripunctata* radiation is still unresolved. Moreover, the studies mentioned previously were unable to recover a well supported phylogenetic hypothesis for relationships among the species groups within the radiation.

> In this paper, we propose a new phylogenetic hypothesis for species of the *tripunctata* radiation of *Drosophila* based on sequences of mitochondrial genes of cytochrome oxidase subunits 1 and 2 (COI and COII) and test for different evolutionary hypotheses. Our aim was to improve the results obtained by Yotoko et al. (2003) and Robe et al. (2005) by adding both taxa and characters. In addition, we tested for monophyly of taxonomic groups (*calloptera*, *cardini*, *guarani* and *tripunctata*), of the *tripunctata* radiation, and of specific clades that appeared on the phylogenetic trees as monophyletic.

2. Materials and methods

2.1. COI and COII sequence data

Specific location of collection and taxonomic placement of each species are given in Table 1. All individuals included in the analysis

^{*} Corresponding author. Fax: +55 19 3521 6235.

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Table 1

Taxonomic placement and collection site of each species collected for DNA extraction and accession numbers of all sequences included in the phylogenetic analyses.

Subgenus	Group	Subgroup	Collection Site ^a	Species	Accession Number	
					COI	COII
Drosophila	calloptera		Mata Ribeirão Cachoeira — Bosque dos Jequitibás	D. atrata D. ornatipenis D. schildi	EF569988 * EF570010 * EF570016 *	EF570024 EF570038 AY162973
	cardini		Serra do Japi Brasilia Bosque dos Jequitibás Serra do Japi	D. cardini D. cardinoides D. neocardini D. polymorpha	EF569991 * EF569992 * EF570006 * EF570014 *	AY162974 AY162975 EF570034 EF570040
	guarani	guaramunu	Serra do Japi Serra do Japi Serra do Japi	D. griseolineata D. guaraja D. maculifrons	EF569995 * EF569996 * EF569998 *	EF570029 EF570030 EF570031
		guarani	Mata Ribeirão Cachoeira Serra do Japi	D. guaru D. ornatifrons	EF569997 * EF570009 *	EF570031 AY162978
	pallidipenis		Serra do Japi	D. pallidipenis	EF570011 *	AY162982
	sticta		Mata Ribeirão Cachoeira	D. sticta	EF570018 *	EF570044
	tripunctata	I	Mata Ribeirão Cachoeira Mata Ribeirão Cachoeira Bosque dos Jequitibás Serra do Japi	D. nappae D. neoguaramunu D. setula SP22 ^b	EF570005 * EF570007 * EF570022 * EF570017 *	AY162983 EF570036 EF570042 EF570043
		ΙΙ	Serra do Japi Mata Ribeirão Cachoeira Serra do Japi Serra do Japi Serra do Japi Serra do Japi Mata Ribeirão Cachoeira	D. cuaso D. medioimpressa D. mediopunctata D. mediosignata D. paraguayensis D. roehrae D. unipunctata	EF569993 EF569999 EF570001 EF570002 EF570012 EF570015 EF570020	EF570027 AY162994 AY162988 AY162985 EF570039 EF570041 EF570047
		III	Serra do Japi Bosque dos Jequitibás Mata Ribeirão Cachoeira Serra do Japi Serra do Japi Mata Ribeirão Cachoeira Serra do Japi Mata Ribeirão Cachoeira Bosque dos Joquitibás	D. bandeirantorum D. bifilum D. frotapessoai D. mediopicta D. mediostriata D. nigricincta D. paramediostriata D. trifilum	EF569989 EF569990 EF569994 EF570000 EF570003 EF570008 EF570013 EF570019	EF570025 EF570026 EF570028 EF570033 EF570034 EF570037 AY162995 EF570046
		IV	Bosque dos jequitidas	D. metzii D. tripunctata	EF570004 FF570023 *	AY 162992 AF519343
	quinaria			D. falleni D. innubila D. quinaria D. recens D. subquinaria	AY541136 AY541192 AY154400 AY154456 AY154457	AF147117 AY541211 AF478428 AF147123 AY154457
	immigrans repleta	immigrans hydei	Mata Ribeirão Cachoeira — —	D. immigrans D. eohydei D. hydei	EF570021* DQ471601 DQ471602	AY162993 AF145889 DQ202020
Sophophora	melanogaster	melanogaster	- - - -	D. melanogaster D. mauritiana D. sechellia D. simulans D. yakuba	NC001709 NC005779 NC005780 NC005781 NC001322	NC005779 NC001709 NC005780 AF474082 NC001322

^a Coordinates for each collection site are: 22°55' S, 47°03' W (Bosque dos Jequitibás, Campinas, SP); 15°46 S 47°55 W (Brasília, DF); 22°50' S, 46°55' W (Mata Ribeirão Cachoeira, Campinas, SP); 23°13' S, 46°53' W (Serra do Japi, Jundiaí, SP).

^b Undescribed species. SP22 and *D. nappae* are sibling species.

* New sequences obtained in this study.

were adult males, identified by the aedeagus, the most reliable method of identification of these species (Vilela, 1992). In addition, prior to DNA extraction, the terminalia of each male was removed and preserved in 70% alcohol. This procedure would allow for future confirmation of species identification and reevaluation in case of taxonomic revisions.

Total DNA of each individual was extracted using a phenol–chloroform protocol (Azeredo-Espin et al., 1991). The primers used for amplification were TL2-N-3014 and C1-J-2195 (COI), and TL-2-J3037 and TK-N-3785 (COII), described in Simon et al. (1994). The amplified products were purified with the QIAquick PCR purification kit. With the exceptions of the 5' fragment of COI of *D. guaru*, *D. trifilum*, *D. maculifrons* and *D. setula*, and COII of *D. mediostriata*—in which case PCR products were cloned into the PCR2.1 cloning vector using a TA Cloning Kit (Invitrogen)—all PCR products were directly sequenced. Sequencing was performed using BigDye (Applied Biosystems) chemistry on either an ABI377A or an ABI3700 automatic sequencer. At least two sequences of each fragment were obtained for each individual to ensure high quality of sequences. Except for the cloned samples (three clones per species), for which we used primers provided by the Cloning Kit, the primers used for sequencing were the same as in PCR. All resulting sequence chromatograms were evaluated and edited with the use of the programs Phred (Ewing et al., 1998), Phrap and Consed (Gordon et al., 1998).

Additional sequences were obtained from GenBank whereas individuals of *D. ornatipenis* were obtained from Tucson Fly Stock Download English Version:

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