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A new species of *Ornithodoros* (Acari: Argasidae) from desert areas of northern Chile

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ABSTRACT

Ornithodoros atacamensis n. sp. is described from larvae collected on the lizard *Liolaemus bisignatus* and from free-living adults collected in desert areas from the Pan de Azúcar and Llanos de Challe National Parks, in Northern Chile. Additionally, unengorged larvae were obtained from fertilized females, which laid eggs in the laboratory. Morphological and mitochondrial 16S rDNA sequence analyses were performed in order to compare this new soft tick species with other congeneric Neotropical representatives. Larvae of *O. atacamensis* are morphologically closely related to *Ornithodoros talaje* sensu stricto, *Ornithodoros puertoricensis*, *Ornithodoros rioplatensis*, *Ornithodoros guaporensis* and *Ornithodoros hasei*, all belonging to the *O. talaje* species group. The larval diagnostic characters for this species are a combination of a large pyriform dorsal plate with a length of approximately 300 μm, 17 pairs of dorsal setae with five central pairs, hypostome with apex pointed and dental formula 2/2 in most rows, 3/3 apically, and capsule of the Haller's organ oval in shape without reticulations. Phylogenetic analyses inferred from the mitochondrial 16S rRNA gene and a Principal Component Analysis based on morphometric characters provide additional support to the description of *O. atacamensis* as an independent lineage within the genus clustering within the *O. talaje* species group.

1. Introduction

The worldwide specific richness of soft ticks (Argasidae) is currently represented by *ca.* 200 species (Vial and Camicas, 2009; Guglielmone et al., 2010; Nava et al., 2010, 2013; Dantas-Torres et al., 2012; Heath, 2012; Venzal et al., 2012, 2013, 2015; Trape et al., 2013; Barros-Battesti et al., 2015). Combining the use of morphologically significant characters and molecular markers, 15 new species of soft ticks have been described in the last 12 years for the Neotropical Zoogeographic Region (Venzal et al., 2015; Barros-Battesti et al., 2015). This fact strongly suggests that the number of species of soft ticks is underestimated in the Neotropics.

Ticks of genus *Ornithodoros* Koch are nidicolous and cave dweller parasites well adapted to survive in arid environments (Hoogstraal, 1985; Oliver, 1989). The northern half of Chile (roughly 18°–33°S) is characterized by large extensions of arid ecosystems, including within its range the Atacama Desert, one of the driest regions of the world (Clarke, 2006). To date, the Chilean diversity of soft ticks is represented by *Otobius megnini* (Dugès, 1884) (González-Acuña and Guglielmone, 2005), two species of *Argas* and six species of *Ornithodoros*, being five of them described for the northern arid regions of the country (González-Acuña and Guglielmone, 2005; González-Acuña et al., 2008; Venzal et al., 2013, 2015). Based on larval and adult morphology, and on phylogenetic analyses of the 16S rRNA mitochondrial gene, here we present the description of a

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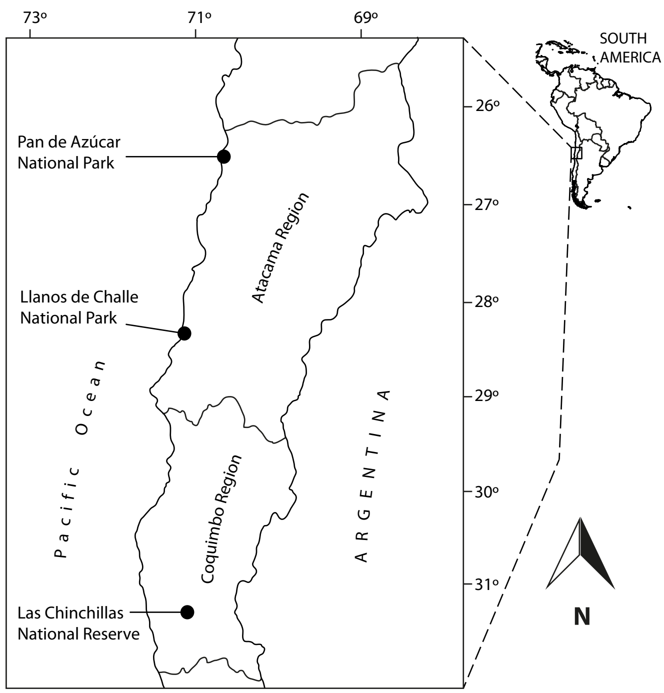

