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Bioecological aspects and integrative taxonomy of sand flies (Diptera: Psychodidae: Phlebotomina)

Aspectos bioecológicos e Taxonomia integrativa de flebotomíneos (Diptera: Psychodidae: Phlebotominae)

Aspectos bioecológicos y taxonomía integradora de los flebótomos (Diptera: Psychodidae: Phlebotominae)

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ABSTRACT

Objective: Describe the characteristics of the environment that influence the development of sand flies, and compile information about the integrative taxonomy of these insects. **Bibliographic review:** Sand flies (Diptera: Psychodidae: Phlebotominae) are insects, some species of which are involved in the transmission of neglected diseases such as leishmaniasis. Thus, greater attention must be paid to ecological factors and anthropogenic changes, as they have a direct influence on the presence of the vector and the adaptation of species, favoring changes in the sylvatic cycle of the disease and consequently promoting an increase in reported cases of the disease. Traditional taxonomic identification of these insects is done by analyzing morphological characters, but the use of DNA sequences has been used by researchers as an integrative approach, since molecular techniques provide reliable, fast and efficient identifications as well as the elucidation of species complexes and the discovery of new taxa, and there are good reference sequences for many species. **Final considerations:** Therefore, it is crucial to understand the diversity and bioecology of vectors, including integrative taxonomy, to understand and support future research to help control leishmaniasis.

Keywords: Ecology, Phlebotomine, Sand flies, Molecular Taxonomy.

RESUMO

Objetivo: Descrever as características dos ambientes que influenciam o desenvolvimento dos flebotomíneos, e compilar informações sobre a taxonomia integrativa destes insetos. **Revisão Bibliográfica:** Os flebotomíneos (Diptera: Psychodidae: Phlebotominae) são insetos, com algumas espécies envolvidas na transmissão de doenças negligenciadas como as leishmanioses. Assim, se faz necessária uma maior atenção aos fatores ecológicos e às alterações antrópicas, pois influenciam diretamente na presença do vetor e na adaptação de espécies, que favorecem mudanças no ciclo silvestre da doença e, consequentemente, favorece um aumento nos casos notificados da doença. A identificação taxonômica tradicional desses insetos é realizada por meio da análise de caracteres morfológicos, no entanto, o uso de sequências de DNA tem sido utilizado pelos pesquisadores como uma abordagem integrativa, uma vez que as técnicas moleculares fornecem identificações confiáveis, rápida e precisa, bem como a elucidação de complexos de espécies e a descoberta de novos táxons, além de existirem boas sequências de referência disponíveis para muitas espécies. **Considerações finais:** Portanto, é essencial ressaltar a importância do entendimento da diversidade e bioecologia dos vetores, incluindo a taxonomia

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integrativa, para compreender e fornecer subsídios para futuras pesquisas que auxiliem no controle das leishmanioses.

Palavras-chave: Ecologia, Phlebotomine, Flebotomíneos, Taxonomia Molecular.

RESUMEN

Objetivo: Describir las características de los ambientes que influyen en el desarrollo de los flebotomíneos y recopilar información sobre la taxonomía integradora de estos insectos. **Reseña bibliográfica:** Los flebotomíneos (Diptera: Psychodidae: Phlebotominae) son insectos, y algunas especies están implicadas en la transmisión de enfermedades desatendidas como la leishmaniosis. Por lo tanto, es necesario prestar mayor atención a los factores ecológicos y a los cambios antropogénicos, ya que influyen directamente en la presencia del vector y en la adaptación de las especies, lo que favorece los cambios en el ciclo selvático de la enfermedad y, en consecuencia, el aumento de los casos declarados de la enfermedad. La identificación taxonómica tradicional de estos insectos se realiza mediante el análisis de caracteres morfológicos, sin embargo, el uso de secuencias de ADN ha sido utilizado por los investigadores como un enfoque integrador, ya que las técnicas moleculares proporcionan identificaciones fiables, rápidas y precisas, así como la elucidación de complejos de especies y el descubrimiento de nuevos taxones, además de existir buenas secuencias de referencia disponibles para muchas especies. **Consideraciones finales:** Por lo tanto, es esencial hacer hincapié en la importancia de comprender la diversidad y la bioecología de los vectores, incluida la taxonomía integradora, con el fin de entender y proporcionar subsidios para futuras investigaciones que ayuden a controlar la leishmaniasis.

