

MORPHOLOGICAL STUDY OF *ORNITHODOROS VIGUERASI* COOLEY AND KOHLS, 1941 (ACARI: IXODIDA: ARGASIDAE), WITH SEQUENCE INFORMATION FROM THE MITOCHONDRIAL 16S rDNA GENE

Santiago NAVA¹, José M. VENZAL², Enrique A. REYES NOVELO³, Atilio J. MANGOLD¹ and Marcelo B. LABRUNA⁴

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¹ Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela and Consejo Nacional de Investigaciones Científicas y Técnicas, CC 22, CP 2300 Rafaela, Santa Fe, Argentina. snava@rafaela.inta.gov.ar. amangold@rafaela.inta.gov.ar

² Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Regional Norte – Sede Salto, Rivera 1350, CP 50000 Salto, Uruguay. dpvuru@hotmail.com

³ Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, CP 97000, Mérida, Yucatán, México. enrique.reyes@uady.mx

⁴ Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Av. Prof. Orlando M. de Paiva 87, 05508-900, São Paulo, Brazil. labruna@usp.br

ABSTRACT — A morphological and molecular study of *Ornithodoros (Subparmatus) viguerasi* (Acari: Argasidae) was carried out. Free-living males and females of this tick species were collected in Calcehtok cavern, Yucatán, Mexico. The morphology of the females of *O. viguerasi* was identical to the holotype female from Cuba. The only difference was related to size; females from Mexico were bigger than the holotype female. The male of *O. viguerasi* was described for the first time from the specimens collected in Mexico. The diagnostic characters are a combination of a genital aperture covered by a semicircular flap, a central sclerotized plate posterior to genital aperture, a transverse and thin plate anterior to the genital aperture located at the level of the anterior margin of coxae I, a pair of sclerotized plates bordering coxae II, III and IV, basis capituli rectangular in shape and protrusible, a hypostome roughly blunt at the apex with small denticles (it appears to be functionless), and article I of the palpi with a medial integumental extension which has a long setae on the medial margin. A comparative morphological analysis of the male described in this work with the paratype specimens from Cuba was also conducted; we conclude that the nymphs described in the original description of *O. viguerasi* correspond to males. Sequences of the mitochondrial 16S rDNA gene of both male and female ticks from Mexico were identical. In a phylogenetic analysis, the monophyly of the subgenus *Subparmatus* could not be resolved.

KEYWORDS — *Ornithodoros viguerasi*; Argasidae; morphology; 16S rDNA; Mexico

INTRODUCTION

The argasid tick *Ornithodoros viguerasi* Cooley and Kohls, 1941 was described from specimens collected from a bat cave in Cuba. This species belongs to the subgenus *Subparmatus* with *Ornithodoros marinkellei*

Kohls, Clifford and Jones, 1969 and *Ornithodoros mormoops* Kohls, Clifford and Jones, 1969 (Kohls *et al.* 1969). The geographical distribution of *O. viguerasi* includes Cuba, Costa Rica, Dominican Republic, Haiti, Jamaica, Mexico, Puerto Rico,

Trinidad & Tobago and Venezuela (Tamsitt and Fox 1970; Jones *et al.* 1972; Hoogstraal 1985; Guglielmo *et al.* 2003). All previous records of host-tick associations for *O. viguerasi* correspond to larvae parasitizing bats: larvae have been collected on *Phyllonycteris poeyi* Gundlach, 1861; *Pteronotus macleayii* Gray, 1839; *Pteronotus quadridens* Gundlach, 1840; *Pteronotus parnellii* Gray, 1843; *Pteronotus gymnonotus* Natterer, 1843; *Pteronotus davyi* Gray, 1838; *Brachyphylla nana* Miller, 1902; *Mormoops megalophylla* Peters, 1864; *Mormoops blainvillei* Leach, 1821; *Erophylla sezekorni* Gundlach, 1861; and *Erophylla bombifrons* Miller, 1899 (Kohls *et al.* 1965; Cerný and Dusbábek 1967; Cerný 1969; Tamsitt and Fox 1970; Jones *et al.* 1972; De la Cruz and Abreu 1984; Kurta *et al.* 2007).

