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Redescription of *Ornithodoros dyeri* (Ixodida: Argasidae) based on morphologic and molecular data

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ABSTRACT

Larvae, nymphs and adults of the cave dwelling tick *Ornithodoros dyeri*, collected in 3 Mexican states, were studied using morphological and molecular methods. The adults and nymphs were characterized by an elongated body in proportion to the width and a dorsum bounded by two contiguous ridges and one third ridge (inner) that was incomplete on each side. The larvae of this species have 14 pairs of dorsal setae, a venter body with nine pairs of setae plus a posteromedian; a moderately large, dorsal plate and piriform, a hypostome arising from a relatively short, subtriangular median extension of the basis capituli, and a capsule of Hallerís organ with reticulations. Based on a maximum likelihood analysis of the sequences of a fragment of approximately 414 bp of the mitochondrial 16S rRNA gene, we showed that *O. dyeri* represents an independent lineage within neotropical species of the Argasidae. The bat species *Mimon cozumelae* and *Peropteryx macrotis* represents a new host record for this argasid.

1. Introduction

Argasidae includes a diverse group of ticks consisting of approximately 200 species (Guglielmone et al., 2010; Nava et al., 2013; Venzal et al., 2012, 2013a, 2015). According to Guglielmone et al. (2010), and following the classification by Hoogstraal (1985), five genera are recognized, i.e., *Antricola* Cooley and Kohls (1941); *Argas* Latreille (1796); *Nothoaspis* Keirans and Clifford (1975); *Ornithodoros* Koch (1844) and *Otobius* Banks (1912). In particular, the genus *Ornithodoros* is represented by 121 species worldwide (Barros-Battesti et al., 2015; Venzal et al., 2015). In Mexico, 32 species of argasids are known, and 20 belong to *Ornithodoros* (Pérez-Ortiz et al., 2014). To increase the knowledge of argasids, the objectives of this work were: to morphologically and molecularly determine the cave dwelling species, *Ornithodoros dyeri* Cooley and Kohls (1940); and to locate this species in a phylogenetic context, based on the mitochondrial 16S gene.

2. Materials and methods

The material used in this study includes newly collected specimens in three localities from Yucatán, Mexico during October 2014: Oquedad 1, carretera Santa Elena-Loltún, Km 56 (20°17′34″N, 89°38′42″W), 98 m.a.s.l., Relative Humidity (RH): 79%, Temperature (T): 30 °C; Oquedad 2, carretera Santa Elena-Loltún, Km 56 (20°17′34″N, 89°38′42″W), 98 m.a.s.l., RH: 79%, T: 30 °C; Cueva El Naranjal, Tekax, 2-X-2014 (20°11′56″N, 89°18′36″W), 61 m.a.s.l., RH: 89%, T: 26 °C. In each locality, bats, bat guano and crevices from the walls were sampled. For the collection of the bats, mist nets were used inside the caves; approximately 300 g of bat guano was collected in plastic boxes, and each one was posteriorly processed in portable Berlese-Tullgren funnels; crevices on the walls were inspected, especially near bat colonies where most of the ticks (nymphs and adults) were found. In addition, specimens deposited in the Colección del Laboratorio de Acarología (LAFC), Facultad de Ciencias, and Colección Nacional de Ácaros (CNAC), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City, were also studied: Coahuila: Cueva del

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Detector Perdido, Monclova, (26°58′27.07320″N, 101°03′29.84040″W); Veracruz: Cueva Rey del Oro, Emiliano Zapata, 215 m, and Yucatán: Cueva (Actún) Chocantes, Tekax (20°12′10″N, 89°17′58″W).

All of the specimens were preserved in vials with 96% ethanol, identified using morphological keys, and compared with the original species description (Cooley and Kohls, 1940; Kohls et al., 1965). Measurements of the nymphs and adults were made with a Zeiss stereomicroscope (475200 9901) and are presented in millimeters. The measurements of the larvae are in micrometers and were made on a microscope Zeiss Axioscope 2 plus microscope, using the AxioVision 4 software. The body wall of the larvae was punctured with an entomological needle, and the specimens were placed in 10% KOH where they were slightly compressed to remove the host blood. Next, the ticks were washed 2 times in distilled water for 20 min and transferred to 10% glacial acetic acid for 10 min and then to isopropyl alcohol for 20 min. Finally, the ticks were cleared in lactophenol and mounted in Hoyerís medium. The specimens for the molecular analyses (larva, female, and male) were also preserved in 96% ethanol.

For the scanning electron microscopy (SEM), the specimens were dehydrated in 100% ethanol and dried to a critical point with liquid carbon dioxide. The dried specimens were mounted on aluminum specimen stubs, coated with a gold palladium alloy, and examined using a scanning electronic microscope Hitachi Stereoscan Model S–2469 N SEM (Hitachi Ltd., Tokyo, Japan).