Palabras clave: Ecología, Phlebotomine, Flebótomos, Taxonomía Molecular.

INTRODUCTION

Phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) are small hematophagous insect that transmit protozoan parasites of the genus *Leishmania* Ross, 1903, the causative agent of leishmaniasis (AKHOUNDI M, et al., 2016). Worldwide, 1,060 species from the sand fly group have been described, 32 of which are fossil. On the American continent, 556 species in 23 genera have been described, 17 of which are fossil. In Brazil, 262 species have been described (GALATI EAB and RODRIGUES BL, 2023).

Leishmaniases are neglected diseases that occurs in 98 countries, with 12 million cases and 20,000 to 40,000 deaths per year. The annual incidence of new cases varies between 0.2 and 0.4 million and between 0.7 and 1.2 million per year for visceral and cutaneous leishmaniasis respectively (PAHO/WHO, 2023).

The correct identification of sand flies at the species level is important to support epidemiological studies. However, due to the complexity of morphological and diagnostic features and the need for skilled professionals for correct identification, it may be non-specific for some groups (RODRIGUES BL and GALATI EAB, 2023).

The use of integrative taxonomy, where more than one tool is used to identify sand flies, is safer. In this context, DNA barcoding is a good option, because it identifies species based on the diversity of a fragment of the mitochondrial *cytochrome c oxidase subunit I* (*coi*) gene, the central aim of which is to use a short genetic sequence from a specific part of the genome to distinguish between species, just as a supermarket scanner distinguishes products using the black stripes of the Universal Product Code (HEBERT PD, 2003).

For Rodrigues BL and Galati EAB (2023) DNA barcoding is a reliable and economical technique for the molecular taxonomy of sand fly. Moreover, these barcode datasets are able to understand closely-related species (the so-called ‘cryptic species’) and their diverse populations within nominal taxa.

In this context, we highlight relevant aspects of sand flies and compile information about the biocology and integrative taxonomy of this group. We explain how important these data are for understanding sand fly diversity and how they can help in the discovery and naming of new species using integrative approaches.

BIBLIOGRAPHIC REVIEW

Life cycle and bioecological aspects of phlebotomine sand flies

These sand flies are holometabolous insects that develop from the egg through the four stages of larva, pupa and adult. The duration of the individual stages can vary depending on the species and environmental conditions. For example, the development cycle of *Lutzomya longipalpis* (Lutz and Neiva, 1912) lasts an average of 27 to 30 days, but humidity and temperature are important factors in the development of its life cycle (YOUNG DG and DUNCAN MA, 1994; LAWYER P, et al., 2017; CECÍLIO P, et al., 2022).

Female sand flies can lay their eggs between five and eight days after their blood meal, but there are also species that feed several times before developing viable eggs. They can lay up to 70 eggs in locations chosen according to humidity and availability of organic matter, and eggs are often found in leaves, cracks, animal burrows and holes in the ground (MORALES A, et al., 2005).

Depending on the species, the eggs are between 0.3 and 0.4 mm in size, oval or ellipsoid and change color from brown to black. The eggs hatch between 4 and 20 days after being laid and can go into diapause under unfavorable conditions, at very low temperatures. The larvae feed on organic matter, particularly vegetable matter, including animal feces and arthropod bodies (LAWYER P, et al., 2017; GALATI EAB, 2018). The pupae are yellowish in color and have very clear eyes. The body consists of 13 segments, the first four of which are fused together to form the cephalothorax, while the remaining segments are highly individualized and form the abdomen. During maturation, the pupa's skin and eyes darken and remain dark until the adults hatch. The pupal stage lasts between six and 13 days, depending on the species, with the adults hatching at night (RIBEIRO LM, et al., 2015).