The description of Cooley and Kohls (1941) was based on a female, nymphs and larvae collected in "Cueva Somorrostro", Cuba. Larvae were found attached to bats, probably *P. poeyi*. Although Guglielmo *et al.* (2003) stated that all stages of *O. viguerasi* were described, the description of adults by Cooley and Kohls (1941) was based on only the female, without inclusion of male specimens. Danielová *et al.* (1982) reported the finding of males and females of *O. viguerasi* on the walls of bat caves in Cuba, but the methodology for tick determination was not explained by these authors and the collection where the ticks were deposited was not indicated. Therefore, it can be affirmed that the male of this tick species is not formally described.

The aim of this work was to carry out the description of males of *O. viguerasi* from specimens recently collected from a bat cave in Mexico, and to compare the morphology of both males and females from Mexico with the morphology of the specimens of *O. viguerasi* described by Cooley and Kohls (1941) and also with those deposited in the United States National Tick Collection (USNTC, Georgia Southern University, Statesboro, Georgia, USA). In addition, information obtained from sequences of the mitochondrial 16S rDNA gene was used to infer the phylogenetic position of this tick in relation to other Neotropical tick species of the family Argasidae, and to confirm that the different stages analyzed in this work belong to the same taxon.

MATERIALS AND METHODS

Free-living argasid ticks (males and females) were collected by the authors during June 2010 in Calcehtok cavern (20°33'02"N, 89°54'43"W), Yucatán, Mexico. After morphological analysis, 16 females were identified as *O. viguerasi* following Cooley and Kohls (1941). Seventeen males were tentatively assigned to *O. viguerasi* due to the presence of sclerotized ventral plates, which is a diagnostic character of adults of the subgenus *Subparmatus*. Additionally, a fragment of circa 420-bp of the mitochondrial 16S rDNA gene was obtained from two males and one female in order to confirm the taxonomic determination by morphological criteria. Both males and females were deposited at the Tick Collection of the Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Rafaela, Santa Fe, Argentina (INTA 2187), at the Coleção Nacional de Carrapatos (CNC) of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil (CNC 1717) and in the Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay (DPVURU 768).

Six males and six females were measured using a stereoscope Nikon® C-PS (all measurements are given in mm, the mean followed by the range in parentheses). Scanning electron photomicrographs of males and females were taken at the Servicio de Microscopía Electrónica, Museo de La Plata, Universidad Nacional de La Plata, Argentina, using a JEOL/JSM 6360 LV® Digital Scanning Microscope, and Sub-Unidad de Microscopía Electrónica de Barrido, Facultad de Ciencias, Montevideo, Uruguay, using JEOL JMS-5900 scanning electron microscope. The holotype female (RML17169), the paratype nymph (RML17164) and other material (RML50482, RML64683, RML17495, RML64680, RML64679, RML64682, RML52459, RML17162, RML64681, RML19351, RML64688, RML51218, RML 45488, RML64684, RML64678, HH16772) of *O. viguerasi* deposited in USNTC were also examined and included in the morphological analysis.

Sequences of the mitochondrial 16S rDNA gene were obtained following the methodology described by Mangold *et al.* (1998). Each of the