To confirm the conspecific identity of the different stages collected, we performed a molecular analysis using a larva and two adults (female and male). The extraction was made using a DNAeasy blood and tissue kit (Qiagen, Valencia, California). We amplified the 16S rDNA sequence using primers 16S + 1 59-CCGGTCTGAACTCAGATCAAGT-39 and 16s-1 59CTGCTCAATGATTTTTTAAATTGCTGTGG-39 (Norris et al., 1996). The PCR was performed in a final volume of 25 μL with the following reaction mixture: 12.5 μL of PCR Master Mix solution (Qiagen), 100 ng of the corresponding oligonucleotides and 30 ng of genomic DNA. The PCR conditions were those of Trout et al. (2009) and amplified the 414 bp. PCR positive products were purified and sequenced in an automatic sequencer (ABI PRISM 310). The sequences obtained in this work and sequences from other *Ornithodoros* species available in Genbank were edited using BioEdit Sequence Alignment Editor (Hall, 1999) and aligned using the CLUSTAL W program (Thompson et al., 1994). The phylogenetic analysis was performed with the maximum-likelihood (ML) method. The tree was generated with the GTR model by using a discrete gamma- distribution (+G). The best-fitting substitution models were determined with the Bayesian Information Criterion using the ML model test implemented in Mega 5 (Tamura et al., 2011). Support for topologies was tested by bootstrapping over 1000 replications. Sequences of *Argas neghmei* Kohls and Hoogstraal (1961); *Argas monachus* Keirans et al. (1973) and *Argas keiransi* Estrada-Peña et al. (2003) were used as out groups.

Ticks were deposited at LAFC and CNAC; accession numbers are indicated below. DNA sequences obtained were submitted to GenBank; accession numbers are indicated in Fig. 4.

3. Results

A total of 25 specimens were collected in the three sites sampled in the present study during 2014. The adults were present only in Km 56, Santa Elena-Loltún road (Cavity II) and inhabited crevices on the caves walls; larvae were found in the three localities and included, two parasitizing bat species: *Mimon cozumelae* Goldman (1914) and *Peropteryx macrotis* Wagner, 1943 (Table 1).

All of the specimens, from the field collections and those deposited at the LAFC and CNAC were identified as *O. dyeri* following Cooley and Kohls (1944) and Kohls et al. (1965). Next, we present a brief characterization of the four developmental stages that were studied based on newly collected material and specimens deposited at LAFC and CNAC.

3.1. *Ornithodoros dyeri*

Female (based on 10 specimens). Idiosome: Body elongated, anterior end pointed, posterior rounded. Integument of margin differing from that of the dorsal and ventral surfaces (Fig. 1A). Length from anterior to posterior margin: 3.83 ± 0.47 (3.32–5.12); width: 1.53 ± 0.19 (1.34–1.92). Dorsal surface bounded by two contiguous ridges, with a third ridge (inner) incomplete on each side; with irregular small ridges and subcircular elevations of different sizes (discs *sensu* Cooley and Kohls, 1940); mammillae absent. Anterior dorsal area with a distinct median hump (Fig. 1A). Eyes absent. Ventral surface with folds with an intricate pattern, but only a definite groove (postanal) is present (Figs. 1B, 2A); genital opening at level of coxae I–II, having a "V" shape depression (Figs. 1B, 2C). Spiracle plate semicircular behind coxae IV; anus elliptical with valves provided with short setae. Setae few and very fine scattered on dorsal and ventral surfaces. Hood absent but with a median ridge extension from the mouth parts to anterior margin (Figs. 1B, 2B).

Capitulum: Basis capituli large with irregular transverse ridges and micromammillated at the base (Figs. 1B, 2B). Cheeks present and with the tip of the median ridge extension give protection to mouthparts (Fig. 2B). Hypostome short, broad and truncated, with sides nearly parallel. One central pore at base of hypostome. Dentition 5/5 covering two-thirds of the hypostome (Fig. 2B). Denticles short with "u" shape. Palps setae and posthypostomal setae barbed.

Legs: Micromammillated (Fig. 2B) and moderate in length. Tarsus I 0.39 ± 0.035 (0.36–0.44) long; tarsus IV 0.49 ± 0.04 (0.44–0.6) long. Coxae I separated, the rest contiguous (Figs. 1B, 2A) . Surface of coxae micromammillated. Tarsi I–IV without protuberances or dorsal humps. Claws stout.

Male (based on 10 specimens). Body as in female, but smaller (Figs. 1C,D and 2D,E); length from anterior to posterior margin: 3.29 ± 0.53 (2.64–4.25); width: 1.33 ± 0.26 (1.11–1.84). Legs as in female; tarsus I 0.36 ± 0.056 (0.27–0.45) long; tarsus IV 0.44 ± 0.06(0.37–0.56) long. Genital opening between coxae I, as a semicircular flap (Fig. 2F).

