



Molecular systematics applied to Phlebotomine sandflies: Review and perspectives



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ABSTRACT

A review of the literature related to the molecular systematics of the Phlebotomine sandflies (Diptera, Psychodidae) is proposed. It shows that molecular systematics is more frequently used to perform evolutionary systematics than to help in the field of alpha taxonomy. On more than 900 living species and subspecies described, 180 (about 20%) have been processed for molecular systematics. The countries of origin where the sandflies processed come from are endemic for leishmaniasis and the ratio of species sampled for molecular systematics studies is high for vector groups and low for species not involved in the transmission of leishmaniasis. The main studies focused on intraspecific topics, others on closely related species, and a few compared genera of sandflies. Mitochondrial markers (more than 50% of the markers studied) are preferred to non mitochondrial markers. The use of mtDNA markers alone to explore phylogenetic relationships is considered as dangerous, especially concerning closely related species.

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1. Introduction

DNA markers are interesting characters for Phlebotomine sandflies (Diptera, Psychodidae) systematics, as well as for many other groups of vectors, parasites, animals, plants, fungi, and bacteria. Their first application to the systematics of Phlebotomine sandflies was done by pioneers in the 1990's (Adamson et al., 1991; Booth et al., 1991; Adamson et al., 1993; Maingon et al., 1993; Zeledon et al., 1993; Booth et al., 1996; Esseghir et al., 1997; Friedrich and Tautz, 1997; Depaquit et al., 1998; Dias et al., 1998). They constitute powerful tools to define populations with evident consequences to characterise vectors and non-vectors, to emphasise cryptic species, to associate males with females in a same species or to propose evolutionary systematics.

These molecular approaches tend to supplant the traditional morphological ones in the field of systematics for several reasons. The latter is longer and more difficult to carry out than the former, and this independently of the application (alpha taxonomy or phylogenetical systematics). Nevertheless, it is clear that phylogenetical studies between not closely related species, belonging to many genera, from different continents, require a perfect

knowledge of the group and can only be considered by some taxonomists who have an important background in the field. Moreover, the expensive cost of molecular techniques has dropped dramatically over the past 15 years. These tools are used routinely in many laboratories and do not require an important background on Phlebotomine sandflies. Lastly, molecular studies are more easily publishable than morphological studies and are appreciated by journal's editors. However, this facility is only apparent and many pitfalls remain.

Three families of molecules were processed for sandflies systematics:

- Proteins like isoenzymes (Miles and Ward, 1978; Caillard et al., 1986; Ryan et al., 1986; Perrotti et al., 1991; Pesson et al., 1991; Zhang and Leng, 1991; Lanzaro et al., 1993; Maingon et al., 1993; Zeledon et al., 1993; Dujardin et al., 1996; Remy-Kristensen et al., 1996; Dujardin et al., 1997; Mukhopadhyay et al., 1998; Munstermann et al., 1998; Benabdennbi et al., 1999; Kassem et al., 1999; Lampo et al., 1999; Arrivillaga et al., 2000; Perrotey et al., 2000; Feliciangeli and Lampo, 2001; Marquez et al., 2001; Mukhopadhyay et al., 2001; Zhang and Leng, 2002; Aransay et al., 2003; Arrivillaga et al., 2003; Torgerson et al., 2003; Belen et al., 2004; Dujardin et al., 2004; Pesson et al., 2004; Meneses et al., 2005; Perrotey et al., 2005; Boussaa et al., 2008a,b; Hernández et al., 2008;

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Arrivillaga and Marrero, 2009; Boussaa et al., 2009) and more recently an analysis of the complete proteome using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) (Dvorak et al., 2014).

- Cuticular hydrocarbons (Ryan et al., 1986; Kamhawi et al., 1987; Phillips et al., 1990a,b; Mahamat and Hassanali, 1998) and chemical molecules involved in the communication between species, like pheromones (Lane et al., 1985; Ward et al., 1991; Zeledon et al., 1993; Dujardin et al., 1997; Mahamat and Hassanali, 1998; Bauzer et al., 2002a,b; Maingon et al., 2003; Hamilton et al., 2005; Watts et al., 2005; Salomon et al., 2010; Vigoder et al., 2010).
- DNA sequences focusing on the heart of this work.