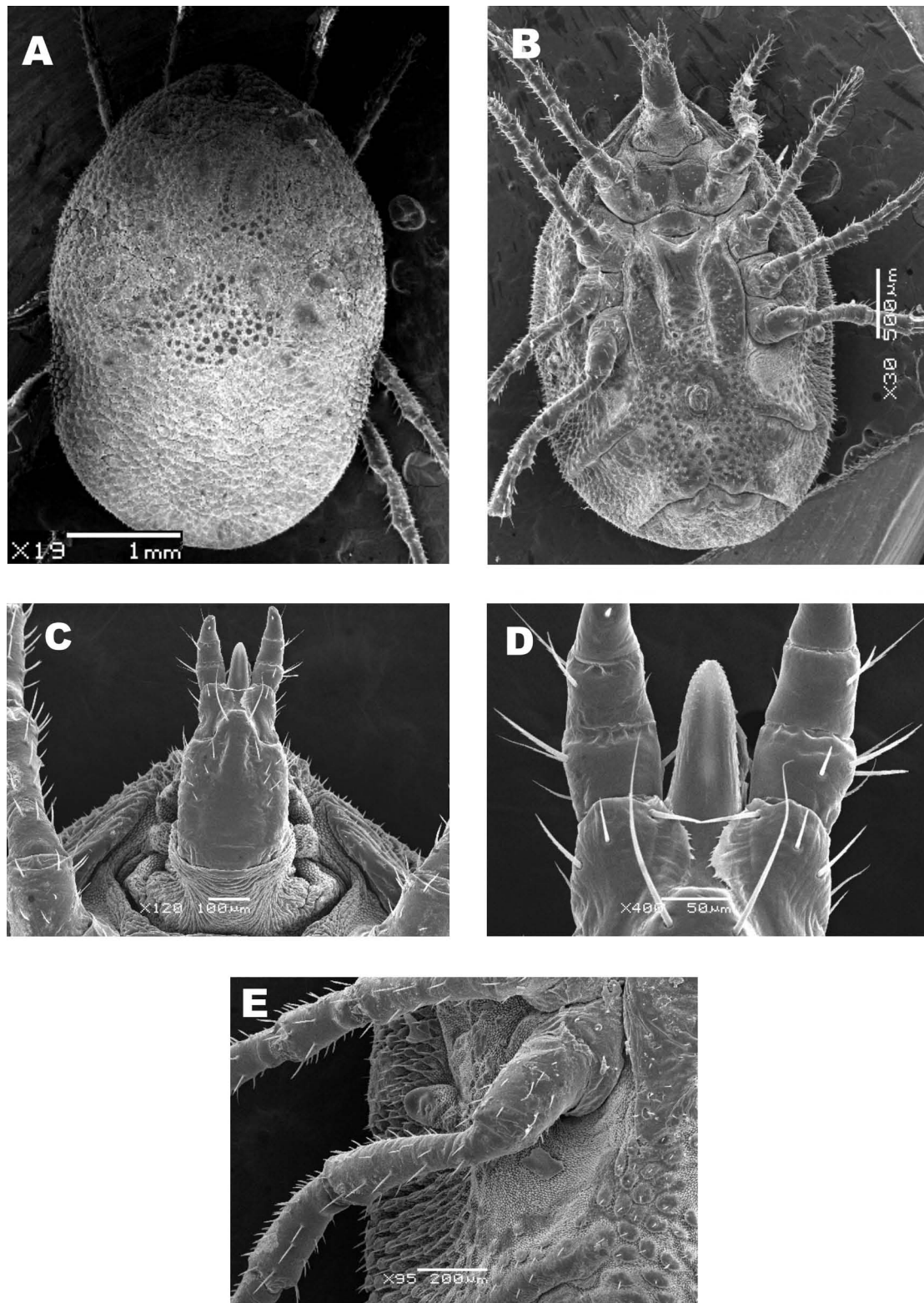


FIGURE 1: Female of *Ornithodoros viguerasi*: A – dorsum. B – venter. C – capitulum. D – hypostome. E – Integument of the body surface (lateral and ventral view).

sequences was aligned with each other and with the corresponding sequences of the *Ornithodoros* species available in GenBank, using the BioEdit Sequence Alignment Editor (Hall 1999) with the CLUSTAL W program (Thompson *et al.* 1994). The phylogenetic analysis was made using neighbor-joining distances (NJ) and maximum parsimony (MP) methods. The NJ tree was generated from the

Tamura-Nei model and gaps were excluded in the pairwise comparison. MP analysis was performed using the close neighbor-interchange (CNI) method with search level 3 and a random addition of trees with 10 replications. Gaps were excluded from the analysis. Support for the NJ and MP topologies was tested by bootstrapping over 1,000 replications. The sequences of *Argas neghmei* Kohls and

