N O T E

DNA Barcoding and First Record of *Martarega hondurensis* (Hemiptera: Notonectidae) in Northwestern Mexico[1]

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Martarega (White) is a genus of aquatic insects of the Notonectidae family and Notonectidae subfamily, ranging from the state of Arizona (USA) to Argentina (Truxal 1949, J. Kans. Entomol. Soc. 22:1–36; Menke and Truxal 1966, Contrib. Sci. 106:1–6; Barbosa et al. 2015, Zootaxa 3947:417–424). The species within this genus are most commonly found in their brachypterous form and frequently inhabit lotic and perennial aquatic ecosystems, which allow them to capture prey through water currents (Truxal 1949; Menke and Truxal 1966).

There are two known species of *Martarega* in Mexico: *M. mexicana* (Truxal) and *M. hondurensis* (Bare), both of which share morphological characteristics but can be differentiated by (1) the position of the nodule of the middle femur and (2) the composition of the hairs from the ventral keel in males (Truxal 1949; Mazzucconi 2011, Aquat. Insects 33:113–126). Based on historical records, *M. mexicana* is reported to inhabit Morelos in central Mexico, Veracruz toward the Gulf of Mexico, and the states of Nayarit, Sonora, and Chihuahua in northwestern Mexico and along the Pacific Ocean coast (Truxal 1949; Menke and Truxal 1966; Bogan et al. 2013, Southwest. Nat. 58:494–497). On the other hand, *M. hondurensis* has been reported in Campeche in the southeast of Mexico (Niesser 1968, Studies on the Fauna of Suriname and other Guayanas 10:110–136) and is reported for the first time herein in Sinaloa, Mexico.

Efforts to determine the faunal composition of aquatic insects in Mexico lack a detailed list of species belonging to the *Martarega* genus (Bond et al. 2014, Parasit. Vectors 7:41; Durán-Rodríguez et al. 2022, Hidrobiológica 32:127–140), which highlights the need to conduct investigations that contribute to a better understanding of

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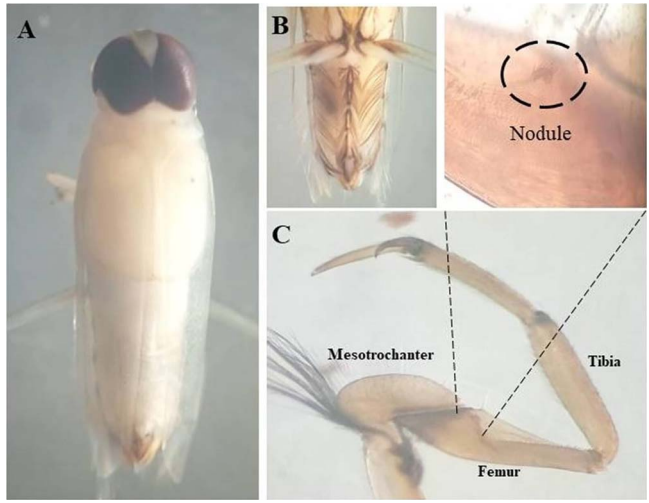

Fig. 1. Morphological and molecular characterization of *M. hondurensis* collected in Sinaloa, Mexico. (A) Dorsal view of a male, (B) ventral view with visualization of the keel with the anteroventral and lateral external hairs, and (C) location of the nodule on the middle femur of the leg.

their diversity and distribution in the country. An analysis that can improve the basic comprehension of their distribution and the methods that complement their identification (e.g., molecular analysis) can significantly advance the conservation and management of these species.

Martarega hondurensis was collected from the following rivers and locations: Culiacan (24°48′N, 107°24′W), San Lorenzo (24°26′N, 107°05′W), and Piaxtla (23°53′N, 106°37′W) in Sinaloa, Mexico. The samples were collected between January and June 2024 using a 100-μm mesh light net. The specimens were placed in transparent plastic jars with approximately 100 mL of 75% ethanol and then examined under a stereomicroscope. They were identified following the descriptions of males by Truxal (1949) and Mazzucconi (2011).

