

Prediction of the secondary structure at the tRNASer (UCN) of *Lutzomyia longipalpis* (Diptera: Psychodidae)

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Abstract. *Lutzomyia longipalpis* is the main vector of *Leishmania infantum*, the etiological agent of visceral leishmaniasis in America and Colombia. Taxonomically belongs to the subgenus *Lutzomyia*, which includes other vector species that exhibit high morphological similarity to the female species difficult to identify vectors in leishmaniasis foci and suggesting the search for molecular markers that facilitate this task, further researchs with mitochondrial genes, chromosome banding, reproductive isolation and pheromones evidence the existence of species complex. The aim of this study was to predict the secondary structure of mitochondrial transfer RNA serine (tRNASer) for UCN codon of *Lutzomyia longipalpis* as molecular marker for identify of this species. Sequences recorded in Genbank of *L. longipalpis* sequences were aligned with tRNA's from previously described species and then tRNASer secondary structure was inferred by software tRNAscan-SE 1.21. The length of tRNASer was 67 base pairs (bp). Two haplotypes were detected in the five sequences analyzed. The *L. longipalpis* tRNASer showed 7 intrachain pairing in the acceptor arm, 3 in the DHU arm, 4 in the anticodon arm and 5 in the T Ψ C. The size of the loops corresponded to 5 nucleotides in the DHU, 7 in the anticodon, 4 in the variable and 7 in the T Ψ C. *L. longipalpis* is distinguished from other species at subgenera

Lutzomyia by the secondary structure and substitutions inferred tRNASer evidenced in the primary sequence.

Key words: *Lutzomyia longipalpis*, molecular taxonomy, tRNASer.

Lutzomyia longipalpis (Lutz & Neiva, 1912), is a neotropical phlebotomine with a wide and discontinuous geographical distribution linked to areas of tropical dry forest, from southern Mexico to northern Argentina (Arrivillaga 2002, Lainson and Rangel 2005), and It is considered the main vector of *Leishmania infantum* (Nicolle, 1908), a pathogen responsible for the clinical manifestations associated with visceral leishmaniasis in America (Grimaldi 1989, Soares and Turco 2003).

In Colombia, it is responsible for the active transmission of *L. infantum* in epidemic outbreaks of visceral leishmaniosis in Santander, Cundinamarca, Córdoba and Huila (Morrison et al 1993, Floréz et al 2006, Fernandez et al 2002, Gonzales et al 2006). Studies of experimental infection have proven their susceptibility to infection of viruses belonging to the genera Vesiculovirus, Orbivirus, Flavivirus, Alphavirus, Bunyavirus and Phlebovirus (Jennings and Bormann, 1980, Tesh and Modi, 1983).

Taxonomically *L. longipalpis* belongs to the subgenus *Lutzomyia* (França, 1924), differentiated mainly in the male by morphological characters such as the presence of curved mushrooms in the paramer, a tufo of accessory mushrooms at the base of the gonocoxite and in the female by a

spermatheca. Barrel-shaped as long as 4x wide (Young and Duncan 1994), this subgenus includes the majority of *Leishmania* spp. vectors. and presents species whose females are morphologically indistinguishable from *L. longipalpis* (Martins 1978; Vigoder et al 2010), making it difficult to identify them using taxonomic keys if there are no males present in the collection. This species presents morphological variations in abdominal tergites (Mangabeira 1969, Ward et al 1985), originating investigations that have shown a complex of species through studies of reproductive isolation (Lanzaro et al 1993), isoenzymes (Lanzaro et al 1998), chromosomal banding (Yin et al 1999), mitochondrial genes (Soto et al 2001, Arrivillaga, et al 2002) and nuclear genes (Peixoto et al 2001, Lins et al 2002, Lins 2008, Hoyos-Lopez et al. 2012), pointing out significant genetic differences at the population level as a consequence of the low flight capacity, little dispersion, and presence of climatic and geographic barriers between the sites where it is found (Morrison et al.1993, Arrivillaga et al., 2002), it has been suggested that these factors could be involved in differences in the transmission of *L. infantum* (Rocha et al., 2011).

In this context, the use of morphological characters associated with spermathecae, the high presence of females, the low number of males in the entomological collections, and the similarity between species of the same subgenus *Lutzomyia*, make difficult the taxonomic identification and vectorial incrimination of species that occur sympatrically in foci of leishmaniasis. An alternative is the search for molecular characters that allow differentiating species morphologically similar to *L. longipalpis* within the subgenus *Lutzomyia*, possible species within the longipalpis complex, and in turn determining the evolutionary relationships between populations (Lanzaro and Warburg 1995).

