

## ***Amblyomma yucumense* Krawczak, Martins & Labruna, 2015 (Acari: Ixodidae) in Argentina**

DANIELA LAMATTINA<sup>1\*</sup>, JOSÉ M. VENZAL<sup>2</sup>, EVELINA L. TARRAGONA<sup>3,4</sup>, ATILIO J. MANGOLD<sup>3</sup> & SANTIAGO NAVA<sup>3,4</sup>

<sup>1</sup>Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Jujuy y Neuquén S/N, CP 3370 Puerto Iguazú, Misiones, Argentina; E-mail: daniela.lamattina@gmail.com

<sup>2</sup>Laboratorio de Vectores y Enfermedades Transmitidas, Facultad de Veterinaria, CENUR Litoral Norte-Salto, Universidad de la República, Rivera 1350, CP 50000 Salto, Uruguay; E-mail: jvenzal@unorte.edu.uy

<sup>3</sup>Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Rafaela, CC 22, CP 2300 Rafaela, Santa Fe, Argentina; E-mail: nava.santiago@inta.gob.ar

<sup>4</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

\*Corresponding author

The Argentinian fauna of hard ticks (Acari: Ixodidae) is currently composed of at least 39 species, being the genus *Amblyomma* the most numerous with 24 species (Nava *et al.* 2017). *Amblyomma yucumense* Krawczak, Martins & Labruna, 2015 was recently described from specimens collected in the Turvo State Park, state of Rio Grande do Sul, southern Brazil (Krawczak *et al.* 2015). To date this is the only place where this tick species has been found. But in this work we report for the first time the presence of *A. yucumense* in Argentina by means of morphological diagnoses and the analysis of partial sequences of the mitochondrial 16S rRNA gene.

The study area is located at Moconá Provincial Park (MPP) (27°9'S, 53°54'W), which is situated in central Misiones province, Argentina, and harbors almost 1000 hectares of Paraná Forest. This area is part of the Atlantic Rainforest ecoregions complex (Plací & di Bitteti 2005). The Park connects the Turvo State Park in Brazil (17,495 has) and the Yabotí Biosphere Reserve (YBR) in Argentina (253,773 has), constituting a large biological corridor.

During an ecological study of ticks and tick-borne diseases in Misiones province, free-living ticks were collected by flagging/dragging and visual search of ticks on the tips of the leaves along animal trails at the MPP and the YBR for three consecutive days in September 2017 (Terassini *et al.* 2010). All specimens were placed in tubes containing 96% ethanol, and identified according to Martins *et al.* (2014), Krawczak *et al.* (2015) and Nava *et al.* (2017). Representative specimens of the *A. yucumense* ticks collected were subjected to DNA extraction in order to amplify a 410-bp fragment of the mitochondrial 16S rDNA gene following Mangold *et al.* (1998). Sequences were aligned with each other and with the corresponding sequences of the Neotropical *Amblyomma* species available in GenBank, using the BioEdit Sequence Alignment Editor (Hall 1999) with the CLUSTAL W program (Larkin *et al.* 2007).

A total of 594 ticks were collected, of which 448 were *Amblyomma brasiliense* Aragão, 1908, 90 *Amblyomma incisum* Neumann, 1906, one *Amblyomma ovale* Koch, 1844, 48 *Haemaphysalis juxtakochi* Cooley, 1946 and seven *A. yucumense* (Table 1).

The male *A. yucumense* (Figs. 1A, 1B) was collected near the Moconá waterfalls. The diagnostic characters are a combination of basis capituli rectangular with cornua on posterior margin, scutum with deep punctuations evenly distributed except in dark-brown median-lateral spots, presence of longitudinal stripes originating in the cervical area and extending posteriorly to the level of the first festoon and a pale stripe indicating a pseudoscutum, coxa I with two triangular spurs sub-equal in

size, the external thinner and slightly longer than the internal, coxa IV with one spur longer than the spurs of coxae II and III, and spiracular plate comma-shaped.

Regarding *A. yucumense* nymphs (Figs. 1C, 1D), three were collected at the PPM and three at the YBR. All nymphs presented an apically rounded hypostome and dentition 2/2, basis capituli hexagonal without cornua, with posterior margin straight, scutum with deep punctations evenly distributed and larger in the laterals, cervical grooves exceeding the level of the posterior margin of the eyes; coxa I with two triangular spurs, the external longer than the internal, and spiracular plates comma-shaped.

**TABLE 1.** Free-living ticks collected on vegetation at Moconá Provincial Park and Yabotí Biosphere Reserve, Misiones, Argentina, September 2017.

	Females	Males	Nymphs	Larvae
<i>Amblyomma brasiliense</i>	21	27	399	1
<i>Amblyomma incisum</i>	2	2	86	
<i>Amblyomma ovale</i>	1			
<i>Amblyomma yucumense</i>		1	6	
<i>Haemaphysalis juxtakochi</i>	5	8	35	

The 16S sequences obtained from the male and the nymph of *A. yucumense* from Argentina (Genbank accession numbers: MH282856) showed 100% identity with that of *A. yucumense* from Turvo State Park in Brazil (GenBank accession number: KJ914670). The male of *A. yucumense* collected in PPM and one nymph of this species collected in YBR are deposited in the Tick Collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Argentina (male: INTA 2379; nymph: INTA 2380).

