



DNA barcoding of Neotropical black flies (Diptera: Simuliidae): Species identification and discovery of cryptic diversity in Mesoamerica

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Abstract

Although correct taxonomy is paramount for disease control programs and epidemiological studies, morphology-based taxonomy of black flies is extremely difficult. In the present study, the utility of a partial sequence of the COI gene, the DNA barcoding region, for the identification of species of black flies from Mesoamerica was assessed. A total of 32 morphospecies were analyzed, one belonging to the genus *Gigantodax* and 31 species to the genus *Simulium* and six of its subgenera (*Aspathia*, *Eusimulium*, *Notolepria*, *Psaroniocompsa*, *Psilopelmia*, *Trichodagmia*). The Neighbour Joining tree (NJ) derived from the DNA barcodes grouped most specimens according to species or species groups recognized by morphotaxonomic studies. Intraspecific sequence divergences within morphologically distinct species ranged from 0.07% to 1.65%, while higher divergences (2.05%–6.13%) in species complexes suggested the presence of cryptic diversity. The existence of well-defined groups within *S. callidum* (Dyar & Shannon), *S. quadrivittatum* Loew, and *S. samboni* Jennings revealed the likely inclusion of cryptic species within these taxa. In addition, the suspected presence of sibling species within *S. paynei* Vargas and *S. tarsatum* Macquart was supported. DNA barcodes also showed that specimens of species that are difficult to delimit morphologically such as *S. callidum*, *S. pseudocallidum* Díaz Nájera, *S. travisi* Vargas, Vargas & Ramírez-Pérez, relatives of the species complexes such as *S. metallicum* Bellardi *s.l.* (e.g., *S. horacioi* Okazawa & Onishi, *S. jobbinsi* Vargas, Martínez Palacios, Díaz Nájera, and *S. puigi* Vargas, Martínez Palacios & Díaz Nájera), and *S. virgatum* Coquillett complex (e.g., *S. paynei* and *S. tarsatum*) grouped together in the NJ analysis, suggesting they represent valid species. DNA barcoding combined with a sound morphotaxonomic framework provided an effective approach for the identification of medically important black flies species in Mesoamerica and for the discovery of hidden diversity within this group.

Key words: DNA barcoding, COI, Simuliidae, medically important black flies, Mesoamerica

Introduction

Black flies (Diptera: Simuliidae) comprise 26 genera and an estimated 2,163 species (2,151 living and 12 fossil) (Adler & Crosskey 2014). In most of these species, the female requires a blood meal for egg maturation, and it is this requirement that makes members of this family important as biting pests and in the transmission of parasites and pathogens of the blood and skin of humans and other warm-blooded animals (Hernández-Triana *et al.* 2011; 2012; Shelley *et al.* 2010). The most important parasites in humans that are transmitted by simuliids are the nematodes *Onchocerca volvulus* (Leuckart), which causes onchocerciasis or "river blindness", primarily in sub-Saharan Africa, and *Mansonella ozzardii* Manson, which causes mansonelliasis or "serous cavity filariasis", primarily in Latin America (Shelley *et al.* 2010). Recently, it has been hypothesized that certain species of black flies, in onchocerciasis endemic areas, may also transmit a neurotropic virus that may be an endosymbiont of the microfilariae that causes nodding syndrome and epilepsy without nodding (Colebunders *et al.* 2014).

vector complexes in combination with their chromosomal banding pattern across their distribution range (Hernández-Triana *et al.* 2011, 2012). The latter highlights the continuing need for research using an integrated taxonomic approach on the Simuliidae in the Neotropical Region.

Although the volume of DNA barcode data in BOLD and GenBank is increasing rapidly, much work is still required to populate these databases for the global fauna of Simuliidae. In Mesoamerica, further collecting is still required to obtain new material for the *Simulium canadense* species group in the subgenus *Trichodagmia* and species in the genus *Tlalocomyia* across Mexico and Guatemala. As well, surveys are needed on the Caribbean Islands, especially in the high mountain ranges in the Greater Antilles. In South America, the high Andean mountain range across Colombia, Perú, and Ecuador and the lowlands in Madre de Dios in Perú are also in need of study, together with studies in the temperate regions of Patagonia in Argentina, where *Cnesia*, *Cnesiamina*, *Gigantodax*, and *Paraustrosimulium* are abundant.

In summary, the COI barcoding gene correctly distinguished 97% of the morphologically distinct species (all life stages) in the family Simuliidae examined from Mesoamerica, demonstrating its value for species identification. It has also been shown that the COI barcoding region is a useful tool in revealing high levels of genetic diversity in known species complexes (for example *S. exiguum s.l.*, *S. lutzianum s.l.*, *S. metallicum s.l.*, *S. ochraceum s.l.*, and *S. virgatum s.l.*), in supporting the existence of putative complexes (*S. paynei*, *S. tarsatum*), and in revealing new complexes (*S. callidum*, *S. puigi*, *S. quadrivittatum*, and *S. samboni*). We believe that available results establish that DNA barcoding is a powerful identification tool and a versatile aid for establishing species boundaries, especially when coupled with morphotaxonomy and cytogenetic analysis. The latter would have a direct impact on control strategies and studies on disease transmission by supporting the correct identification of vector and/or pest species.

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