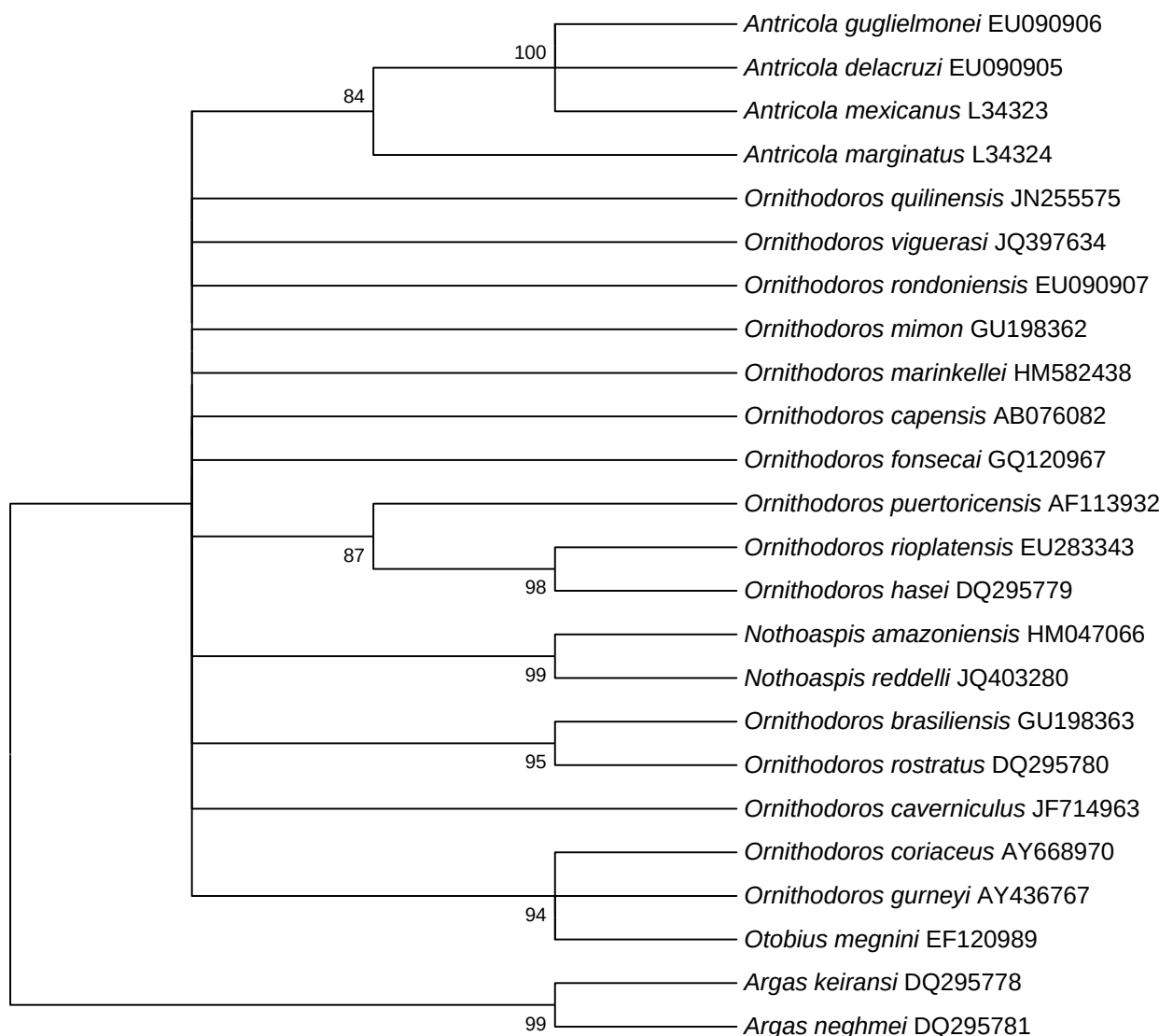


FIGURE 2: Neighbor-joining condensed tree based on 16S rDNA partial sequences. GenBank accession numbers are indicated. Only bootstraps > 70% are presented.

Hoogstraal, 1961 and *Argas keiransi* Estrada-Peña, Venzal and González-Acuña, 2003 were employed as outgroups. All analyses were performed using Mega 4.0 (Tamura *et al.* 2007). The classification scheme of Argasidae followed in this work was that presented by Guglielmone *et al.* (2010).