Fig. 1. Map of Chile showing the localities where *Ornithodoros atacamensis* n. sp. specimens were collected.

new species of *Ornithodoros* associated with reptiles in desert areas of northern Chile.

2. Material and methods

2.1. Ticks

Ticks were collected from the environment and from lizards in three localities from northern Chile (Fig. 1). Reptiles were captured according to the licenses given by the Servicio Agrícola and Ganadero (SAG) and the Corporación Nacional Forestal (CONAF) (documents 6007/2014 and 033/2014 respectively). Free-living adults (10 females and 17 males) belonging to the genus *Ornithodoros* were collected from rock crevices in Pan de Azúcar National Park (PANP, 26°17′S; 70°38′W; 2014), and a total of 139 larvae of *Ornithodoros* were collected on *Liolaemus bisignatus* (Philippi 1860) (Squamata: Liolaemidae) in PANP (n = 53) and Llanos de Challe National Park (n = 86) (LCNP, 28°11′S; 71°09′W; 2011–2014), both sites located in the Atacama region. Also, two *Ornithodoros* larvae were found on *Callopistes maculatus* Gravenhorst 1838 (Squamata: Teiidae) in Las Chinchillas National Reserve (LCNR, 31°30′S; 71°06′W; 2011–2014), Coquimbo region. Larvae from reptiles were collected in 70–90% ethanol and adult ticks were brought alive to the laboratory and placed in incubators at 25 °C and 80% of relative humidity. Under these conditions, two females laid eggs and we obtained the unfed larvae. Ten laboratory-reared and 39 field-collected larvae were clarified in 20% KOH solution, mounted in semi-permanent slides for microscopy using Hoyer's medium, and measured using a Nikon Eclipse E200 optical microscope. All measurements are given in millimeters (mm), with the mean followed by the standard deviation and range in parentheses. Terminology for larval chaetotaxy and measurements followed Venzal et al. (2008, 2013).

Slightly engorged females, males and unengorged larvae were prepared according to the methods presented by Corwin et al. (1979) and used to obtain scanning electron microscopy images. The micrographs were obtained using a JEOL JMS-5900 scanning electron microscope (JEOL, Tokyo, Japan). Terminology for the description of adult characters followed Cooley and Kohls (1944) and Nava et al. (2013). One of the females from which unfed larvae were obtained in the laboratory, one field-collected larva from PANP and one larva from PNLC were submitted to DNA extraction using the Guanidine Isothiocyanate technique (Sangioni et al., 2005).

A principal component analysis (PCA) based on Pearson correlation matrix was performed for 41 morphometric variables (Table 1) of unengorged larvae in order to discriminate the relationship between phenotypically closely species. To this effect, measurements of *Ornithodoros guaporensis* Nava et al., 2013, *Ornithodoros hasei* (Schulze, 1935), *Ornithodoros puertoricensis* Fox 1947, *Ornithodoros rioplatensis* Venzal et al., 2008, and *Ornithodoros talaje* sensu stricto (s. s.) (Guérin-Méneville, 1849) were included.

In order to obtain unengorged adult specimens for allowing a better visualisation of morphological characters, field-collected *Ornithodoros* adults were maintained unfed in the incubator for 14 months. Thereafter, females (n = 2) were examined using a Zeiss Stemi SV11 stereomicroscope, and their external morphology was compared with other morphologically related Neotropical Ornithodorinae. To this effect, we analysed the following unengorged females deposited in the tick collection "Coleção Nacional de Carrapatos" (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, Brazil: *O. guaporensis* from Bolivia (n = 2; CNC-2305); *O. rioplatensis* from Uruguay (n = 2; CNC-3254), and *O. puertoricensis* from Panama (n = 4; CNC-3255). Photographs of examined females were taken with a Canon PowerShot SX700HS camera. We only analysed females because the external morphology of adult argasids is very similar for both sexes (with the exception of the genital area).