Recently, the mitochondrial transfer gene for serine (UCN) has been proposed as a molecular marker for the genetic characterization and diagnosis of the species (Vivero et al 2007, Pérez-Doria 2008, Pérez-Doria 2011), being explored in 11 species of phlebotomine with emphasis on species with importance in the transmission of leishmaniosis. In the present article we describe, for the first time, the primary and secondary structure of the mitochondrial transfer RNA for serine that recognizes the codon UCN (tRNA^{Ser}) of *L. longipalpis* from different localities to differentiate it from other species of the subgenus *Lutzomyia*.

Materials and methods

To describe the primary and secondary structure of the gene for tRNA^{Ser}, sequences obtained from specimens of *L. longipalpis* collected by the program of study and control of tropical diseases (PECET), of different geographical origin and registered in Genbank under the access numbers were used: AF403498 (Brazil), AF403497 (Brazil), AF403496 (Costa Rica), AF403495 (Costa Rica), AF403494 (Cundinamarca-Colombia), AF403493 (Cundinamarca - Colombia), AF403492 (Cundinamarca - Colombia). The sequences were aligned with the ClustalW algorithm in Bioedit (Hall 1999) and then analyzed in MEGA 4.1 (Tamura et al 2007) to determine the nucleotide composition and polymorphic sites of the gene. The secondary structure of the tRNA^{Ser} of *L. longipalpis* was obtained with the program tRNAscan-SE 1.21 (Lowe and Eddy 1997) and was manually graphed. Additionally, the sequences of *L. longipalpis* were compared with the sequences described for the genus *Lutzomyia* by Vivero (2007), Pérez-Doria (2008) and Pérez-Doria (2011).

Results & Discussion

The mitochondrial genome of insects has 13 protein-coding genes, a 12S rRNA gene, a 16S rRNA gene, a control region and 22 tRNA genes (Hoy 2006; Behura 2011), one for each amino acid, with the exception of leucine and serine, which individually possess two types of molecules that recognize the codon AGY and UCN, the latter is the one described in the present study (Hanada et al 2000).

The tRNA^{Ser} (UCN) of *Lutzomyia longipalpis* has a length of 67 bp, presenting a composition of 43.1% adenine, 7.5% cytosine, 10.7% guanine and 38.8% thymine, highlighting the high proportion of A - T (81.9%) as has been recorded in the mitochondrial genome of arthropods and insects (Crease 1999, Behura 2011). No overlap was observed between the last cytochrome B codon and the first codon of tRNA^{Ser}, and an intergenic spacer of 8 bp was present between them. This finding contrasts with the economic profile of the mitochondrial genome, the overlap between both genes and the absence of the intergenic spacer in *Lutzomyia trinidadensis* (Newstead 1922), *Lutzomyia dubitans* (Sherlock 1962), *Lutzomyia panamensis* (Shannon 1926) and *Lutzomyia evansi* (Nuñez- Tovar 1924) (Vivero et al 2007, Pérez-Doria et al 2008, Pérez-Doria et al 2011).

The modeled secondary structure of the tRNA^{Ser} of *L. longipalpis* is made up of the amino acid acceptor arm, the arm and the magnifying glass dihydrouridine (DHU), the arm and the magnifying glass of the anticodon, the variable magnifying glass, the arm and the magnifying glass ribotimidine-pseudouridinacytosine (TψC) (Figure 1). In the acceptor arm of the amino acid, seven matings were observed, six of which corresponded to pair A - U (adenine - uracil) and one to G - C (guanine - cytosine). This acceptor arm of *L. longipalpis* has a similar structure to

that inferred for *Lutzomyia hartmanni* (Fairchild and Hertig 1957), highlighting that during the in vivo maturation, the last two bases U - U (uracil - uracil) from the 3' end of the tRNA^{Ser} are replaced by the CCA trinucleotide (cytosine - cytosine - adenine), forming the binding site of the respective amino acid (Pérez-Doria et al 2011).