RESULTS

Sixteen females collected in Calcehtok cavern were identified as *O. viguerasi* according to the description of Cooley and Kohls (1941) and after the comparison with the holotype deposited in USNTC. The specimens were determined by the following unique combination of morphological characters (Figures 1a, 1b, 1c, 1d, 1e): presence on the venter of a transverse band posteriorly to the level of coxa IV; dorsum covered by numerous mammillae irregular in size and shape; postero-lateral margins with mammillae elevated, columnar in shape, about twice as high as their diameter, with a single hair; hood present but not well-developed; basis capituli rectangular in shape; hypostome roughly blunt with small denticles present only at the apex; article I of the palpi longer than article II, with a medial integumental extension covering the basal portion of the hypostome and the presence of a long setae on the medial margin; sclerotized plates bordering coxae II, III and IV; a pair of sclerotized plates well developed, positioned anteriorly to the genital aperture, between coxae I. The measurements of the females collected in Calcehtok cavern were: total body length 4.10 (3.70 – 4.53), maximum body width 2.69 (2.26 – 2.86), genital aperture length 0.25 (0.19 – 0.30), genital aperture width 0.52 (0.36 – 0.61), palpi length 0.41 (0.36 – 0.47), tarsus I length 0.63 (0.55 – 0.68), tarsus IV length 0.79 (0.68 – 0.89).

DNA sequences of the 16S rDNA gene of two males (the morphological description is detailed below) and one female of *O. viguerasi* were obtained (GenBank accession numbers JQ397632, JQ397633 and JQ397634). These three sequences were identical. The rooted NJ tree derived from 16S sequences is presented in figure 2. MP reconstructions showed similar topologies and are not shown. A close association between *O. viguerasi* and the other Neotropi-

cal species of the genus *Ornithodoros* included in the analysis was not found.

Description of the male (Figures 3A, 3B, 3C, 3D, 4A, 4B)

Body — Outline oval, pointed anteriorly, broadly rounded posteriorly, total length 3.3 (2.9 – 4.0), greatest width 2.1 (1.8 – 2.4).

Dorsum (Figure 3A) — Surface covered by numerous mammillae, irregular in shape, larger on posterior and marginal fields; most mammillae with a single short seta (Figure 4b); small disks present on the lateral margins and more numerous on the anterior half of the body.

Venter (Figures 3B, 4A) — Mammillae much less numerous than in dorsum, absent in areas surrounding genital aperture and coxae; genital aperture between coxae I and covered by a semicircular flap; a central sclerotized plate posterior to genital aperture, extending from the level of coxa I to the level of the posterior margin of coxa II; a transverse and thin plate anterior to the genital aperture, located at the level of the anterior margin of coxae I and surrounded by a few mammillae; a pair of sclerotized plates bordering coxae II, III and IV, with few short setae; preanal groove distinct on the sides but interrupted in the middle, transverse postanal groove continuous, not interrupted in the middle, and median postanal groove short and reaching the transverse postanal groove.

Capitulum (Figures 3A, 3B) — Basis capituli rectangular in outline, with 1 pair of posthypostomal setae, sometimes visible dorsally because it appears to be protrusible; palpi length 0.30 (0.26 – 0.33), article I of the palpi longer than article II, with a medial integumental extension covering the basal portion of the hypostome; integumental extensions with a long setae on the medial margin. Hypostome roughly blunt at the apex and broader at the base, with small denticles, minute at apex, gradually enlarging in size posteriorly.

Legs — All coxae inserted in the anterior half of body, covered by few and short setae; coxae II–IV contiguous, I and II separated; tarsi long, narrow apically, but with numerous setae; tarsus I length 0.41 (0.36 – 0.45), tarsus IV length 0.36 (0.33 – 0.41).