Nymph (measurements n = 10, except for tarsus IV n = 9). Body as in female, but smaller (Fig. 2G,H); length from anterior to posterior margin: 3.19 ± 0.46 (2.15–3.6); width: 1.31 ± 0.11 (1.1–1.48). Dorsal surface with discs conforming 3 groups as follows: anterior group with 3 lateral pairs, 2 central pairs, and one impair disc anterior to the central pairs; middle group conformed by 3 subgroups, the first with 1 central pair and 2 laterals which are small and contiguous; the second group with one central and 1 lateral pair, and the third subgroup with 2 lateral pairs. The posterior group is arranged in 3 parallel lines of discs; the lateral lines have approximately 7 discs each, and the central line near of 10 small discs (Fig. 2G). Legs as in female; tarsus I 0.36 ± 0.03 (0.32–0.4) long; tarsus IV 0.42 ± 0.06 (0.3–0.48) long. Genital primordium is a depression at level of opening sexual that resembles the V-shaped depression as in females (Fig. 2I).

Larva (based on 12 of 23 collected specimens; ticks near or fully engorged were not measured due to they were damaged during the mounting process). Idiosome: Length including capitulum 1119.83 ± 209 (901–1218), length without capitulum 762 ± 160.5 (623–919); width 613.5 ± 139.3 (425–948). Dorsum of body with 14 pairs of setae (Fig. 3A), seven antero-lateral (Al): Al_1 64.27 ± 6.62 (51.12-71.38); Al_2 63.13 ± 5.72 (55.37–73.98); Al_3 61.56 ± 5.43(49.51–68.94); Al_4 54.21 ± 5.52

Table 1
New host and locality records for *Ornithodoros dyeri* in Mexico obtained through new field collections and review of specimens deposited in scientific collections.

Mexican state	Locality	Date	Accession numbers	Stage	Site of collection
Coahuila	Cueva del Detector Perdido	02/08/2008	CNAC 009228	1♀, 3♂, 2N	Cave walls
Veracruz	Cueva Rey del Oro	05/06/1992	LAF000129	1N	Cave walls
	Cueva Rey del Oro	11/06/1992	LAF000127 LAF000130 LAF000131 LAF000133 LAF000134	16♀, 9♂, 10N	Cave walls
	Cueva Rey del Oro	20/01/1993	LAF000128	1♀	Cave walls
Yucatán	Cueva Chocantes	10/04/1995	LAF000126	1N	Cave walls
	Km 56, Santa Elena-Loltún road (Cavity I)	01/10/2014	LAF000205 LAF000206	4L	*ex Mimon cozumelae*
	Km 56, Santa Elena-Loltún road (Cavity II)	01/10/2014	LAF000207 LAF000208	16L	*ex Mimon cozumelae*
	Km 56, Santa Elena-Loltún road (Cavity II)	01/10/2014	LAF000211	1♀, 1♂	*ex Peropteryx macrotis*
	Cueva El Naranjal	02/10/2015	LAF000209 LAF0002010	3L	*ex Peropteryx macrotis*

N = Nymphal stage; L = Larval stage.

