

READERS' OPINION AND DISCUSSION

Trichophoromyia auraensis is a putative vector

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The sandfly *Trichophoromyia auraensis* has recently evolved as a proven vector of *Leishmania* (*Viannia*) endemic to state of Acre in the north of Brazil. This note is intended to propose a correction in the report of the first occurrence of natural infection of *Leishmania* (*Viannia*) in this species. We and the other scientific groups reinforced that *Tr. auraensis* is a possible vector involved in the transmission of American cutaneous leishmaniasis in Acre, Brazil.

Key words: *Leishmania* - disease vectors - Acre - Brazil - *Trichophoromyia auraensis*

About the article: First description of *Leishmania* (*Viannia*) infection in *Trichophoromyia auraensis* (Psychodidae: Phlebotominae) in the transmission area of American cutaneous leishmaniasis in Acre state, Amazon Basin, Brazil.

American cutaneous leishmaniasis (ACL) is a disease highly endemic to the state of Acre, Brazil. This state is located on the Brazilian border and is surrounded by Peru and Bolivia, both of which have a high number of ACL cases reported. The state of Acre (Brazil) was the unit of the federation with the highest incidence of ACL in 2010, reaching 141.64 cases per 100,000 inhabitants. However, very few studies have been conducted on the development of sandfly ecology in this state. A quick search on Scopus demonstrated that only seven studies researching on this topic in the state of Acre have been published thus far. Valdivia et al. (2012), who found *Trichophoromyia auraensis* (Mangabeira) infected with *Leishmania* (*Viannia*) in Madre Dios, the Peruvian state that borders Acre, indicated that it is the main vector of *Leishmania* (*Viannia*) in the Southwest Amazon Basin. In Acre, both the microregion of Rio Branco and a municipality (Assis Brasil) located in the microregion of Brasileia, are considered endemic areas of epidemiological relevance for ACL. According to the Notifiable Diseases Information System (SINAN 2014), 387 cases were reported in the municipality between 2007 and 2013, corresponding to an annual mean detection coefficient of 93 ACL cases per 10,000 inhabitants.

Owing to its epidemiological importance, our research groups chose this area for conducting some epidemiological studies focusing on the ecological aspects of sandflies and *Leishmania*. In this region, 13 new occurrences were registered and two new species of sandflies were described (Teles et al. 2013, Oliveira et al. 2015). Moreover, the species of *Leishmania* in human cases were characterised by polymerase chain reaction (PCR) (Teles et al. 2015).

In July 2016, our team published an epidemiological study conducted on almost seven thousand collected sandflies in Memórias do Instituto Oswaldo Cruz (MIOC) (Teles et al. 2016). The females were grouped by pools and examined for the DNA tracks of *Leishmania*. We found that specimens of *Lutzomyia davisi* and *Lu. auraensis*/*Lu. ruifreitasi* contained the DNA tracks of *Leishmania* from the *L. braziliensis* complex at high concentrations. We registered this as the first report of the infection of *Lu. auraensis*/*Lu. ruifreitasi* by *L. (V.) guyanensis* and *L. (V.) braziliensis* in Brazil.

This note is to make a scientific correction; recently, in MIOC, January 2017, de Araujo-Pereira et al. (2017) assigned the first description of *Leishmania* (*V.*) infection in *Tr. auraensis* in the Rio Branco municipality of Acre state. The note of de Araujo-Pereira et al. (2017), despite not inedited to the occurrence, is important because it reinforces that it is possible to find the infected populations of *Tr. auraensis* in some hundreds of kilometres of the endemic regions considered as ACL hot spots such as Assis Brasil, in Acre. This species also occurs in other Brazilian states such as Amazonas, Pará, Rondônia and Mato Grosso, which are characterised by high population, highways, intense deforestation, and intense migration caused by economical attractiveness. Therefore, these states should be studied more deeply to understand the role of this sandfly in the transmission of ACL.