The number of matings in the DHU arm was three: two pairs G - C (guanine - cytosine) and one U - A (uracil - adenine), and the T Ψ C arm presented five matings, of which four corresponded to pair U - A (uracil - adenine) and a pair G - C (guanine - cytosine). The anticodon arm presented four canonical matings: three corresponding to the pair U - A (uracil - adenine) and one G - C (guanine - cytosine), the presence of a non - canonical mating U - U (uracil - uracil) was also observed. this arm. The size of the DHU, T Ψ C, variable and anticodon loupes corresponded to five, seven, four and seven nucleotides respectively, also presenting two nucleotides (adenine-adenine) between the acceptor arm and the DHU magnifying glass, and a nucleotide (adenine) between the latter and the anticodon arm.

The tRNA^{Ser} of *L. longipalpis* is distinguished from the others recorded (Vivero et al 2007, Pérez-Doria 2008, Pérez Doria 2011) mainly by the substitutions in positions 14, 45 and 51; in position 52 a change to guanine is observed, evidenced by a Costa Rican haplotype different to the other analyzed sequences, this change corresponds to adenine to guanine in the T Ψ C magnifying glass and does not originate changes in the secondary structure. By way of conclusion, the primary and secondary structure of the tRNA^{Ser} of *L. longipalpis* differs from those previously recorded for the genus *Lutzomyia*, which indicates its usefulness to differentiate it taxonomically, however it is recommended to extend the use of this marker to species that are evolutionarily close to *L. longipalpis* within the subgenus *Lutzomyia*. Exploration of other molecular markers as cytochrome oxidase I – COI is a tool with remarkable results to

differentiate mosquito species and sandflies (Hoyos et al. 2015a, Hoyos et al. 2015b, Toro-Cantillo et al. 2018) in rural areas where conditions favor the transmission of Leishmaniasis (Hoyos et al. 2013, Hoyos et al. 2016, Toro-Cantillo et al. 2017)

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References

ARRIVILLAGA, J.; D, NORRIS.; M, FELICIANGLI.; G, LANZARO. 2002. Phylogeography of the neotropical sand fly *Lutzomyia longipalpis* inferred from mitochondrial DNA sequences. Infection, Genetics and Evolution 2: 83 – 95.

BEHURA, S.; LOBO, N.; HAAS, B.; DEBRUYN, B.; LOVIN, D.; SHUMWAY, M *et al.* 2011. Complete sequences of mitochondria genomes of *Aedes aegypti* and *Culex quinquefasciatus* and comparative analysis of mitochondrial DNA fragments inserted in the nuclear genomes. Insect Biochemistry and Molecular Biology 41: 770 – 777.

CREASE, T.J. 1999. The complete sequence for the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233: 89 – 99.

FERNÁNDEZ J.; CHARRY, T.; BELLO,F.; ESCOBAR, J.; LOZANO, C.; AYALA, M *et al.* 2002.Prevalencia de Leishmaniosis Visceral canina en municipios de Huila Colombia. Revista de Salud Pública4:278 – 285.

FLOREZ ,M.; J, MARTINEZ.; R, GUTIERREZ.; K, LUNA.; H, SERRANO.; C, FERRO.; V, ANGULO.; M, SANDOVAL. 2006. *Lutzomyia longipalpis* (Diptera: Psychodidae) at a suburban

focus of visceral leishmaniasis in the Chicamocha Canyon, Santander, Colombia. *Biomédica* 26: 109 – 120.

GONZALES, C.; O, CABRERA.; L, MUNSTERMANN .; C, FERRO. 2006. Distribución de vectores de *Leishmania infantum* en Colombia. *Biomédica* 26 (Suppl I): 64 – 72.

GRIMALDI, G.; R, TESH & D, MACHAMON. 1989. A review on the geographic distribution and epidemiology of Leishmaniasis in the new world. *American Journal of Tropical Medicine and Hygiene* 41: 687 – 725.

HALL, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95 – 98.

HANADA, T.; SUZUKI, T.; WATANABE, K. 2000. Translation activity of mitochondrial tRNA with unusual secondary structure. *Nucleic Acids Symposium Series* 44: 249 – 250.

HOY, M. 2006. Insect molecular genetics: an introduction to principles and applications. Academic press, San Diego, 544 p.

Hoyos R, Uribe S, Velez I. 2012. Typification of Colombian specimens of *Lutzomyia longipalpis* (Diptera: Psychodidae) by “Barcoding”. *Rev Colomb Entomol* 38: 134-140.

Hoyos Lopez, R., Vivero Gomez, R. J., CONTRERAS, M. A., & SOTO, S. U. (2013). Phlebotomines sandflies (Diptera: Psychodidae) on a rural area from Santa Fe de Antioquia, Colombia. *Revista Colombiana de Entomología*, 39(1), 51-55.

