Molecular identification of *Deinocerites atlanticus* (Adames, 1971) (Diptera: Culicidae) using cytochrome oxidase I

Angie Toro-Cantillo & Richard Hoyos-López

Grupo de Investigación en Enfermedades Tropicales y Resistencia Bacteriana, Universidad del Sinú, Facultad de Ciencias de la Salud, Córdoba, Colombia

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*Deinocerites atlanticus* (Adames, 1971) is a widely distributed mosquito, which has been reported in Panama, Colombia and Venezuela\(^1\). The immature stage of this genus develops in the water accumulated inside holes, excavated by crabs; and adult mosquitoes are generally active at night. The females are considered opportunistic and ingest blood from a variety of vertebrates, including mammals, reptiles, amphibians, and birds\(^2\)\(^\sim\)\(^3\). Very few studies have been carried out on the behaviour, ecology, and biology of this species; and the role they play as a vector in transmitting possible diseases in vertebrates is largely unknown.

Normally, these mosquitoes are not considered as a threat to public health, but some species are occasionally infected with arboviruses, including Venezuelan equine encephalitis virus (VEEV) and St. Louis encephalitis virus (SLEV)\(^4\). In Colombia, the species *De. atlanticus* has been studied to determine if the environmental conditions were favourable for the reappearance of VEEV in a rural area of the Manaure municipality (Guajira)\(^5\). Recently, VEEV was detected in specimens belonging to *De. atlanticus* in a rural area from San Bernardo del Viento (Córdoba, Colombia)\(^6\). In this study, we used the DNA barcode methodology for molecular identification, in the mosquito species *De. atlanticus* collected in the rural area of San Antero, a coastal municipality from the Cordoba region (Colombia), because it has allowed to supply certain limitations that the morphological identification of the species presents; complementing, corroborating and recognizing new species, which are stored in databases such as BOLD or GenBank, as well as studying species complexes and knowing the biological richness of the species that circulate in different geographical areas.

Mosquitoes used in this study were collected between July and September 2016 in a rural area from San Antero (Tijo, 9°21′59.63″ N, 75°48′51.91″ W) (Córdoba, Colombia). Adults were collected using manual aspirators and Shannon traps close to rural forest patches, farms and mangroves. Three Shannon traps were installed (distributed one per site, *i.e.* rural forest patches, farms, and mangroves) at a height of 1.5 m from the ground level. They operated from 1800 to 2100 hrs. Each trap recorded date, time, coordinates, and type of coverage. A total of 100 specimens belonging to Culicidae family were collected, of which 20 were selected for this study, as they were in good condition and according to the morphological characteristics belonged to the species *De. atlanticus*, using pictorial keys\(^6\)\(^\sim\)\(^7\). Legs from specimens identified as *De. atlanticus* were removed, the DNA was extracted using the protocol described by Atencia *et al.* Amplification of the ~680 nt fragment of the DNA barcode region from the *COI* gene was achieved using the primer pair LCO-1490/HCO-2198\(^9\)\(^\sim\)\(^10\). PCR-mix contained, 1 × NH\(_4\)SO\(_4\) buffer, 1 mM each DNTP, 5 mM of MgCl\(_2\), 0.5 µM each primer, 0.4 U of taq polymerase (Bioline, Maryland) and 4 µl of DNA template. Water of molecular biology quality was added to the mixture to make the total volume up to 50 µl. The PCR thermocycler parameters included a single cycle at 94 °C for 10 min followed by 35 cycles of 95 °C for 60 sec, 50 °C for 60 sec and 72 °C for 60 sec, terminating with a 72 °C cycle for 5 min as the final extension step and a 4 °C hold. PCR products were visualized on 1% agarose gels, containing ethidium bromide (diluted to 1/50), using a Dark Reader lector (Imagen, Alexandria). PCR products were sequenced using the same LCO/HCO primers utilizing the sequencing services of Macrogen company (Seoul, Korea). Sequences were manually edited in Bioedit v7.2.0 software (http://www.mbio.ncsu.edu/BioEdit/bioedit.htm) and consensuses in the FASTA format were aligned in ClustalW\(^11\). Genetic distances were estimated in MEGA\(^12\) v6.0 using the Kimura 2-parameter model (K2P)\(^13\), and molecular operational taxonomic units (MOTU’s) were identified according to calculated genetic distances and clusters within a dendrogram inferred by the neighbour-joining (NJ) algorithm\(^14\) (K2P model bootstrap = 1000 replications)\(^15\). *Culex* (Melano-
conion) erraticus (Say, 1823) was used as an outgroup because they are good references for inter-species genetic distances. Genetic diversity parameters were estimated by polymorphic sites, a number of haplotypes, haplotype diversity, nucleotide diversity, and Tajima neutrality tests using DnaSP v5.0 software.