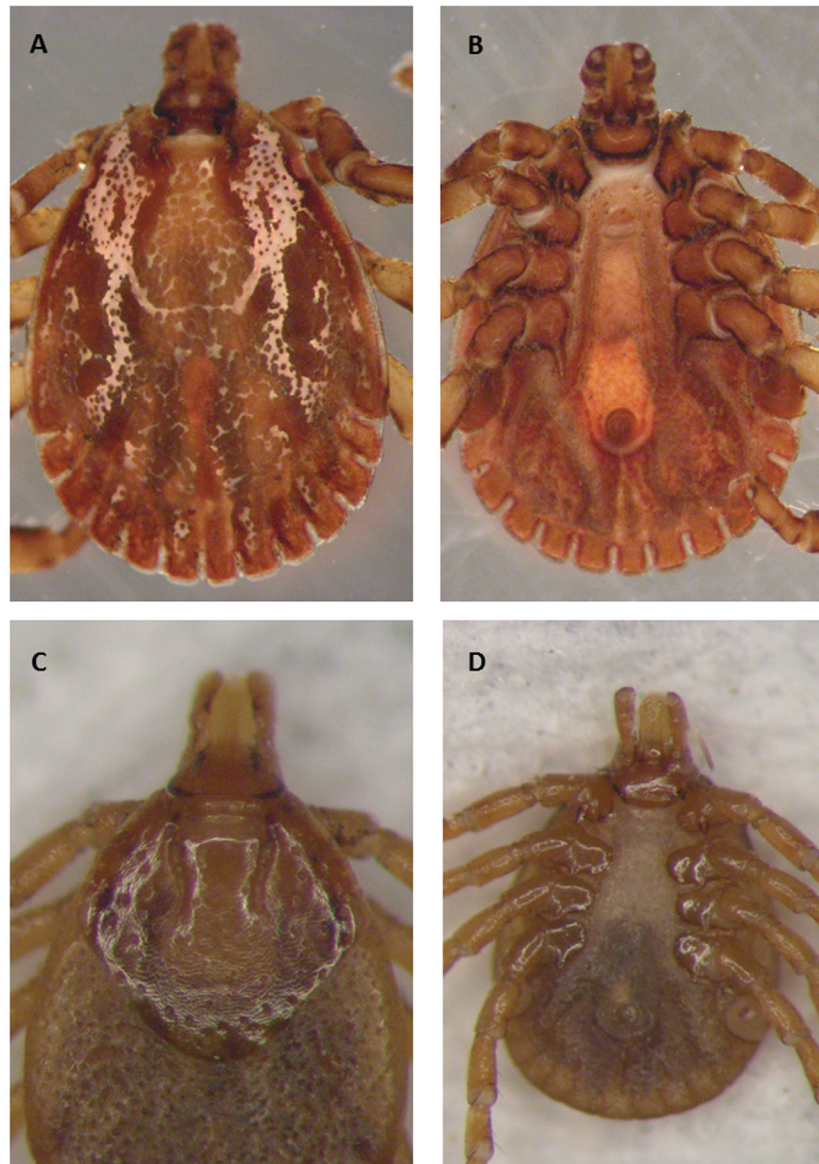
The combination of the morphological characters described above and the analysis of 16S sequences led us to conclude that some of the ticks collected at MPP and YBR belong to *A. yucumense*. This finding constitutes the first record of *A. yucumense* in Argentina.

As stated by Krawczak *et al.* (2015), *A. yucumense* is phylogenetically related to *A. coelebs* and *A. dubitatum*, two tick species that were previously found in Misiones province (Lamattina *et al.* 2014). The principal morphological differences between *A. yucumense* and *A. dubitatum* males are the absence of a pseudoscutum and a larger distance separating coxa I spurs in the latter (space wider than external spur width) (Krawczak *et al.* 2015). An additional difference between these two species that was noted after examining the male collected in MPP is the shape of the spiracular plate, which is comma-shaped in *A. yucumense* and oval in *A. dubitatum*. Regarding nymphs, the cervical groove length/scutal length ratio is higher in *A. dubitatum* (Krawczak *et al.* 2015).

Main differences between *A. yucumense* and *A. coelebs* males are the absence, in the latter, of long light brown or orange stripes extending from the pseudoscutum to the marginal groove in *A. yucumense*. In this species there are dark brown elevated spots without deep punctations in the scutum, which are absent in *A. coelebs*. A larger distance also separates coxa I spurs in *A. coelebs* males. Regarding nymphs, cervical groove is short in *A. coelebs*, and punctations in the scutum are deep and evenly distributed in both central and lateral fields, unlike *A. yucumense* nymphs, which scutum presents deeper punctations in the lateral fields.