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- REPLY**
- Comments about the finding of *Trichophoromyia auraensis* (Mangabeira 1942) sandfly specimens infected by *Leishmania* (*Viannia*) parasites in Acre state
- Since 2011, our group has been conducting field studies in the state of Acre, trying to contribute with new findings about phlebotomine sandflies fauna and potential leishmaniasis vectors in the state. In an epidemiological context, Amazon is undergoing rapid changes in environmental conditions that directly affect the population of sandfly vectors and therefore disease transmission. Although considered a region with an increasing incidence of cutaneous leishmaniasis, knowledge of the geographical distribution of phlebotomine sandflies associated with disease transmission is still scarce for some regions, such as Acre state, where the cutaneous manifestation of the disease reached high prevalence in the last years (Martins & Silva 1964, Arias & Freitas 1982, Arias et al. 1984, Silva-Nunes et al. 2008, Azevedo et al. 2008, Teles et al. 2013, de Araujo-Pereira et al. 2014, Oliveira et al. 2015).
- From 2011 to 2012, we centralised the sandfly captures in residential areas within forested regions and recreational areas of Rio Branco municipality, the capital of Acre, and found a very diverse fauna in urban and peri urban areas of the city, as diverse as that encountered in areas of primary forest. A total of 16 Phlebotominae genera and 23 species was taxonomically identified, being *Trichophoromyia auraensis* and *Nyssomyia whitmani* the most abundant species in both forested and peridomiliary environments. Taking into account the high prevalence of *Th. auraensis* in Rio Branco (more than 50% of the total captured insects), we reported in 2014, this species as a putative vector in the region (de Araujo-Pereira et al. 2014).
- The manuscript by Oliveira et al. (2015) describes *Trichophoromyia ruifreitasi*, a new phlebotomine sandfly species from Acre, morphologically similar to *Th. auraensis*, that was identified between male specimens collected in the municipality of Assis Brasil (Oliveira et al. 2015, Teles et al. 2016). As mentioned by Oliveira et al. (2015), “*This species is similar to Th. auraensis* (Mangabeira), but the two species can be easily differentiated by the distribution of setae on their parameres, and by the presence of a dorsal lobe in the parameres of the new species”. In this study, the authors detailed the morphological characteristics of male specimens of *Th. ruifreitasi* identified in the municipality of Assis Brasil (Oliveira et al. 2015). In contrast to the investigation by Teles et al. (2016) performed in the municipality of Assis Brasil, in the study areas of Rio Branco, we identified all male specimens of the *Trichophoromyia* genus as *Th. auraensis* (de Araujo-Pereira et al. 2014). The study by Oliveira et al. (2015) already mention our investigation about the sandfly fauna in Rio Branco, in which we report *Th. auraensis* as a putative vector in this Amazonian region (de Araujo-Pereira et al. 2014). Valdivia et al. (2012) were the first to identify *Th. auraensis* as a natural carrier of *Leishmania* (*Viannia*) *lainsoni* and *Leishmania* (*Viannia*) *braziliensis* in Madre de Dios, an Amazon region in Peru bordering the state of Acre. Similar to that observed in Rio Branco, in this region of Peru, *Th. auraensis* was also the most abundant sandfly species (63%), and the minimum infection prevalence was estimated to be 0.6% by kDNA-polymerase chain reaction (kDNA-PCR) performed in insect pools. Using a FRET-based real time PCR, the authors identified four *Th. auraensis* pools positive for *L. (V.) lainsoni* and *L. (V.) braziliensis* (Valdivia et al. 2012).
- Proceeding with our investigation in Rio Branco, we further analysed a sampling of non-blood-fed female sandflies for the presence of *Leishmania* DNA in each insect, individually. The choice for performing the molecular diagnosis separately, by individual specimen instead of pools of insects, was due to the complexity and diversity of the sandfly fauna in the Amazon Basin. Using a multiplex PCR directed simultaneously to *Leishmania* kDNA and to the IVS6 cacophony gene region in neotropical sandflies, coupled to hybridisation with a *L. (Viannia)* biotinylated probe (de Pita-Pereira et al. 2005), total positivity of 6.94% was found in the municipality of Rio Branco. The molecular characterisation of *Leishmania* spp. was performed by sequencing the 234 bp fragment obtained by amplifying a region of the *hsp70* gene from each positive sample, previously assayed for the presence of *Leishmania* kDNA (da Graça et al. 2012). *L. (V.) braziliensis* DNA was identified in *Th. auraensis*, *Evandromyia saulensis* and *Pressatia* sp. individuals. For the other positive samples of *Th. auraensis* and *Ev. saulensis*, the identification was restricted to the *Viannia* genus. These data were recently published in Memórias do Instituto Oswaldo Cruz (de Araujo-Pereira et al. 2017). The finding of *L. (Viannia)* DNA in two *Ev. saulensis*, with the confirmation of *L. braziliensis* in one specimen, corresponds to the first record of possible infection associated with this sandfly. Moreover,

we registered in Rio Branco, Acre state, *Th. auraensis* and *Pressatia* sp. infected by *L. (Viannia)* parasites (in three out of nine positive *Th. auraensis* individuals and in the only positive *Pressatia* sp., the genetic material was confirmed as *L. braziliensis*). The role of these species as vectors of parasites responsible for New World tegumentary leishmaniasis remains to be established for better understanding the risk of the disease transmission in the Neotropics (de Araujo-Pereira et al. 2017).

In our opinion, both studies (Teles et al. 2016, de Araujo-Pereira et al. 2017) were published in very close periods, almost in parallel. Our manuscript was submitted in June 2016 and the one by the group of Teles was published in July 2016, during the peer-revision process of our manuscript.

Finally, we highlighted differences on some technical and critical aspects between the two studies. In Teles et al. (2016), the collected sandfly females from Assis Brasil, Acre state, were assembled in groups (2-20 specimens per group), and each pool was composed by the same species, date and place of capture. Because females of *Th. auraensis* and *Th. ruifreitasi* are probably indistinguishable, the authors named them *Th. auraensis/Th. ruifreitasi* [Lu. *auraensis*/Lu. *ruifreitasi*, according to Young and Duncan (1994)]. Teles et al. (2016) found a minimal infection prevalence of 2.05% in Lu. *auraensis*/Lu. *ruifreitasi* (16 out of 58 pools) by parasites from the *L. braziliensis* complex. If one considers the limitation to morphologically differentiate between sandfly females of both species, our study in Rio Branco was advantageous, since there was no overlap among *Th. auraensis* and *Th. ruifreitasi* in the study areas. All male specimens of the *Trichophoromyia* genus were previously identified as *Th. auraensis* (de Araujo-Pereira et al. 2014), and were individually analysed for the presence of *Leishmania* DNA (de Araujo-Pereira et al. 2017).

In general, both studies (Teles et al. 2016, de Araujo-Pereira et al. 2017) are complementary, being performed in two municipalities of the state of Acre with high prevalence of cutaneous disease and thus bringing important contribution in demonstrating the diversity of sandfly species with potential involvement in the cutaneous leishmaniasis transmission cycle in the investigated areas.

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