A total of 20 sequences were studied, of which eight belonged to rural forest patches, six to farms and six belonged to mangroves. The sequences were compared with the sequences available in the GenBank database to verify the taxonomic identification. The sequences obtained have a length of 694 nt for De. atlanticus and correspond to positions 295 to 945 of the mitochondrial gene cytochrome oxidase I (Reference sequence in GenBank: AF425846.1 – Ae. aegypti). Comparison revealed that, all 20 sequences were compatible with genus Deinocerites, with 100% similarity to the sequences of De. atlanticus stored in the GenBank database. No insertion-deletion events were evident in the sequences analyzed, and the presence of stop codons, characteristic of nuclear copies of mitochondrial genes (NUMT’s), was not detected.

In total five haplotypes were identified, showing a haplotype diversity of 0.68 and eleven polymorphic sites (Table 1); the intra-species genetic distances under Kimura-2 model parameters were low (0.007), indicating the presence of conspecific individuals of the same species. Interspecies genetic distances for De. atlanticus–Cx. erraticus (0.105) corresponded to recorded estimates for species differentiation in mosquitoes and matched with species-specific MOTUs in the NJ dendrogram (Fig. 1).

The taxonomic identification and correct differentiation of Culicidae is a priority in vector incrimination and disease prevention; however, the high morphological similarity between species and the existence of cryptic species complexes makes the use of molecular markers necessary in order to resolve these taxonomic problems and provide for the rapid recognition of vectors. The sequences reported in this study could be of help in metagenomic studies searching for arboviruses, taxonomic confirmation, and in the determination of role that mosquitoes play as vectors in transmitting arboviruses in vertebrates.

Comparing the sequences of this study with those stored in the barcode of life database (BOLD), inconsistencies with the species were evident; however, access to the sequences stored in BOLD is private making it difficult to access the relevant information of the sequences studied. In contrast, the sequences stored in GenBank presented a 100% concordance for the species De. atlanticus.

The municipality of San Antero is characterized by an altitude of <100 m, containing multiple mangroves with well-irrigated soils due to streams, causing formation of several ponds, which are suitable spaces for the proper development of this species of mosquito, since its development occurs in water accumulated in holes excavated by crabs. Their biting activity may extend to humans. However, many essential aspects of De. atlanticus aff. biology remains unknown. For De. atlanticus, arbovirus

![Fig. 1: Neighbour-joining dendrogram estimated with sequences of cytochrome oxidase I obtained from De. atlanticus and Cx. erraticus (Kimura-2-parameter, bootstrap = 1000 replicates). The branch values indicate the bootstrap clusters in same MOTU (values >50). The final alignment was for 657 nucleotides.](image)

**Table 1. Haplotype distribution in sequences of Deinocerites atlanticus collected from a Municipality of Córdoba (Colombia)**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Haplotype distribution</th>
<th>Sampling area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap_1</td>
<td>[1, 16] MF179306_De_atlanticus, MF179302_De_atlanticus</td>
<td>Farms</td>
</tr>
<tr>
<td>Hap_2</td>
<td>[2–6, 9–14] MF179288_De_atlanticus, MF179289_De_atlanticus, MF179290_De_atlanticus, MF179291_De_atlanticus, MF179292_De_atlanticus, MF179295_De_atlanticus, MF179296_De_atlanticus, MF179297_De_atlanticus, MF179298_De_atlanticus, MF179299_De_atlanticus, MF179300_De_atlanticus</td>
<td>Rural forest patches, Mangroves</td>
</tr>
<tr>
<td>Hap_3</td>
<td>[7] MF179293_De_atlanticus</td>
<td>Rural forest patches</td>
</tr>
<tr>
<td>Hap_4</td>
<td>[8, 15, 17, 19] MF179294_De_atlanticus, MF179301_De_atlanticus, MF179303_De_atlanticus, MF179304_De_atlanticus</td>
<td>Farms</td>
</tr>
<tr>
<td>Hap_5</td>
<td>[18, 20] MF179304_De_atlanticus, MF179306_De_atlanticus</td>
<td>Rural forest patches</td>
</tr>
</tbody>
</table>
detection is just one aspect of vector incrimination, and more data are needed to define vector competence, identify specific habitats, biting behaviour, and blood feeding patterns. In Colombia, records of the species De. atlanticus, have been reported in a study, that examined whether the environmental conditions were favourable for the reappearance of VEEV in a rural area of the Municipality of Manaure (Guajira). This study observed that De. atlanticus was the most abundant species (91%) of the collected specimens, making it a vector of importance in the study area, as the prevalent environmental conditions and epidemiological situation could favour the reappearance of the VEEV virus in this locality.

Ethical statement
There is no any ethical consideration in this study.

Conflict of interest
There is no any conflict of interest to declare in this study.

REFERENCES


Correspondence to: Ms. Angie Toro-Cantillo, Cra. 1w No. 38-153, 4536534, Barrio Juan XXIII, Montería, Córdoba, Colombia.
E-mail: angiecantillo09@gmail.com

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