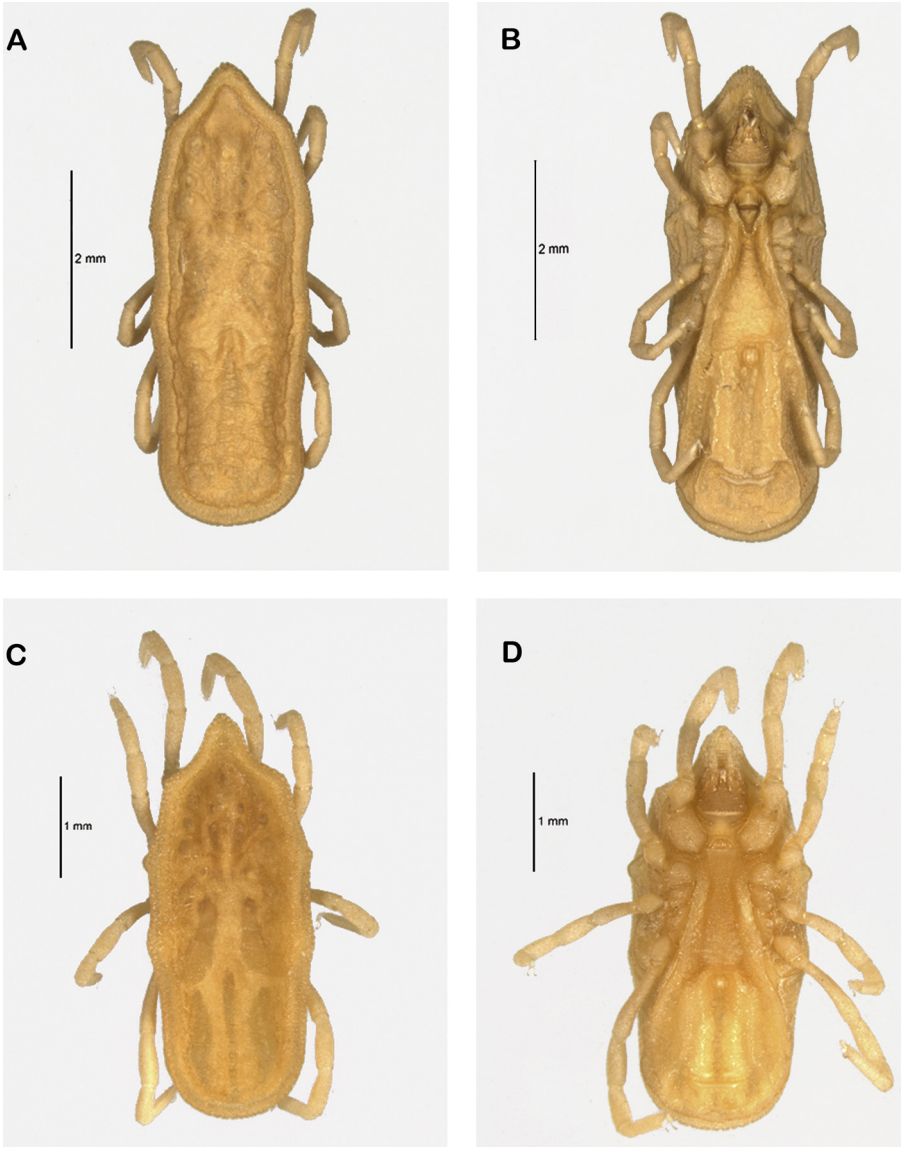

Fig. 1. Optical micrographs of *Ornithodoros dyeri*. Female: (A) dorsal view, (B) ventral view. Male: (C) dorsal view, (D) ventral view. Scale in millimeters.