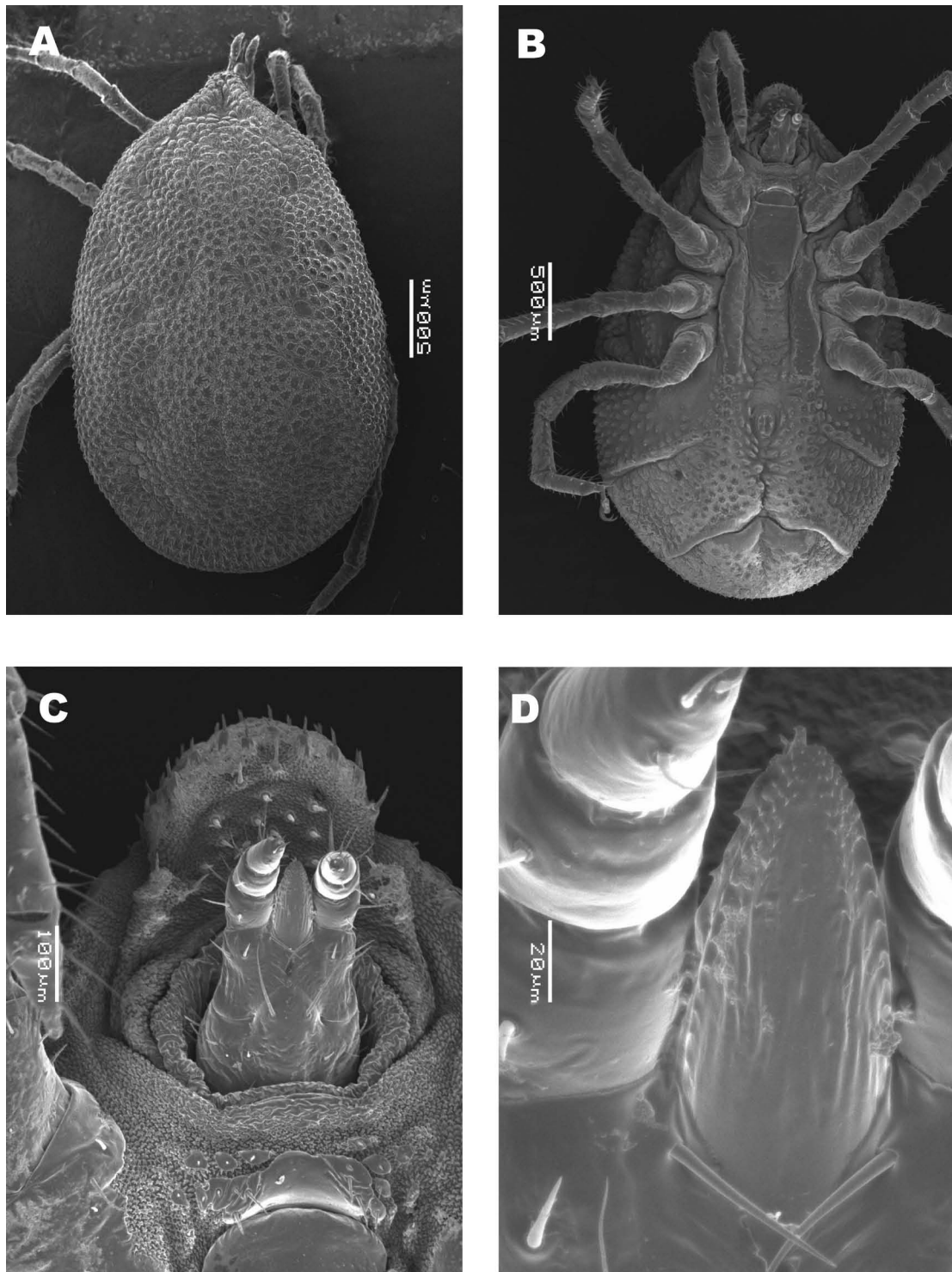


FIGURE 3: Male of *Ornithodoros viguerasi*: A – dorsum. B – venter. C – capitulum. D – hypostome.

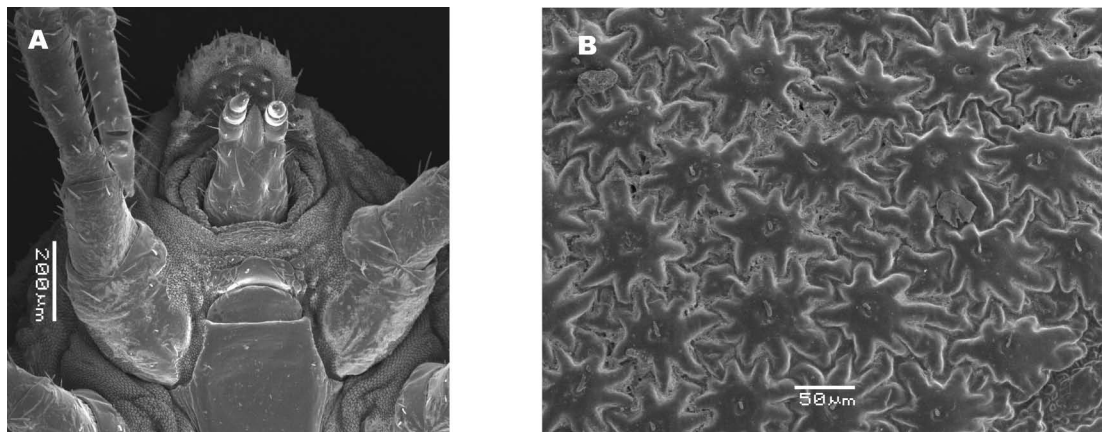


FIGURE 4: Male of *Ornithodoros viguerasi*: A – venter (view of genital aperture). B – integument of the of body surface (posterodorsal).