Adult sand flies have a small body that varies in color from yellow to dark brown and are heavily covered with bristles. They have a hopping flight and live in cold, damp places, preferably near the ground, close to vegetation on roots and/or tree trunks, and can be found in animal burrows, houses and stables. It is difficult to estimate the survival rate of sand flies in the wild. However, it is estimated that females can survive up to three gonotrophic cycles on average. Males live much shorter lives in the wild, around one week (CECÍLIO P, et al., 2022).

One of the most important factors that can change the life cycle of sand flies are climatic factors such as temperature, humidity and precipitation, which are directly related to the interaction with the *Leishmania* protozoa, their geographical distribution and development. Resistance to extreme temperatures varies from species to species. A notable example of this is *Lu. longipalpis*, which has been shown to be adapted to unfavorable temperature conditions (AGUIAR GM and VIEIRA VR, 2018; MARTINS KA, et al., 2023).

Various human-induced actions favor changes in the sylvatic cycle of the disease, including deforestation for agriculture, mining, logging, road construction or the expansion of urban areas. These actions have led to an increase in reported cases, which means that the spread of leishmaniasis is often linked to environmental problems that have a negative impact on vegetation cover, such as a reduction in food sources, which can lead to the migration and adaptation of vectors and reservoirs to urbanized and anthropized sites (PINTO MORAES JL, et al., 2020).

Some American species such as *Lu. longipalpis*, *Nyssomyia whitmani* (Antunes and Coutinho, 1939) and *Evandromyia evandroi* (Costa Lima and Antunes, 1936) are already strongly adapted to urbanization and the urban environment, which is due to anthropogenic interventions (LEONARDO FS and REBÊLO JMM, 2004; OLIVEIRA-DE-ANDRADE AR, et al., 2014). The species of the *Lu. longipalpis* complex do not occur in conserved areas without human settlements (CARABALLO V and ARRIVILLAGA J, 2010), and for some *Leishmania* species that used to occur exclusively in the wild, such as *L. (Leishmania) amazonenses* (Lainson and Shaw, 1972), there is now evidence of their urbanization (CALDART ET, et al., 2017).

The breeding of domestic animals around homes and poor hygienic conditions attracts natural and wild reservoirs that cause outbreaks and epidemics of leishmaniasis (HOYOS-LÓPEZ R, et al., 2016). Thus, animal shelters favor the process of colonization of species adapted to environments with human presence, since a large amount of food is available to the adult sand flies and their larvae can survive under suitable conditions (SILVA FS, et al., 2012).

Some studies on bioecology and food sources have shown that sand flies are more abundant in environments where animals are present (GUIMARAES-E-SILVA AS, et al., 2017; RIBEIRO DA SILVA RC, et al., 2019). In addition, other factors can influence the occurrence of vectors near residential areas, such as deforestation, which leads to a deterioration in forest quality and affects the structure of sand fly communities, reducing species richness (REBÊLO JMM, et al., 2019; PINTO MORAES JL, et al., 2020).

Medical Importance: Leishmaniasis

Leishmaniases are a group of infectious diseases caused by protozoa of the order Kinetoplastida, the family Trypanosomatidae and the genus *Leishmania* and have a significant impact on public health. The species of this parasite are divided into the subgenera *Leishmania* and *Viannia* Lainson and Shaw, 1987, the latter of which is only found in the New World. Two basic clinical forms for this disease are described: cutaneous leishmaniasis (CL), the most common form, which is characterized by lesions on the skin and/or mucous membranes. And visceral leishmaniasis (VL), the most severe form of the disease in humans, mainly affects the spleen, liver and bone marrow, but can also affect the lungs, intestines and lymph nodes and is fatal if left untreated (OPS/OMS, 2023).