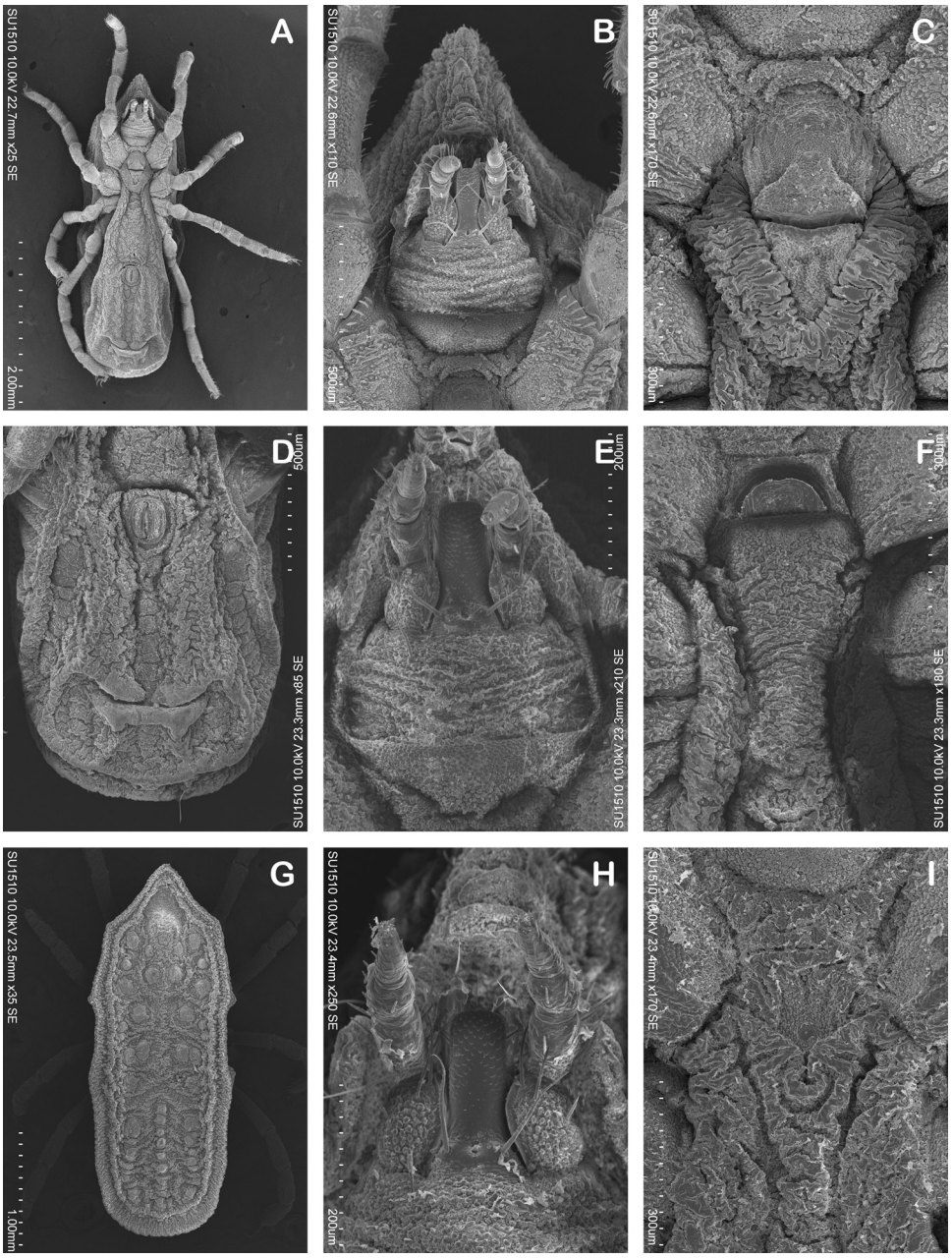

Fig. 2. Scanning electron microscopy of *Ornithodoros dyeri*. Female: (A) ventral view, (B) capitulum, (C) genital opening. Male: (D) postanal groove, (E) capitulum, (F) genital opening. Nymph: (G) dorsal view, (H) hypostome, (I) depression at level of sexual opening. Scale in micrometers.

(42.95–62.88); Al_5 53.4 ± 3.02 (46.65–57.36); Al_6 46.6 ± 5.9 (39.28–56.3); Al_7 54.8 ± 4.8 (47.56–62.59); three central pairs (C): C_1 57.24 ± 6.40 (49.14–65.79); C_2 48.45 ± 6.36 (44.1–58.73); C_3 47.11 ± 4.6 (39.86–56.8), and four posterolateral setae (Pl): Pl_1 50.24 (n = 9) ± 5.6 (44.61–58.14); Pl_2 47.7 ± 3.9 (44.4–54.27); Pl_3 45.84 ± 4.61 (37.72–50.95); Pl_4 43.5 ± 3.34 (37.08–49.44). Dorsal plate moderately large, piriform (Fig. 3A), length 219.72 ± 11.40 (206.34-248.63); width 127.35 ± 9.30 (111.17–142.2). Venter of body with nine pairs of setae (includes one pair on anal valves) plus a posteromedian seta (PM) (Fig. 3B): 36.21 (n = 11) ± 5.86 (28.81–43.83), three pairs of sternal setae (St): St_1 49.6 ± 8.2 (34.54–63.01); St_2 48.41 ± 5.5 (38.44–54.71); St_3 47 ± 5.64 (36.8–54.21); four pairs of circumanal setae (Ca): Ca_1 35.04 (n = 11) ± 5.01 (27.45–40.61); Ca_2 41.4 (n = 11) ± 5.6 (33.5–51.15); Ca_3 49.5 ± 9.05 (41.07–59); Ca_4 49 ± 5.17 (38.11–55.15), and a postcoxal setae (Pc): 32.1 ± 5.31(26.05–43.52).

Capitulum: Basis of capituli measuring 270 ± 16.16 (230–294), from basis capituli to insertion of hypostome; length to basis capituli to apex of hypostome: 404.22 (n = 5) ± 14.6(385–426); width 198.01 ± 8.4(180.5–207). Two pairs of posthypostomal setae, Ph_1 and Ph_2. Distance between Ph_1 setae 19.3 ± 2.7 (13–22.86) and between Ph_2 setae 85.2 ± 4.3 (77.8–91.8). Hypostome long and pointed. Hypostome arises from a sclerotized, subtriangular median extension, measuring 311.21 (n = 4) ± 17 (286.34–322) from apex to Ph_1, and 127.6 (n = 5) ± 7.11 (121.4–137.5) to point of insertion of hypostome in basis capituli. Dentition 3/3 in the anterior three fourths, and 2/2 posteriorly to base (Fig. 3C). Palpal length 278.5 ± 8.73(267.1–292), segment I length/width: 80 ± 5.9 (67.18–88.45)/32.52 ± 2.17 (29–37); segment II 78 ± 7.9 (65–90)/33.7 ± 2 (30.52–38.3); segment III 76.7 ± 2.6 (73.6–80.12)/32.4 ± 1.34 (30.64–35); segment IV 49.24 ±3.9 (43.85–54.77)/18.21 ± 1.5 (15.85–22.1).