2.2. Phylogenetic analysis

Obtained sense and antisense sequences of the mitochondrial 16S rRNA gene were assembled, manually edited, and an alignment with other sequences of Neotropical congeneric species available in GenBank. Alignment was performed using the Clustal W program (Thompson et al., 1994), and reconnection branch swapping with missing data; all positions were equally weighed. A Bayesian analysis was performed using MrBayes v3.1.2 with 1,000,000 generations and trees being sampled every 1000 generations, running 4 times beginning with random starting trees. The General Time Reversible model (GTR) was used combined with the models of gamma distribution (G) (Huelsenbeck and Ronquist, 2001). The first 25% of the trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability (BPP). Sequences of *Ixodes holocyclus* Neumann 1899 (AB051845) and *Ixodes uriae* White 1852 (AB030017) were used as out-groups.

3. Results

3.1. Descriptions

Ixodida Leach, 1815
Argasidae Canestrini, 1890
Ornithodoros Koch, 1844

3.2. Ornithodoros atacamensis *n. sp. Muñoz-Leal, Venzal & González-Acuña*

3.2.1. *Larva (Figs. 2–3)*

Body: Idiosoma subcircular . Length including capitulum: 0.801 ± 0.028 (0.761–0.834), and not including capitulum: 0.474 ± 0.020 (0.438–0.499), width: 0.489 ± 0.035 (0.435–0.536). Dorsum: Dorsal plate pyriform, length 0.302 ± 0.010 (0.283–0.318),

Table 1
Average and range (mm) of measurements from *O. atacamensis*, *O. puertoricensis*, *O. rioplatensis*, *O. guaporensis*, *O. hasei*, and *O. talaje* used for de PCA. The number of measured ticks is indicated in parenthesis.