The use of the adjective molecular does not refer in routine to the use of any molecules for systematics. In fact, in its current application, it is restricted to DNA markers. We follow this convention in this article proposing an analysis of the literature concerning the molecular systematics of Phlebotomine sandflies.

2. Material and methods

The key words selected to find references were: molecular, systematics, phylogeny, barcoding, Psychodidae, Phlebotomine, sandfly, sand fly, sandflies, sand flies, *Phlebotomus*, *Lutzomyia*, and *Sergentomyia*. The databases selected were PubMed, (National Center for Biotechnology Information (NCBI), National Library of Medicine, NIH) (<http://www.ncbi.nlm.nih.gov/pubmed>), Armed Forces Pest Management Board Literature Retrieval System (<http://www.afpmb.org/content/literature-retrieval-system>), and my personal database. The last search was conducted on August, 22nd 2014.

For each publication, the molecular markers selected, the taxa processed, their geographic origin (countries) and the taxonomic goal have been registered. Concerning the latter approach, we divided the goals in alpha taxonomy and evolutionary systematics subdivided in different levels of analysis: intraspecific, interspecific within a genus, and intergeneric.

We used the abbreviations for the genera and subgenera of sandflies (Marcondes, 2007).

3. Results

The publications included in the present study focus on different goals.

Regarding the taxonomy, a few publications focus on alpha-taxonomy. A first category includes description of new taxa for Science using molecular biology to be sure that males and females belong to the same taxa (Depaquit et al., 2007, 2008a, 2009; Muller et al., 2007; Léger et al., 2012, 2014; Zapata et al., 2012a; Randrianambinintsoa et al., 2013). A second category includes publications describing new species for Science on one gender only and sequences are provided as a tool for a future description of the hitherto unknown gender (Depaquit et al., 2004b; Randrianambinintsoa et al., 2012; Randrianambinintsoa and Depaquit, 2013). Thirdly, in four publications, DNA sequences have been used to associate males and females in existing species (Depaquit et al., 2004a; Khadri et al., 2008; Parvizi et al., 2010b; Zhang et al., 2013). Molecular markers have been sequenced several times to identify species (Mukhopadhyay et al., 2000; Depaquit et al., 2005a; Florin et al., 2010; Manonmani et al., 2010; Parvizi et al., 2010b; Latrofa et al., 2011a,b; Tiwary et al., 2012; Minter et al., 2013) whereas cyt b sequences, associated to isoenzymes revealed a new species not named (Pesson et al., 2004).

The other publications are related to evolutionary systematics. They focus on different taxonomic levels (Fig. 1).

A total of 83 intraspecific studies has been recorded in the literature (Esseghir et al., 1997; Marcondes, 1997; Marcondes et al., 1997; Dias et al., 1998; Aransay et al., 2000, 2003; Di Muccio et al., 2000; Esseghir and Ready, 2000; Mukhopadhyay et al., 2000; Yin et al., 2000; Marquez et al., 2001; Soto et al., 2001; Arrivillaga et al., 2002, 2003; Bauzer et al., 2002a,b; Depaquit et al., 2002, 2004a, 2005b, 2008b, 2013, 2014; Hodgkinson et al., 2002, 2003; Testa et al., 2002; Bottecchia et al., 2004; Margonari et al., 2004; Pesson et al., 2004; Yahia et al., 2004; Elnaïem et al., 2005; Meneses et al., 2005; Perrotey et al., 2005; de Queiroz Balbino et al., 2006; Dvorak et al., 2006, 2011; Mazzoni et al., 2006, 2008; de Souza Rocha et al., 2007; Hamarsheh et al., 2007; Moin-Vaziri et al., 2007a,b; Baron et al., 2008; Bounamous et al., 2008, 2014; Lins et al., 2008, 2012; Araki et al., 2009, 2013; Bejarano et al., 2009; Vivero et al., 2009; Zhang et al., 2009, 2013; Ferroglio et al., 2010; Franco et al., 2010; Khalid et al., 2010, 2012; Mahamdallie et al., 2010; Parvizi et al., 2010a,b; Salomon et al., 2010; Belen et al., 2011; Florin et al., 2011; Kruger et al., 2011; Boudabous et al., 2012; Cohnstaedt et al., 2012; Kumar et al., 2012; Randrianambinintsoa et al., 2012; Scarpassa and Alencar, 2012, 2013; Zapata et al., 2012a,b; Gajapathy et al., 2013; Jafari et al., 2013; Kasap et al., 2013; Minter et al., 2013; Pech-May et al., 2013; Peyrefitte et al., 2013; Santos et al., 2013; Seblova et al., 2013; Yamamoto et al., 2013; Contreras Gutierrez et al., 2014; Valderrama et al., 2014).