The most commonly reported form of the disease is CL, which according to the World Health Organization is one of the six most important infectious diseases because it is so frequently diagnosed and can lead to deformities. Several pathogen species are known to cause skin manifestations: *L. (V.) guyanensis* (Floch, 1954), transmitted by the sand fly vectors *Ny. umbratilis* (Ward and Fraiha, 1977), *Ny. anduzei* (Rozeboom, 1942) and *Ny. whitmani*; *L. (L.) amazonensis* is transmitted by the vectors *Bichromomyia Flaviscutellata* (Mangabeira, 1942) and *Lu. longipalpis*; *L. (V.) braziliensis* (Vianna, 1911): *Ny. whitmani*, *Ny. intermedia* (Lutz and Neiva, 1912), *Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971, *Ps. complexus* (Mangabeira, 1941), *Ny. neivai* (Pinto, 1926), *Ev. edwardsi* (Mangabeira, 1941) and *Migonemyia migonei* (França, 1920); *L. (V.) lainsoni* (Silveira, Shaw, Braga and Ishikawa, 1987) by *Trichophoromyia ubiquitalis* (Mangabeira, 1942); *L. (V.) shawi* (Lainson, Braga and de Souza, 1989) by *Ny. whitmani*; *L. (V.) naiffi* (Lainson and Shaw, 1989): *Ps. paraensis* (Shannon, 1926), *Ps. amazonensis* (Root, 1934) and *Ps. ayrozai* (Barretto and Coutinho, 1940) (OPS/OMS, 2023).

The other form of the disease, VL, commonly known as kala-azar, is a severe systemic infectious and parasitic disease with worldwide distribution and is one of the top six neglected tropical diseases with a reported number of deaths. In Brazil, the causative agent is *L. (L.) infantum*, transmitted by the sand flies *Lu. longipalpis* and *Lu. cruzi* (Mangabeira, 1938). In the urban environment, the dog (*Canis familiaris*) is the host and main reservoir of *L. (L.) infantum*, while in the wild, foxes (*Dusicyon vetulus* and *Cerdocyon thous*) and marsupials (*Didelphis albiventris*) are the main reservoirs of this parasite (WERNECK, 2016; OPS/OMS, 2023). The first attempts to classify *Leishmania* were made between 1916 and 1961, using monophyletic linear classifications based solely on extrinsic characters. A first classification was proposed in 1908 by Nicolle, who separated *L. infantum* from *L. donovani* (Laveran and Mesnil, 1903). Later, Biagi proposed the separation of various *Leishmania* species from the New World (LAINSON R, 2010).

The first morphological classifications were based on izoenzymes, after which a new classification based on molecular data emerged, dividing the *Leishmania* species into two main phylogenetic lineages: Euleishmania, which comprises four subgenera: *Leishmania (L. donovani)* (Laveran and Mesnil, 1903), *Viannia (L. braziliensis)* (Vianna, 1911), *Sauroleishmania (L. tarentolae)* Wenyon, 1921 and *L. complex enriettii (L. enriettii)* Muniz and Medina, 1948 and Paraleishmania, which includes *L . hertigi* Herrer, 1971, *L. deanei* Lainson and Shaw, 1977, *L. herreri* (Zeledon, Ponce and Murillo, 1979), *L. equatorensis* Grimaldi et al, 1992 and *L. colombiensis* (Kreutzer, Corredor et al., 1991) (CUPOLILLO E, et al., 2000).

Another important factor is that despite decades of research to develop a vaccine against leishmaniasis, there is still no safe and effective vaccine for humans. Therefore, the prevention and control of leishmaniasis requires a combination of intervention strategies, such as ecological, mechanical, biological or chemical controls. Vector control is an important strategy to prevent the disease as it reduces and interrupts the transmission of the disease and reduces the density of sand flies' populations (MOREIRA POL, et al., 2023).