The identified species were processed for molecular analysis by extracting total DNA using the Wizard SV Genomic DNA purification System (Promega, Madison, WI) commercial kit following the manufacturer's instructions. PCR analysis followed using the GoTaq Green Master Mix (Promega) commercial kit and sense oligonucleotides UEA 7/C1-j-2369 (5′-TACAGTTGGAATAGACGTTGA TAC-3′) and anti-sense UEA 10/TL2-N3014 (5′-TCCAATGCACTAATCTGCCA TATTA-3′), which amplified the partial region of a gene that codes for the Cytochrome C Oxidase Subunit I (COI) (Lorenz et al. 2015, Infect. Genetic. Evol. 35:144–152; Mohammed et al. 2024, J. Infect. Dev. Ctries 18:1220–1226). The following conditions were applied for the amplification process: 4 min of denaturation at 95°C, followed by 34 cycles (95°C for 40 s, 55°C for 40 s, and 72°C for 50 s) and a final extension step of 75°C for 5 min.

After purification by the Wizard SV Gel and PCR Clean-Up System (Promega) kit, the PCR products were sequenced by the Sanger method (Macrogen, Inc., Seoul, South Korea). The sequences were edited in MEGA v. 11 (Tamura et al. 2021, Mol.

Table 1. COI gene sequence of *M. hondurensis* and genomic differentiation analysis.

Nucleotide Sequence of *Martarega hondurensis*

TCAGCCACTGTAATTATTGCTGTACCAACTGGAATTAAAATCTTTAGATGATTA
GCTACACTACATGGATCTGTAGTAAATTATAGACCATCAATTATATGAGCAA
TTGGATTTGTATTTCTATTTACTATAGGAGGGTTAACAGGAGTTGTGTTAGC
AAACTCCTCAATTGACATTATTTTACACGACACTTACTATGTAGTAGCACAT
TTCCACTATGTTCTATCCATAGGAGCTGTTTTCGCAATTATTGGAAGATTCA
TTCAATGATATCCTATAATAACAGGAATAACTATAAATCCTAAATGATTAAAA
ATACACTTTATTATTATATTCACAGGAGTGAATATAACATTCTTTCCACAACA
TTTCTTAGGACTAAGAGGAATACCTCGACGATACTCTGATTATCCAGATAG
CTATACAACATGAAATGTTGTATCATCTATAGGATCAACTATTTCAATTGTAG
GAGTAATAATTATAATATTTATTATCTGAGAAAGTATAATTAGTAAACGACAA
GTTTTATTTAACGACAATATAAACACAAGAATTGAATGATTACAAAAGTTCCC
GCCAGCTGAACACTCATATTCAGAACTACCAATACTTAGAAGATTCTAATAT
GGCAGATT

Species	GenBank Access	Percent Identity
Buenoa pallens	HM357720.1	88.89
Notonecta amplifica	MZ305077.1	83.20
N. chinensis	NC036671.1	81.86
Eurydema liturifera	NC044763.1	79.82
E. maracandica	NC037042.1	79.72
Phrynovelia bimaculata	JX961658.1	79.75
Patomometra zhengi	MN577445.1	79.53
P. montandoni	MN560137.1	78.78
Potamometropsis ikarus	EU871335.1	79.00
Mesovelia vittigera	JQ362942.1	78.12
Poecilocoris druraei	MZ269306.1	77.90

Biol. Evol. 38:3022–3027) and aligned with deposited sequences from the GenBank genomic database using the Basic Local Alignment Search Tool (BLAST). A species was considered homologous when the nucleotide identity was over 99% (Li et al. 2020, Front. Genet. 11:602863). A sequence matrix of DNA corresponding to the COI region of the aquatic insect species, including the Notonectidae family, was created and subsequently aligned using ClustalW en MEGA v11 (Tamura et al. 2021).

Phylogenetic trees were built based on the estimations from maximum likelihood (ML) and Bayesian (BI), according to the evolutionary model GTR+G+I selected for their Bayesian Information Criterion (BIC) (Luo et al. 2010, BMC. Evol. Biol. 10:1–13). The ML was elaborated using MEGA v11 (Tamura et al. 2021) with 1,000 bootstrap replicas. The BI analysis was made using MrBytes v3.2.7 (Ronquist and Huelsenbeck