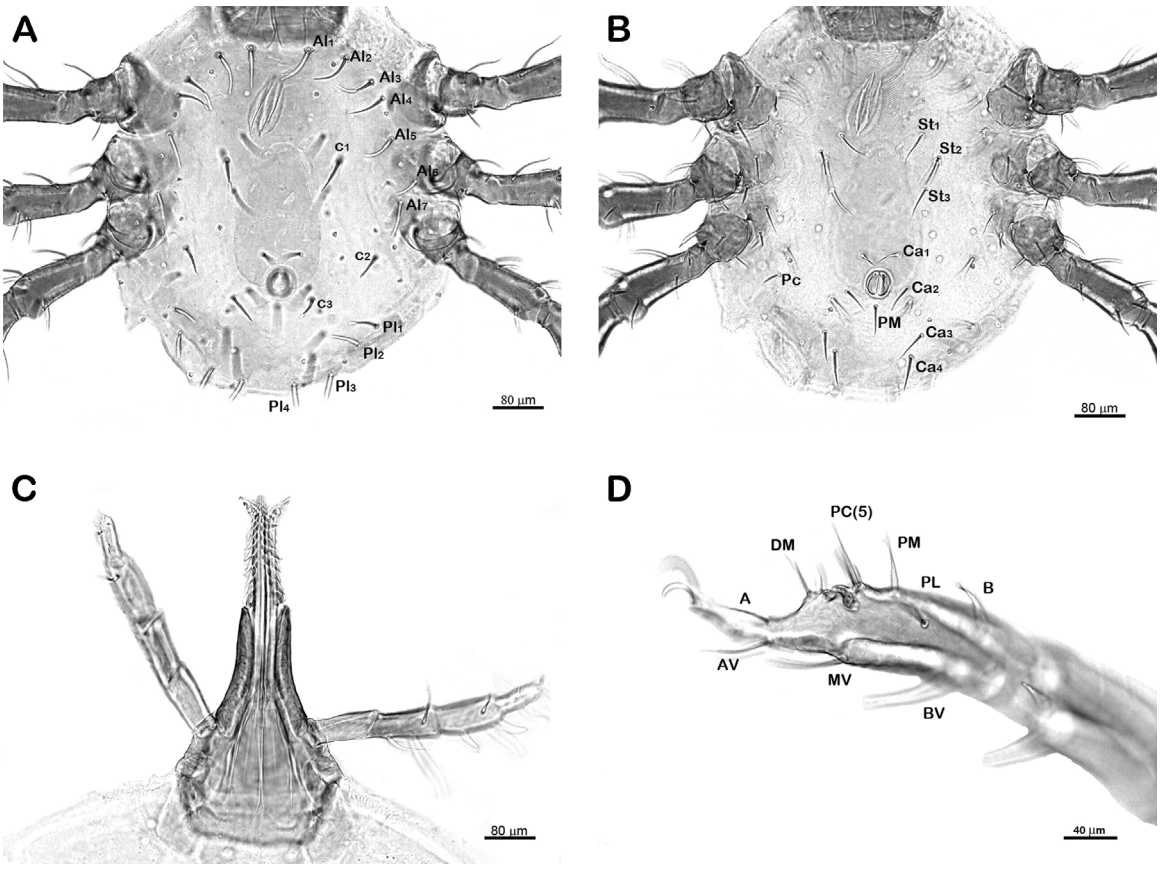

Fig. 3. Optical micrographs of *Ornithodoros dyeri*. Larva: (A) dorsal view, (B) ventral view, (C) hypostome, (D) tarsus I. Scale in millimeters.

Legs: Capsule of Hallerís organ with reticulations. Tarsus I length 197.52 (n = 11) ± 22.9 (151.72–226); Tarsus I width 55 ± 2.6 (49.7–57.9); setal formula 1 pair apical (A), 1 distomedian (DM), 5 paracapsular (PC), 1 posteromedian (PM), 1 basal pair (B), 1 pair apicoventral (AV), 1 pair midventral (MV), 1 pair basiventral (BV), 1 pair posterolateral (PL) (Fig. 3D).

Fragments of approximately 414 bp were obtained for the mitochondrial 16S rRNA gene from the three analyzed specimens: one larva (GenBank accession number: submitted), one female (GenBank accession number: submitted), and one male (GenBank accession number: submitted). The pairwise differences between the sequences varied from 0 to 0.5% meanwhile, differences among the sequences of *O. dyeri* and the rest of the species included in the analysis were always higher than 13%. In spite of the morphological similarities observed between *O. dyeri* and *O. peropteryx*, the molecular analysis reveals that the genetic distance (15.2%) located them in separate clades.

3.2. New locality and host records

As result of this study, we present 6 new locality records for *O. dyeri*, which increase its distribution in 3 Mexican States and in 2 new species of hosts (Table 1).