	O. atacamensis (10)	*O. puertoricensis* (10)	*O. rioplatensis* (10)	*O. guaporensis* (10)	*O. hasei* (5)	*O. talaje* (5)
Dorsal plate: length	0.302 (0.238–0.318)	0.219 (0.202–0.236)	0.262 (0.242–0.276)	0.290 (0.271–0.305)	0.255 (0.191–0.296)	0.251 (0.245–0.262)
Dorsal plate: width	0.202 (0.194–0.217)	0.172 (0.163–0.183)	0.177 (0.166–0.187)	0.220 (0.212–0.232)	0.152 (0.142–0.171)	0.177 (0.170–0.185)
Dorsal setae (pairs): total	17	18	20	20	19	17
Dorsal setae (pairs): dorsolateral	12	14	16	16	15	13
Dorsal setae (pairs): central	5	4	4	4	4	4
Central setae: C_1 length	0.067 (0.063–0.073)	0.094 (0.073–0.105)	0.094 (0.085–0.100)	0.094 (0.083–0.100)	0.083 (0.073–0.086)	0.083 (0.078–0.085)
Central setae: C_2	0.058 (0.051–0.064)	0.086 (0.073–0.093)	0.081 (0.073–0.090)	0.088 (0.085–0.093)	0.069 (0.069–0.071)	0.075 (0.073–0.078)
Central setae: C_3	0.060 (0.055–0.065)	0.085 (0.068–0.095)	0.078 (0.073–0.085)	0.082 (0.075–0.090)	0.070 (0.064–0.078)	0.071 (0.066–0.078)
Central setae: C4	0.058 (0.053–0.063)	0.086 (0.071–0.095)	0.079 (0.063–0.090)	0.092 (0.088–0.097)	0.075 (0.069–0.086)	0.068 (0.063–0.073)
Sternal setae: St_1	0.046 (0.040–0.051)	0.057 (0.049–0.063)	0.064 (0.061–0.066)	0.069 (0.066–0.073)	0.060 (0.054–0.069)	0.055 (0.049–0.061)
Sternal setae: St_2	0.043 (0.039–0.050)	0.059 (0.049–0.071)	0.061 (0.052–0.068)	0.055 (0.049–0.061)	0.056 (0.054–0.059)	0.060 (0.053–0.063)
Sternal setae: St_3	0.044 (0.041–0.046)	0.056 (0.044–0.063)	0.063 (0.056–0.068)	0.059 (0.058–0.061)	0.057 (0.054–0.061)	0.053 (0.053–0.056)
Circumanal setae: Ca_1	0.041 (0.033–0.050)	0.051 (0.044–0.058)	0.054 (0.045–0.061)	0.053 (0.044–0.061)	0.054 (0.049–0.059)	0.047 (0.037–0.053)
Circumanal setae: Ca_2	0.047 (0.041–0.052)	0.068 (0.056–0.078)	0.067 (0.060–0.075)	0.071 (0.066–0.073)	0.066 (0.064–0.071)	0.055 (0.050–0.057)
Circumanal setae: Ca_3	0.057 (0.050–0.064)	0.094 (0.078–0.105)	0.089 (0.078–0.097)	0.102 (0.097–0.107)	0.078 (0.073–0.081)	0.070 (0.067–0.073)
Postcoxal setae: Pc	0.031 (0.028–0.033)	0.046 (0.039–0.053)	0.051 (0.046–0.058)	0.040 (0.034–0.046)	0.059 (0.049–0.069)	0.038 (0.036–0.041)
PMS	0.040 (0.025–0.048)	0.067 (0.049–0.078)	0.057 (0.052–0.063)	0.074 (0.068–0.080)	0.055 (0.049–0.061)	0.053 (0.049–0.056)
Length of basis capituli[a]	0.125 (0.105–0.140)	0.116 (0.098–0.129)	0.121 (0.112–0.134)	0.133 (0.124–0.139)	0.141 (0.133–0.147)	0.133 (0.127–0.137)
Length of basis capituli[b]	0.152 (0.130–0.169)	0.128 (0.110–0.149)	0.136 (0.117–0.164)	0.156 (0.146–0.166)	0.182 (0.176–0.186)	0.154 (0.142–0.160)
Length of basis capituli[c]	0.342 (0.315–0.365)	0.367 (0.323–0.392)	0.370 (0.350–0.392)	0.441 (0.431–451)	0.362 (0.346–0.376)	0.326 (0.320–0.330)
Width of basis capituli	0.202 (0.180–0.220)	0.156 (0.146–0.171)	0.161 (0.150–0.171)	0.180 (0.173–0.193)	0.236 (0.228–0.240)	0.194 (0.185–0.205)
Palpal length	0.244 (0.236–0.254)	0.282 (0.263–0.293)	0.254 (0.250–0.262)	0.323 (0.310–0.332)	0.252 (0.240–0.282)	0.220 (0.215–0.227)
Length article I	0.005 (0.052–0.061)	0.052 (0.049–0.054)	0.054 (0.049–0.062)	0.063 (0.056–0.083)	0.063 (0.054–0.069)	0.052 (0.050– 0.057)
Length article II	0.086 (0.082–0.092)	0.108 (0.100–0.112)	0.089 (0.080–0.097)	0.129 (0.122–0.134)	0.080 (0.073–0.086)	0.069 (0.062–0.075)
Length article III	0.086 (0.082–0.090)	0.094 (0.085–0.100)	0.084 (0.075–0.090)	0.106 (0.085–0.114)	0.067 (0.061–0.073)	0.075 (0.072–0.077)
Length article IV	0.037 (0.035–0.039)	0.037 (0.034–0.041)	0.036 (0.032–0.040)	0.035 (0.029–0.039)	0.046 (0.044–0.049)	0.030 (0.030–0.032)
Width article I	0.025 (0.024–0.027)	0.025 (0.024–0.027)	0.026 (0.025–0.027)	0.027 (0.024–0.032)	0.032 (0.029–0.037)	0.031 (0.030–0.032)
Width article II	0.035 (0.034–0.037)	0.033 (0.032–0.034)	0.036 (0.035–0.037)	0.032 (0.024–0.036)	0.036 (0.034–0.037)	0.037 (0.035–0.037)
Width article III	0.035 (0.034–0.037)	0.033 (0.032–0.034)	0.032 (0.030–0.032)	0.033 (0.032–0.034)	0.034 (0.029–0.039)	0.033 (0.030–0.035)
Width article IV	0.022 (0.022–0.022)	0.020 (0.019–0.022)	0.021 (0.020–0.022)	0.020 (0.019–0.024)	0.023 (0.022–0.024)	0.018 (0.017–0.020)
Hypostome: length[d]	0.219 (0.205–0.230)	0.252 (0.232–0.266)	0.243 (0.237–0.250)	0.309 (0.305–0.315)	0.212 (0.203–0.218)	0.197 (0.187–0.200)
Hypostome: length[e]	0.182 (0.178–0.190)	0.255 (0.207–0.251)	0.231 (0.255–0.237)	0.277 (0.268–0.285)	0.176 (0.174–0.179)	0.169 (0.162–0.175)
Hypostome: width base	0.049 (0.046–0.051)	0.052 (0.049–0.054)	0.054 (0.050–0.057)	0.047 (0.044–0.049)	0.061 (0.059–0.064)	0.053 (0.045–0.055)
Apical dental formula	3	3	3	3	3	3
Median dental formula	2	3	3	2	3	2
Basal dental formula	2	2	2	2	2	2
Denticles in hypostomal row 1	21 (19–22)	27	24 (23–25)	26 (26–27)	18	18 (17–19)
Denticles in hypostomal row 2	20 (18–22)	25	22 (21–24)	25 (25–26)	18 (17–18)	17 (16–17)
Denticles in hypostomal row 3	11 (10–12)	15	12 (11–13)	12 (11–13)	12 (11–12)	10 (9–11)
Tarsus I: length	0.171 (0.165–0.176)	0.176 (0.171–0.180)	0.176 (0.171–0.183)	0.174 (0.168–0.180)	0.175 (0.171–0.184)	0.182 (0.171–0.190)
Tarsus I: width	0.057 (0.053–0.063)	0.059 (0.054–0.061)	0.058 (0.056–0.061)	0.058 (0.056–0.058)	0.063 (0.059–0.069)	0.060 (0.058–0.063)