Even though *A. yucumense*, *A. dubitatum* and *A. coelebs* phylogenetically group together and are morphologically similar (Krawczak *et al.* 2015), they seem to have distinct ecological preferences. *Amblyomma coelebs* has only been found in northern Misiones province, particularly in the Iguazú National Park, where it is the most abundant and most aggressive species (Lamattina *et*

al. 2018), while the most numerous records of *A. dubitatum* come from the central area of the province, where *Hydrochoerus hydrochaeris* populations are more abundant. Until now, the distribution of *A. yucumense* in Argentina is restricted to MPP and YBR.



**FIGURE 1.** A. Male of *Amblyomma yucumense*, dorsal view. B. Male of *Amblyomma yucumense*, ventral view. C. Nymph of *Amblyomma yucumense*, dorsal view. D. Nymph of *Amblyomma yucumense*, ventral view.

*Amblyomma brasiliense*, *A. incisum*, *A. ovale* and *H. juxtakochi* had been previously reported in northern Misiones province (Lamattina *et al.* 2014, 2018), thus the findings in this study expand their distribution ranges to the south. Future studies will focus on determining niche characteristics and niche preferences of each ixodid species found in Misiones province.

From the human health standpoint, further studies are necessary to detect tick-borne pathogens in *A. yucumense* in Argentina, and to gather information on host usage and population dynamics.

We acknowledge Santiago Bellitti, Leonardo Rangel, Nilso Molina and Mara Urdapilleta for their assistance in the field, and the Ministerio de Ecología y Recursos Naturales Renovables of Misiones province for providing sampling permission. Financial support was given by INMeT to DL and by INTA, Asociación Cooperadora INTA Rafaela, CONICET and Agencia Nacional de Promoción Científica y Tecnológica (PICT 526) to ELT, AJM and SN.

## References

- Hall, T.A. (1999) BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Krawczak, F.S., Martins, T.F., Oliveira, C.S., Binder, L.C., Costa, F.S., Nunes, P.H., Gregori, F. & Labruna, M.B. (2015) *Amblyomma yucumense* n. sp. (Acari: Ixodidae), a Parasite of Wild Mammals in Southern Brazil. *Journal of Medical Entomology*, 52, 28–37.  
<https://doi.org/10.1093/jme/tju007>
- Lamattina, D., Tarragona, E.L., Costa, S.A., Guglielmono, A.A. & Nava, S. (2014) Ticks (Acari: Ixodidae) of northern Misiones Province, Argentina. *Systematic and Applied Acarology*, 19, 393–398.  
<https://doi.org/10.11158/saa.19.4.2>
- Lamattina, D., Venzal, J.M., Costa, S.A., Arrabal, J.P., Flores, S., Berrozpe, P.E., González-Acuña, D., Guglielmono, A.A. & Nava, S. (2018) Ecological characterization of a tick community across a landscape gradient exhibiting differential anthropogenic disturbance in the Atlantic Forest ecoregion in Argentina. *Medical and Veterinary Entomology*, doi:10.1111/mve.12295.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.  
<https://doi.org/10.1093/bioinformatics/btm404>
- Mangold, A.J., Bargues, M.D. & Mas-Coma, S. (1998) Mitochondrial 16S rRNA sequences and phylogenetic relationships of Rhipicephalus and other tick genera among Metastriata (Acari: Ixodidae). *Parasitology Research*, 84, 478–484.  
<https://doi.org/10.1007/s004360050433>
- Martins, T.F., Labruna, M.B., Mangold, A.J., Cafrune, M.M., Guglielmono, A.A. & Nava, S. (2014) Taxonomic key to nymphs of the genus *Amblyomma* (Acari: Ixodidae) in Argentina, with description and redescription of the nymphal stage of four *Amblyomma* species. *Ticks and Tick-borne Diseases*, 5, 753–770.  
<https://doi.org/10.1016/j.ttbdis.2014.05.007>
- Nava, S., Venzal, J.M., González-Acuña, D., Martins, T.F. & Guglielmono, A.A. (2017) *Ticks of the Southern Cone of America: Diagnosis, Distribution and Hosts with Taxonomy, Ecology and Sanitary Importance*. Elsevier, Academic Press, London, 348 pp.
- Plací, G. & Di Bitetti, M. (2005) Situación ambiental en la Ecorregión del Bosque Atlántico del Alto Paraná (Selva Paranaense) *Una visión de biodiversidad para la Ecorregión del Bosque Atlántico del Alto Paraná: Diseño de un Paisaje para la Conservación de la Biodiversidad y Prioridades para las Acciones de Conservación*, World Wildlife Fund, Washington, DC, pp. 197–225.
- Terassini, F.A., Barbieri, F.S., Albuquerque, S., Szabó, M.P., Camargo, L.M.A. & Labruna, M.B. (2010) Comparison of two methods for collecting free-living ticks in the Amazonian forest. *Ticks and Tick-borne Diseases*, 1, 194–196.  
<https://doi.org/10.1016/j.ttbdis.2010.08.002>

*Submitted: 13 Apr. 2018; accepted by Lidia Chitimia-Dobler: 17 Apr. 2018; published: 11 May 2018*