

Phylogenetic relationships and evolutionary patterns of the genus *Psammolestes* Bergroth, 1911 (Hemiptera: Reduviidae: Triatominae): tools for vector control of Chagas disease

Carolina Hernández¹, Mateo Alvarado¹⁺, Fabian C. Salgado-Roa^{2,3+}, , Nathalia Ballesteros¹, Nicol Rueda-M², Jader Oliveira^{4,5}, Kaio Cesar Chaboli Alevi⁴, Joao Aristeu da Rosa⁴, Plutarco Urbano⁶, Camilo Salazar^{2*}, Juan David Ramírez^{1*}

¹Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMIBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia.

²Grupo de Genética Evolutiva y Filogeografía, Departamento de Biología, Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia.

³ School of BioSciences, The University of Melbourne, Parkville, VIC, 3052 Australia.

⁴Universidade Estadual Paulista (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara, Sao Paulo 01000, Brazil.

⁵ Universidade de São Paulo (USP), Faculdade de Saúde Pública, São Paulo, SP, Brazil.

⁶Grupo de Investigaciones Biológicas de la Orinoquia, Fundación Universitaria Internacional del Trópico Americano (Unitrópico), Yopal, Colombia.

*Correspondence: juand.ramirez@urosario.edu.co; camilo.salazar@urosario.edu.co

+ Both authors contributed equally

The kissing bugs of the subfamily Triatominae distinguish themselves among the subfamilies of Reduviidae due to their hematophagous behavior, but especially for being vectors of *Trypanosoma cruzi*, which causes Chagas disease. This subfamily comprises 156 species and all the extant species are potential vectors of *T. cruzi*. Current vector control strategies could benefit from a deep understanding of the vector's biology, ecology, and evolution. For this reason, understanding factors that shape vector species diversity and their speciation processes are essential for establishing successful control strategies. The first step to accomplish this goal involves a complete characterization of the number of lineages inside of genera, their phylogenetic relationships, and its evolutionary patterns. The evolutionary history of biodiversity in South America has been poorly studied in the seasonal dry tropical forest (SDTF). Here we studied the diversification of *Psammolestes*, a genus endemic of the SDTF and naturally infected with *T. cruzi*.

We collected 92 individuals of the three *Psammolestes* species, from 12 localities in Venezuela, Colombia, and Brazil. DNA extraction was performed using the DNeasy® Blood & Tissue kit. We performed PCR and sequencing of seven loci: 6 nuclear and 1 mitochondrial locus. Phylogenetic relationships were recovered using maximum likelihood

and Bayesian inference. Divergence times were determined using CYTB locus. Population genetics analyses, tests of neutrality, haplotype networks and STRUCTURE were performed. Geographical diversification was explored testing for isolation by distance, linear regression between the genetic distance and the geographical distances and Monmonier's algorithm. Number of lineages were established with two methods: Bayesian Phylogenetics and Phylogeography method and the multi-rate Poisson Tree Processes method. Niche modelling were performed using BIOMOD2.

Our multilocus analyses recovered *P. coreodes* and *P. tertius* in a monophyletic clade sister to *P. arthuri*. Species delimitation tests recovered these lineages as different species despite the shared genetic variation observed between *P. coreodes* and *P. tertius* in five genes. Also, genetic variation of the genus clustered in three groups that were consistent with the three morphospecies. Our demographic model predicted a scenario of divergence in absence of gene flow, suggesting that mixed haplotypes may be the result of shared ancestral variation since the divergence of the subtropical-temperate species *P. coreodes* and *P. tertius*. In contrast, the tropical species *P. arthuri* was highly differentiated from the other two in all tests of genetic structure, and consistently, the Monmonier's algorithm identified a clear geographical barrier that separates this species from *P. coreodes* and *P. tertius*.

We found three genetically structured lineages within *Psammolestes* that diverged in absence of gene flow in the late Miocene. This result supports a scenario of species formation driven by geographical isolation rather than by divergence in the face of gene flow associated with climatic oscillations in the Pleistocene. Also, we identified the Amazon basin as a climatic barrier that separates tropical from subtropical-temperate species, thus promoting allopatric speciation after long range dispersion. Finally, each species of *Psammolestes* occupies different climatic niches, suggesting that niche conservatism is not crucial for species differentiation. These findings influence the current vector surveillance programs of Chagas disease in the region, and it could be used for the design the new strategies for vector control of triatomines.