DISCUSSION

The previous record of *O. viguerasi* in Mexico corresponds to larvae collected on *M. megalophylla* in Juxtlahuaca cave, Colotlipa, Guerrero (USNTC, RML 45488). Therefore, the finding of *O. viguerasi* from Calcehtok cavern reported in this study constitutes the first record of adults in Mexico and expands its geographical distribution. Morphologically, the females of *O. viguerasi* were identical to the female described by Cooley and Kohls (1941). The only difference was the size, because females from Mexico are bigger than the holotype female.

The diagnostic characters for the male of *O. viguerasi* are a combination of a genital aperture covered by a semicircular flap, a central sclerotized plate posterior to genital aperture, a transverse and thin plate anterior to the genital aperture located at the level of the anterior margin of coxae I, a pair of sclerotized plates bordering coxae II, III and IV, basis capituli rectangular in shape and protrusible, hypostome roughly blunt at the apex with small denticles (it appear to be functionless), and article I of the palpi with a medial integumental extension which has a long setae on the medial margin. The presence of ventral plates, a genital aperture covered by a flap, and the medial integumental extension of the article I of the palpi, are a combination of characters that also are observed in another species of the subgenus *Subparmatus*, *O. marinkellei* (Labruna *et al.* 2011). However, *O. viguerasi* is easily differentiable from *O. marinkellei* due to the shape and disposi-

tion of the ventral plates, the difference in the size of the denticles in the hypostome and the idiosomal surface, which is entirely covered by smooth sclerotized plaques in *O. marinkellei*.

In the description of the nymph of *O. viguerasi*, Cooley and Kohls (1941) included discrete characters and morphometric data. These authors stated that the nymphs are misleading in appearing to have a genital aperture because of the presence of a sclerotized semicircular flap between coxae I. However, after the comparative analysis of the male described in this work with the nymphal specimen deposited as paratype in USNTC (RML17164) (Figure 5), we conclude that the nymph described by Cooley and Kohls (1941) corresponds to a male specimen. In the same way as in *O. marinkellei*, *O. viguerasi* has a semicircular plate that covers the genital aperture (Figure 4a), and probably Cooley and Kohls (1941) mistakenly interpreted this character as the absence of a genital aperture. In the phylogenetic analysis of the available 16S rDNA sequences, the evolutionary relationships between *O. viguerasi* and the other Neotropical species of argasid ticks remain unresolved. In Calcehtok cavern, *O. viguerasi* ticks were found sharing similar environmental conditions with other argasid ticks parasitizing bats such as *Nothoaspis reddelli* Keirans and Clifford, 1975, *Antricola marginatus* (Banks, 1910) and *Antricola mexicanus* Hoffmann, 1958 (S. Nava, J.M. Venzal and M.B. Labruna, unpublished data). However, in spite of this ecological similarity, these



FIGURE 5: Ventral view of the specimen described as the nymph of *O. viguerasi* by Cooley and Kohls (1941) (deposited as paratype in USNTC, RML17164).

taxa did not group together in the phylogenetic trees. *Ornithodoros viguerasi* has a wide distribution, from southern Mexico to Venezuela. Considering this fact, it would be appropriate to carry out an analysis on the intraspecific genetic variation among ticks belonging to different populations of *O. viguerasi* to verify that this taxon represents a single specific entity along its entire distribution. Finally, the monophyly of the subgenus *Subparmatatus* was not resolved with the phylogenetic analyses carried out with 16S rDNA sequences, as *O. viguerasi* and *O. marinkellei* did not group together. These results suggest that the morphological character which defines the subgenus *Subparmatatus* (basis capituli ventrally with a pair of cornua-like extensions posteriorly in larvae) may not be a synapomorphy and calls for a careful analysis of the group. Unfortunately, for now, no public sequence data is available for additional genes that could be used to perform more in-depth analyses and to increase the power of phylogenetic inferences on the evolution of Neotropical ticks.

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