[a] Length of basis capituli to Ph1.
[b] Length of basis capituli to insertion of hipostome.
[c] Length of basis capituli to tip hipostme.
[d] Measured to point of Ph1.
[e] Measured to point of insertion of hipostome in basis capituli.

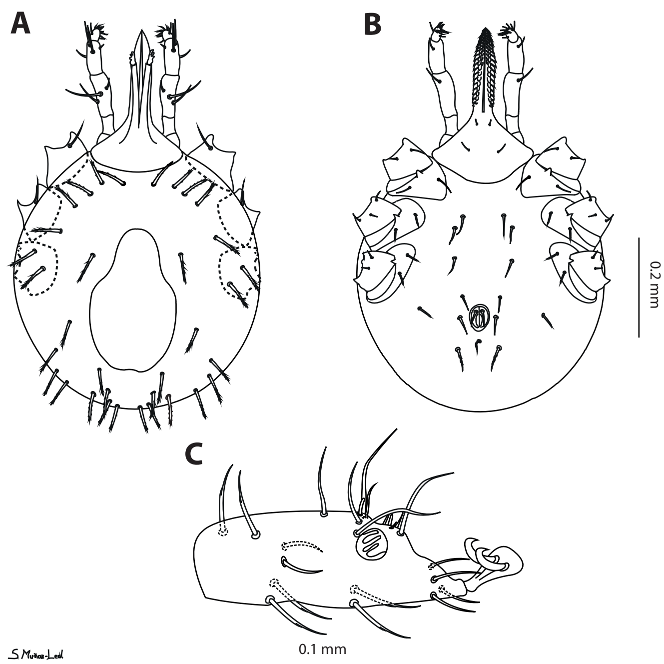

Fig. 2. *Ornithodoros atacamensis* n. sp. drawing of larva. (A) Dorsal. (B) Ventral. (C) Tarsus I. Scale in millimeters.