Hoyos-Lopez R, Roman Pardo S, Castaño, JC, Gallego-Gómez JC. 2015b. DNA barcode for typing of immature mosquitoes from Armenia and Circasia (Quindío, Colombia). *Revista Colombiana de Entomología* 41(2): 218-227.

Hoyos-López, R., Bolaños, R., Contreras-Gutierrez, M., & Carrero-Sarmiento, D. (2016).

Phlebotomine sandflies (Diptera: Psychodidae) in a sub-Andean forest from the Norte de Santander, Colombia. *Journal of vector borne diseases*, 53(1), 70.

JENNINGS, M & BOORMAN, J. 1980. The susceptibility of *Lutzomyia longipalpis* (Lutz and Neiva), Diptera, Psychodidae, to artificial infection with three viruses of the Phlebotomus fever group. *Annals of Tropical Medicine and Parasitology* 74: 455 – 462.

LAINSON, R. & E. RANGEL. 2005. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis; with particular reference to Brazil - A Review -. *Memórias do Instituto Oswaldo Cruz* 100 (8): 811 – 827.

LANZARO, G.; K, OSTROVSKA .; M, HERRERO.; P, LAWYER.; A, WARBURG. 1993. *Lutzomyia longipalpis* is a species complex: genetic divergence and interspecific hybrid sterility among three populations. *American Journal of Tropical Medicine and Hygiene* 48: 839 – 847.

LANZARO, G. & A, WARBURG. 1995. Genetic variability in phlebotomine sandflies: possible implications for leishmaniasis epidemiology. *Parasitology Today* 11: 151 – 154.

LANZARO, G.; ALEXANDER, B.; MUTEBI, J.; MONTOYA, J.; WARBURG, A. 1998. Genetic variation among natural and laboratory colony populations of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae) from Colombia. *Memórias do Instituto Oswaldo Cruz* 93: 65 – 69.

LINS, R.; OLIVEIRA, S.; SOUZA, N.; QUEIROZ, R.; JUSTINIANO, S.; WARD, R.; et al .2002. Molecular evolution of the cacophony IVS6 region in sandflies. *Insect Molecular Biology* 11: 117 – 122.

LINS, R.; SOUZA, N.; PEIXOTO, A. 2008. Genetic divergence between two sympatric species of the *Lutzomyia longipalpis* complex in the paralytic gene, a locus associated with insecticide resistance and lovesong production. *Memórias do Instituto Oswaldo Cruz* 103: 736 – 740.

LOWE, TM.; EDDY, S. 1997.tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* 25:955 – 964.

MANGABEIRA, O. 1969. Sobre a sistemática e biologia do *Phlebotomus* no Ceará. *Revista brasileira de malariologia e doenças tropicais* 21: 3 – 26.

MARTINS, A.V.; A, FALCÃO.; J, SILVA.; E, DIAS. 1984. Nota sobre *Lutzomyia* (*Lutzomyia*) *cruzi* (Mangabeira.; 1938).; com a descrição da fêmea (Diptera.; Psychodidae.; Phlebotominae). *Memórias do Instituto Oswaldo Cruz* 79: 439 – 442.

MORRISON, A.; C, FERRO.; A, MORALES.; R, TESH.; M, WILSON. 1993. Dispersal of the Sand Fly *Lutzomyia longipalpis* (Diptera: Psychodidae) at an Endemic Focus of Visceral Leishmaniasis in Colombia. *Journal of Medical Entomology* 30: 427 – 435.

PEIXOTO, A.; GOMES, C.; DE AMORETTY, P.; LINS, R.; MEIRELES-FILHO, A.; DE SOUZA, N.; et al. 2001. New molecular markers for phlebotomine sand flies. *International Journal for Parasitology* 31: 635 – 639.

PÉREZ-DORIA, A.; BEJARANO, E.; SIERRA, D.; VELEZ, I. 2008. Descripción del ARN de transferencia mitocondrial para Serina (UCN) de *Lutzomyia colombiana*. *Revista Brasileira De Entomologia* 52: 591 – 594.

PÉREZ-DORIA, A.; BEJARANO, E. 2011.tRNASer (UCN) mitocondrial de *Lutzomyia hartmanni* predicción de la estructura secundaria del tRNASer (UCN) mitocondrial del

flebotomíneo *Lutzomyia hartmanni*(Diptera: Psychodidae). Acta Biológica Colombiana 16: 87 – 94.