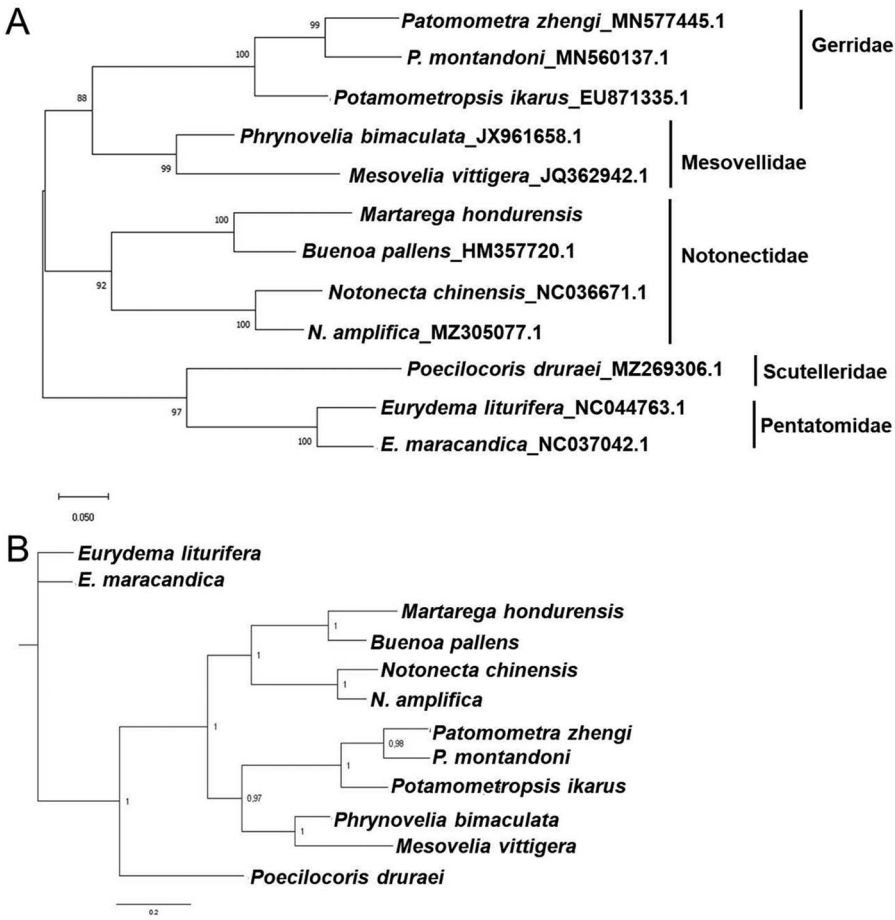

Fig. 2. Phylogenetic analysis of *M. hondurensis* based on estimations from maximum likelihood (A) and Bayesian (B) evolutionary model GTR+G+I.

2003, Bioinformatics 19:1572–1574) with 10 million generations, 4 Monte Carlos chains, and sampling every 1,000 trees. The convergence of the chain was determined using Trecer v1.7.2 (Rambaut et al. 2018, Syst. Biol. 67:901–904). Of the total samples from the trees, 25% were omitted. The remaining samples were used to generate a consensus tree, edited and visualized in FigTree v1.4.3 (Rambaut et al. 2018).

Of the 38 *M. hondurensis* brachyptera that were collected, 15 were female and 23 were males. The latter were identified by the presence of hairs on the anteroventral and lateral surfaces of the keel (Fig. 1A, B), in addition to a nodule situated on the middle femur, located at the level of the joints between the mesotrochanter and the femur (Fig. 1C). From the molecular analysis, it was determined that there were no homologous species of *M. hondurensis* (nucleotide similitude <89%) (Table 1). The results of the phylogenetic tree (maximum likelihood and Bayesian) matched with the taxonomic classification of the species and, thus, they were

grouped within their respective families, forming monophyletic clades for each taxonomic group (Fig. 2A, B). *Martarega hondurensis* was grouped correctly within the Notonectidae family and, based on the analysis, presented a close relationship to *Buenoa pallens* (Champion), followed by the genus *Notonecta* (Fig. 2A, B).

The brachypterous form is frequently observed within species from the *Martarega* genus, including *M. hondurensis* (Gittelman 1974, J. Kans. Entomol. Soc. 47:145–155). This genus has been poorly studied, and *M. hondurensis* is found within the literature as one of the most studied species. Nevertheless, ecological studies and clarification of its geographical distribution are needed.

Molecular topologies indicate that the analyzed fragment in this study is adequate for the molecular identification of *M. hondurensis*, which has been documented for other species of insects (Lorenz et al. 2015, Infect. Genetic. Evol. 35:144–152; Mohammed et al. 2024). It is important to note that, even though sequences based on COI molecular markers for *Martarega* exist, including *M. hondurensis*, this is the first time this specific sequence has been reported. This represents a major contribution that can strengthen investigations of the species richness of *Martarega*, allowing clarification of its geographical distribution and its ecological and evolutionary aspects in endemic areas of the Americas, including Mexico.