Currently, there is an integrated vector management strategy that takes a more flexible, rational and comprehensive approach. This approach enables the simultaneous control of several insects that transmit important infections in endemic areas, using different control methods and strategies as well as interdisciplinary measures. Control methods include ecological, mechanical, biological and chemical approaches, with the latter being used only in specific cases tailored to the epidemiological situation. To ensure an appropriate selection of these measures, the biology and behavior of the vectors as well as the advantages and disadvantages of the methods in the local context must be taken into account, including community acceptance (PAHO/WHO, 2023).

Integrative taxonomy

Filippo Bonanni (1691) was the first to describe a sand fly and named it *Culex minumus aculeatus*. However, after the proposal of the binomial system by Linnaeus (1758) Scopoli JA (1786) described the first sand fly, a female found in Italy named *Bibio papatasi*; it was later included in the genus *Flebotomus* (tribe Phlebotomidae, family Phlebotominae) by Rondani and Berté, named *Flebotomus* in Rondani C (1840) and named *Phlebotomus* by Agassiz (1846). The period with the highest number of species descriptions was from the 1930s to the 1990s, with two particularly notable periods, one in the 1940s and the other in the 1970s (GALATI EAB and RODRIGUES BL, 2023).

Theodor O (1948) and Lewis DJ, et al. (1977) proposed a subdivision of sand flies into two genera for the Old World species, *Phlebotomus* (Rondani) and *Sergentomyia* (France), and three genera for the New World species, *Lutzomyia* (France), *Brumptomyia* (France and Parrot) and *Warileya* (Hertig). The genus *Chinius* (Leng, 1987) belongs to a separate taxon used for some Chinese sand fly species with primitive characteristics (LENG YJ, 1987). According to the revision by Young DG and Duncan MA (1994), the neotropical sand flies were subdivided into *Lutzomyia* França, 2924, *Brumptomyia* França and Parrot, 1921 and *Warileyia* Hertig, 1940. Although this classification approach is not the most up-to-date, it is still accepted by the majority of sand fly taxonomists.

In 2003, Galati proposed a new classification system that revised the existing proposals for the sand flies of the New World. The system recognized 464 species of Neotropical sand flies, which were divided into 23 genera, 20 subgenera, three species groups and 28 series. This included a complete revision and reorganization of the subfamily Phlebotominae, which was subsequently divided into two tribes, Hertigiini (subtribes Hertigiina and Idiophlebotomina) and Phlebotomini (subtribes Phlebotomina, Australophlebotomina, Brumptomyiina, Sergentomyiina, Lutzomyiina and Psychodopygina) (GALATI EAB, 2018). This proposal was published after the publication of the dichotomous key by Young and Duncan (1994) and is currently accepted by the Brazilian Ministry of Health. This update allows the inclusion of new species or new records in Brazil, as well as the revision of characteristics to improve identification. In addition, the classification proposed by Galati (2018) includes the genus *Brumptomyia* with an identification key for females previously considered indistinguishable. In Brazil, the most recent identification keys are those of Shimabukuro PHF, et al. (2011) and Galati EAB (2018). According to the new classifications and their most recent updates, there are 23 genera of New World sand flies and 9 genera of Old World (GALATI EAB, 2018; GALATI EAB and RODRIGUES BL, 2023).

Morphological identification is a laborious taxonomic approach that requires attention and skill from the researcher, and morphological keys are often outdated and incomprehensible, leading to incorrect identifications and taking a long time, sometimes years, to describe a species and/or genus (GALATI EAB, et al., 2017). Therefore, molecular techniques are widely used, especially DNA sequencing analyses, which allow simultaneous processing of samples, offer reliable identifications and provide good reference sequences for many species (YSSOUF A, et al., 2016).