ROCHA, L.; FALQUETO, A.; DOS SANTOS, C.; GRIMALDI, G.; CUPOLILLO, E. 2011. Possible Implication of the Genetic Composition of the *Lutzomyia longipalpis* (Diptera: Psychodidae) Populations in the Epidemiology of the Visceral Leishmaniasis. Journal of Medical Entomology 48: 1016 – 1022.

SOARES, R & TURCO, S. 2003. *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae): a review. Anais da Academia Brasileira de Ciências 75: 301 – 330.

TAMURA, K.; J, DUDLEY.; M, NEI.; S, KUMAR. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596 – 1599.

TESH, R & MODI, G. 1983. Development of a continuous cell line from the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae), and its susceptibility to infection with arboviruses. Journal of Medical Entomology 20: 199 – 202.

Toro-Cantillo, A., Atencia-Pineda, M., & Lopez, R. H. (2017). Flebotomíneos (Diptera: Psychodidae) colectados en área rural de San Bernardo del Viento (Córdoba Colombia). Revista MVZ Córdoba, 22(supl), 6044-6049.

Toro-Cantillo, A, Hoyos-López R (2018). Molecular identification and Genetic Diversity of *Lutzomyia gomezi* (Diptera: Psychodidae) using DNA-barcodes in Cordoba, Colombia. Tropical Biomedicine 35 (1).

URIBE, S.; T, LEHMANN.; E, ROWTON.; I, VELEZ.; C, PORTER. 2001. Speciation and population structure in the morphospecies *Lutzomyia longipalpis* (Lutz and Neiva) as derived from the mitochondrial ND4 gene. *Molecular, Phylogenetic and Evolution* 18: 84 – 93.

VIVERO, R.; CONTRERAS, M.; BEJARANO, E. 2007. Analysis of the primary and secondary structure of the mitochondrial serine transfer RNA in seven species of *Lutzomyia*. *Biomédica* 27: 429 – 438.

VIGODER, F.; ARAKI, A.; BAUZER, L.; SOUZA, N.; BRAZIL, R.; PEIXOTO, A. 2010. Love songs and period gene polymorphisms indicate *Lutzomyia cruzi* (Mangabeira, 1938) as a sibling species of the *Lutzomyia longipalpis* (Lutz and Neiva, 1912) complex. *Infection, Genetics and Evolution* 10: 734 – 739.