3.3. Previous records

Present distribution of *O. dyeri* has been referred as Nearctic and Neotropical (Guglielmone et al., 2003); this species has been recovered from bat retreats, and parasitizing several bats of three families (Emballonuridae, Phyllostomidae and Vespertilionidae). EL SALVADOR: near Santa Rosa, *Balantiopteryx plicata* Peters (1867) (Kohls et al., 1965). USA: Picacho Mountain, near Picacho, bat guano (Cooley and Kohls, 1940); mine tunnel near Yuma, Arizona (Cooley and Kohls, 1944); near Needles, California, crevices in a bat cave (Cooley and Kohls, 1944). MEXICO: Cueva de la Chepa, Chiapas (Mazzotti, 1941); Cueva de la Fábrica, 5 km O de Coquimatlán, Colima (Mazzotti, 1941); Cueva de Taninul, San Luis Potosí (Hoffmann, 1962); Isla Pescadero, Golfo de California, *ex Myotis vivesi* Menegaux, 1901 (Kohls et al., 1965); Isla Partida (Cardonosa Island), Golfo de California, *ex M. vivesi* and bat roost (Kohls et al., 1965); Pond Island, Golfo de California, *ex M. vivesi* and bat roost (Kohls et al., 1965); cave at Sabinas Hidalgo, Nuevo León, bat guano (Kohls et al., 1965); near Tequisistlán, Oaxaca, *ex B. plicata* (Kohls et al., 1965); Puerto Angel, Oaxaca, *ex B. plicata* (Kohls et al., 1965); Quintana Roo, *ex Trachops cirrhosus* Spix (1823) (Wolfgang and Polaco, 1985).

4. Discussion

Klompen and Oliver (1993), in their phylogenetic analysis of the relationships at the generic and subgeneric level in the family Argasidae, included *O. dyeri* as a member of the genus *Carios*. However, the monophyly of *Carios*, as presented in Klompen and Oliver (1993), has a low support as recognized by the authors. For this reason, we prefer to be conservative because the phylogenetic relationships within the family are still controversial, even when the phylogeny is analyzed with molecular tools (Estrada-Peña et al., 2010; Burger et al., 2014).

The distribution of *O. dyeri* includes localities of both the Neotropical and Nearctic realms associated with Chiroptera where it can be found directly parasitising bats or living in bat retreats such as crevices on cave walls (Kohls et al., 1965; Guglielmone et al., 2003). Previous to this study, this species was found in localities belonging to seven Mexican states (Mazzotti, 1941; Hoffmann, 1962; Kohls et al., 1965; Wolfgang and Polaco, 1985); with the data