Other approaches using DNA are Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and microsatellite marker analysis. PCR-RFLP is an economic method and can be used to distinguish major vectors from nearby sand flies, but the information provided by restriction fragments may not be sufficient when analyzing species-rich data sets. Microsatellites, on the other hand, are highly polymorphic, making them suitable for population structure studies. However, they require the development of multiple primer sets that are generally capable of amplifying fragments for specific species and their close relatives, and microsatellites may underestimate true nucleotide diversity compared to sequence-based measurements (RODRIGUES BL and GALATI EAB, 2023).

Another newer method using a new protein-based approach, named ‘matrix-assisted laser desorption ionization time-of-flight mass spectrometry’ (MALDI-TOF MS), has been proposed as a promising method for the molecular identification of sand flies. This method offers significant advantages, mainly due to its quickness and cost-effective, making it an attractive option for future entomological surveillance in sand flies and leishmaniasis endemic areas. This approach is based on the comparison of the mass spectra of unknown samples with a database of reference spectra of known species. This technique has shown promise due to its high speed of analysis, ease of use and relatively low cost compared to other molecular identification methods (PAREYN M, et al., 2020).

DNA barcoding is one of the most widely used techniques for molecular identification using a fragment of the gene encoding the mitochondrial DNA (mtDNA) protein: *Cytochrome C oxidase subunit I (coi).* This region is of great importance for facilitating biodiversity inventory and species identification (HEBERT PD, et al., 2003). The coi gene has been sequenced in about 270 species worldwide and was the most commonly used gene for insects in the past (SIMON C, et al., 1994).

According to Pinto IS et al. (2023), DNA barcoding is a useful strategy for the molecular identification of sand flies from the western Brazilian Amazon, as this tool efficiently detected the cryptic diversity of *Pintomyia serrana* (Damasceno and Arouck, 1949), which can be confirmed in future studies using an integrative approach. In addition, notable coi barcode datasets were generated, such as *Th. auraensis* (Mangabeira, 1942), *Ny. shawi* and *Ps. paraensis*, which play a role in the transmission of *Leishmania* spp. in the Brazilian Amazon.

Of the techniques mentioned above, PCR-RFLP and DNA barcoding are currently most commonly used for the molecular identification of sand flies (RODRIGUES BL and GALATI EAB, 2023).

In general, knowledge of sand fly systematics has expanded with the introduction of several new methods such as chromosome analysis, multivariate morphometrics, laboratory rearing and colonization of sand flies, isoenzymatics, molecular and phylogenetic analysis and, more recently, mass spectrometry. These advances have led to better identification and classification of sand fly specimens (AKHOUNDI M, et al., 2016). Although these techniques have many advantages, it must be pointed out that the use of molecular markers, especially DNA barcoding, should not replace classical/morphological taxonomy. On the contrary, it is important to use them integratively and combine them with the knowledge of cryptic diversity to support new research projects aimed at better identification of morphological differences (POSADA-LÓPEZ L, et al., 2023).

FINAL CONSIDERATIONS

Knowledge of the bioecology of the vectors is of great importance because it helps to understand the dynamics of the life cycle of the transmitting insects, which is crucial information for identifying effective control strategies. In addition, it is important to understand the impact of anthropogenic changes on the transmission cycle of the disease, considering that environmental changes caused by human activities can affect the distribution and abundance of sand fly vectors and the interaction between them, the hosts and the parasite that causes leishmaniasis. Another important aspect is the inclusion of integrative taxonomy in sand fly research, because it plays a fundamental role in the rapid and accurate identification of species, especially cryptic species or isomorphic females. In addition, molecular taxonomy contributes to the clarification of species complexes and the discovery of new taxa. This approach is an important contribution to future research in the fight against leishmaniasis. The study of the bioecology of vectors and the application of integrative taxonomy are therefore complementary approaches that provide valuable information for the control of leishmaniasis and contribute to the understanding of the transmission patterns of the disease and the development of more effective prevention strategies.

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