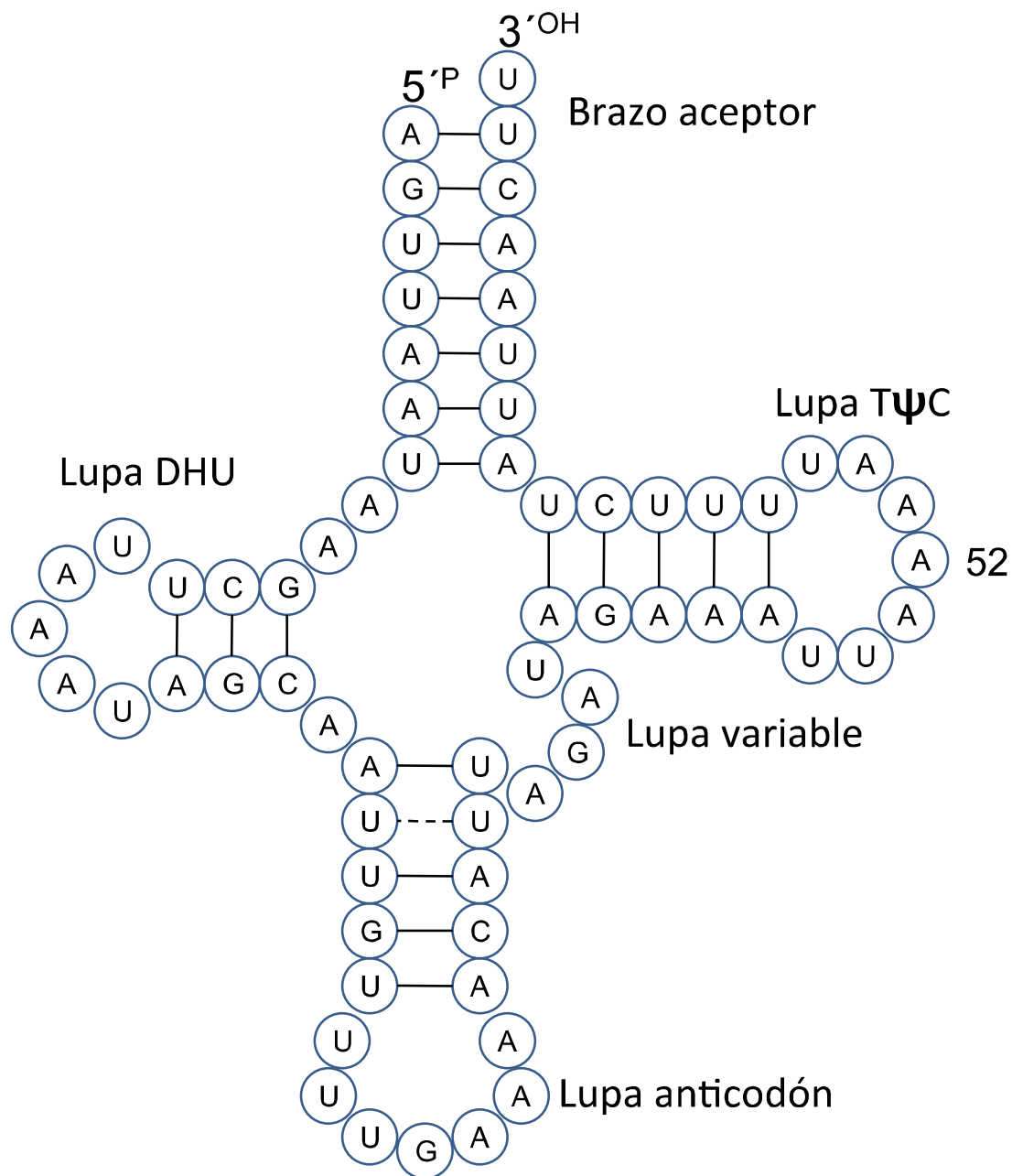
WARD, R.; A, RIBEIRO.; L, RYAN.; A, FALCO.; E, RANGEL. 1985. The distribution of two morphological forms of *Lutzomyia longipalpis*(Lutz & Neiva) (Diptera: Psychodidae). *Memórias do Instituto Oswaldo Cruz* 80: 145 – 148.

YIN, H.; J, MUTEBI.; S, MARRIOTT.; G, LANZARO. 1999. Metaphase karyotypes and G-banding in sandflies of the *Lutzomyia longipalpis* complex. *Medical & Veterinary Entomology* 13: 72 – 77.

YOUNG, D. & M. A. DUNCAN. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Memoirs of the American Entomological Institute*, 54: 1 – 881.

ZAMORA-DELGADO, Julián; CASTAÑO, Jhon Carlos; HOYOS-LÓPEZ, Richard. DNA barcode sequences used to identify *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in La

Tebaida (Quindío, Colombia). Revista Colombiana de Entomología, 2015, vol. 41, no 2, p. 212-217.



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1111111111222222222233333333334444444444555555555566666666667
123456789012345678901234567890123456789012345678901234567890
Lutzomyia trinidadensis AGTTAATAAGCTTTA-ATAGCAATTGTTTTGAAAACATTAGATAAAAAATTTAAAAATTTCTATTAACCTT
Lutzomyia panamensis .....A.A.A.....--.....
Lutzomyia cayenennsis cayenennsis .....A.T.C.....--.A.....
Lutzomyia dubitans .....-.....C--C.....
Lutzomyia gomezi .....-.....--C.....
Lutzomyia rangeliana .....A.A.....T.....AA.T.....--.....
Lutzomyia evansi .....-.....C.C.....--.....
Lutzomyia pia .....-.....C.....G...CC.G...--.....
Lutzomyia tihuiliensis .....A.-.....G...ACC...--.....
Lutzomyia hartmanni .....A.-.....A.....G...CATT...-A.....
Lutzomyia columbiana .....-.....G...C.....--.....G...
Lutzomyia longipalpis Llcolo1 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llcolo2 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llcolo3 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llbra1 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llbra2 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llcost1 .....A.-.....G.....A...--.....
Lutzomyia longipalpis Llcost2 .....A.-.....G.....AG...--.....

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Figure 2. Multiple alignment of the sequences registered in Vivero (2007), Pérez-Doria (2008) and Pérez-Doria (2011). The nucleotides are represented by the first letter of the respective name. The points indicate homology and the dashes correspond to insertion-deletion events.