A total of 55 papers comparing different species or subspecies within a genus has been recorded (Booth et al., 1991, 1994; Adamson et al., 1993; Maingon et al., 1993; Esseghir et al., 1997; Marcondes et al., 1997; Aransay et al., 2000; Depaquit et al., 2000, 2002, 2005b, 2008a,b, 2014; Di Muccio et al., 2000; Esseghir and Ready, 2000; Mukhopadhyay et al., 2000; Soto et al., 2001; Lins et al., 2002; Mazzoni et al., 2002, 2006, 2008; Testa et al., 2002; Torgerson et al., 2003; Beati et al., 2004; Pesson et al., 2004; Moin-Vaziri et al., 2007a; Bounamous et al., 2008, 2014; Perez-Doria et al., 2008a; Absavaran et al., 2009; Kuwahara et al., 2009; Vivero et al., 2009; Azpurua et al., 2010; Franco et al., 2010; Khalid et al., 2010, 2012; Parvizi et al., 2010a,b; Belen et al., 2011; Kruger et al., 2011; Latrofa et al., 2011a,b; Kumar et al., 2012; Randrianambinintsoa et al., 2012, 2013; Jafari et al., 2013; Kasap et al., 2013; Minter et al., 2013; Pech-May et al., 2013; Randrianambinintsoa and Depaquit, 2013; Scarpassa and Alencar, 2013; Yamamoto et al., 2013; Zhang et al., 2013; Contreras Gutierrez et al., 2014).

Lastly, a total of 22 publications comparing taxa belonging to different genera has been recorded (Esseghir et al., 1997; Depaquit et al., 1998, 1999; Aransay et al., 2000; Esseghir and Ready, 2000; Lins et al., 2002, 2012; Mazzoni et al., 2002; Torgerson et al., 2003; Vivero et al., 2007, 2009; Terayama et al., 2008; Kuwahara et al., 2009; Azpurua et al., 2010; Kruger et al., 2011; Latrofa et al., 2011b; Kumar et al., 2012; Jafari et al., 2013; Minter et al., 2013; Yamamoto et al., 2013; Bounamous et al., 2014; Contreras Gutierrez et al., 2014).

The methods used for DNA sequences analyses are mainly sequences alignment and Neighbor-Joining, maximum parsimony and probabilistic methods like maximum likelihood and Bayesian inferences. Two focuses on the secondary structure of the molecular marker (Vivero et al., 2007, 2009; Perez-Doria et al., 2008b).

Some studies used Random Amplified polymorphic DNA (RAPD) (Adamson et al., 1993; Maingon et al., 1993; Dias et al., 1998; Mukhopadhyay et al., 2000; Margonari et al., 2004; Meneses et al., 2005; de Queiroz Balbino et al., 2006; Dvorak et al., 2006, 2011; de Souza Rocha et al., 2007; Seblova et al., 2013), Restriction Fragment Length polymorphism (RFLP) (Terayama et al., 2008; Latrofa et al., 2011a; Tiwary et al., 2012; Minter et al., 2013;

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