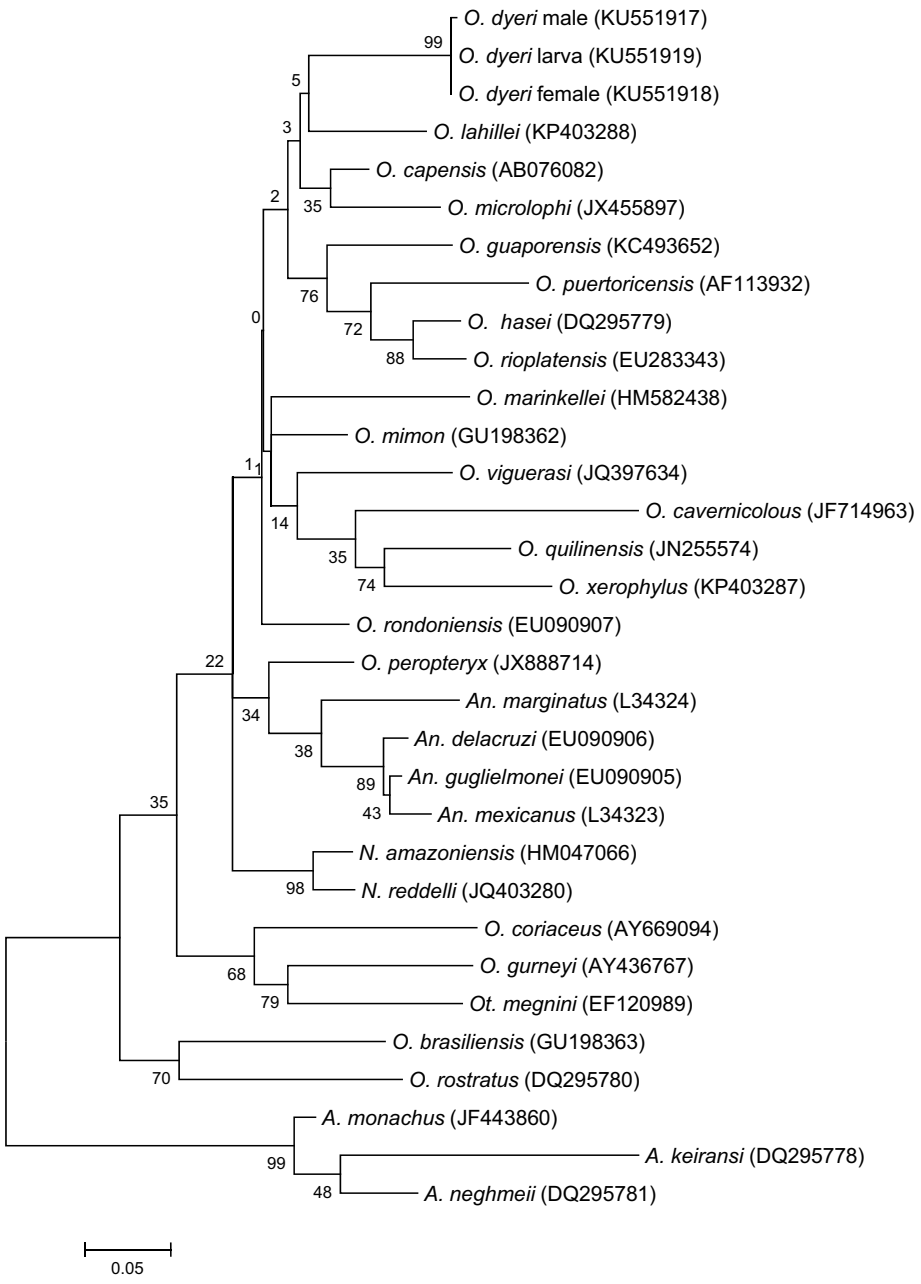

Fig. 4. Maximum-likelihood tree based on 16S rDNA partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are indicated in brackets. An., *Antricola*; O., *Ornithodoros*; N., *Nothoaspis*; Ot., *Otobius*; A., *Argas*.

presented herein, the current distribution of *O. dyeri* was expanded to three additional states with two situated in the Neotropics (Veracruz and Yucatán) and one in the Nearctic region (Coahuila). In the same way, the two new hosts recorded for this tick (*M. cozumelae* and *P. macrotis*) increased to five for the number of bat species associated with this argasid.

The morphological study conducted on the Mexican adult and nymphal stages confirm that *O. dyeri* is characterized as having an elongated body in proportion to width with a dorsum bounded by two contiguous ridges and a third ridge (inner) incomplete on each side. These traits have been considered as diagnostic by Cooley and Kohls (1940) and Cooley and Kohls (1944). In general, argasids have several nymphal stages throughout their life cycle (Sonenshine, 1991); however, some species of *Ornithodoros* such as *O. peropteryx* Kohls, Clifford and Jones, 1969 (Venzal et al., 2013b), *O. faccini* Barros-Battesti, Landulfo and Luz, 2015 (Barros-Battesti et al., 2015) and probably others (Venzal et al., 2013b) only have one nymphal instar. The life cycle of *O. dyeri* remains unknown; notwithstanding, the presence of a depression at the level of sexual opening that resembles the "V"-shaped depression present in females of this species suggests that the specimens described in this study correspond to the stage previous to adult as referred by Cooley and Kohls (1940). On the other hand, in the original description of *O. dyeri* Cooley and Kohls (1940) established the presence of "discs" (defined by these authors as any structural modification of the body wall at the point of attachment of the dorsoventral muscles) in this species; however, in the compilation of morphological characteristics for nymphs of the Neotropical species of *Ornithodoros* (*Alectorobius*) presented by Landulfo et al. (2013), this traits is referred as absent for *O. dyeri*. In the present study, we observed numerous subcircular elevations (discs *sensu* Cooley and Khols, 1940) on the dorsal surface of adults and more conspicuously in the nymphal instar.

However, according to Kohls et al. (1965) larvae of this species are distinguished by a set of characters, i.e., the presence of 14 pairs of dorsal setae (11 dorsolateral and three central pairs), a venter body with nine pairs of setae (including one pair on the anal valves), plus a posteromedian (9 + 1), a moderately large dorsal plate and piriform, hypostome arising from a relatively short, subtriangular median extension of the basis capituli, and capsule of Hallerís organ with reticulations. From the number of pairs of the dorsal setae (14–16) and the presence of reticulation in the Hallerís organ, the larvae of *O. dyeri* most closely resembles that of those of *O. yumatensis* Cooley and Kohls (1941), *O. knoxjonesi* Jones and Clifford (1972) and *O. peropteryx*; notwithstanding, the number of setal pairs on the ventral idiosome of the 4 species (9 + 1, 8 + 1, 8 + 1 and 8 + 1, respectively) enables their distinction (Kohls et al., 1965, 1969; Jones and Clifford, 1972). Additionally, in *O. knoxjonesi* and *O. peropteryx*, the hypostome is narrow near mid-length (Venzal et al., 2013b); meanwhile, the hypostome of *O. dyeri* lacks this constriction.

The molecular analysis of the 16S rDNA sequences showed that *O. dyeri* represents an independent lineage within Neotropical species of the Argasidae (Fig. 4). However, the validity of the subgenus *Alectorobius*, where this species was included by Clifford et al. (1964) based on morphological traits, is doubtful after our study; at least 3 other species included in this subgenus are located in different clades, which, indicated a paraphyletic origin. A similar conclusion was obtained by Dantas-Torres et al. (2012), who established that the definition of *Alectorobius* is obsolete, because this subgenus originally included larvae with a pointed hypostome (but now include species whose larvae present hypostome bluntly pointed anteriorly) and a capsule of Haller's organ without reticulation (and now some species have reticulations in this organ), among other characteristics. More taxon sampling, in particular molecular data of a more conservative gene, is required to evaluate its validity and the necessity of its emendation.

Conflict of interest

Authors declare to have no conflict of interest.

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