width: 0.202 ± 0.007 (0.194–0.217). Dorsal surface provided with 17 pairs of setae, 7 anterolateral, 5 central and 5 posterolateral. Anterolateral setae (Al): Al_1 length 0.067 ± 0.003 (0.063–0.074), Al_2 length 0.065 ± 0.004 (0.056–0.071), Al_3 length 0.061 ± 0.005 (0.052–0.067), Al_4 length 0.063 ± 0.003 (0.059–0.066), Al_5 length 0.063 ± 0.003 (0.059–0.067), Al_6 length 0.063 ± 0.003 (0.056–0.066), Al_7 length 0.066 ± 0.003 (0.060–0.071). Central setae (C): C_1 length 0.067 ± 0.003 (0.063–0.073), C_2 length 0.058 ± 0.004 (0.051–0.064), C_3 length 0.060 ± 0.003 (0.055–0.065), C_4 length 0.058 ± 0.003 (0.053–0.063, C_5 length 0.056 ± 0.005 (0.049–0.064). Posterolateral setae (Pl): Pl_1 length 0.058 ± 0.004 (0.053–0.067), Pl_2 length 0.063 ± 0.003 (0.059–0.066), Pl_3 length 0.059 ± 0.003 (0.053–0.062), Pl_4 length 0.059 ± 0.004 (0.052–0.066), Pl_5 length 0.058 ± 0.002 (0.054–0.061).

Venter: Ventral surface provided with 7 pairs of setae plus 1 pair on anal valves, posteromedian seta present. Three pairs of sternal setae (St): St_1 length 0.046 ± 0.004 (0.040–0.051), St_2 length 0.043 ± 0.003 (0.039–0.050), St_3 length 0.044 ± 0.002 (0.041–0.046); three pairs of circumanal setae (Ca): Ca_1 length 0.041 ± 0.005 (0.033–0.050), Ca_2 length 0.047 ± 0.004 (0.041–0.052), Ca_3 length 0.057 ± 0.004 (0.050–0.060); posteromedian setae (Pm) length 0.040 ± 0.007 (0.025–0.048), postcoxal setae (Pc) length 0.031 ± 0.002 (0.028–0.033).

Capitulum: Basis capituli pentagonal, posterior margin slightly curved. Length from posterior margin of basis capituli to posthypostomal setae Ph_1 0.126 ± 0.011 (0.105–0.140), length from posterior margin of basis capituli to insertion of hypostome 0.153 ± 0.014 (0.130–0.169), length from posterior margin of basis capituli to apex of hypostome 0.342 ± 0.015 (0.315–0.365), width 0.202 ± 0.013 (0.180–0.220). Two pairs of posthypostomal setae; Ph_1 length 0.012 ± 0.001 (0.011–0.012), Ph_2 length 0.031 ± 0.003 (0.024–0.034), distance between Ph_1 setae 0.027 ± 0.003 (0.023–0.031), distance between Ph_2 setae 0.067 ± 0.004 (0.060–0.075). Palpi total length 0.244 ± 0.006 (0.236–0.254), segmental length/width from I–IV: (I) 0.055 ± 0.003 (0.052–0.061)/0.025 ± 0.001 (0.024–0.027), (II) 0.086 ± 0.003 (0.082–0.090)/0.035 ± 0.001 (0.034–0.037), (III) 0.086 ± 0.003 (0.082–0.090)/0.035 ± 0.001 (0.034–0.037), (IV) 0.038 ± 0.001 (0.035–0.039)/0.022 ± 0.001 (0.021–0.022). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9. Hypostome: length from Ph_1 to apex 0.219 ± 0.008 (0.205–0.230), length from insertion of hypostome in basis capituli to apex 0.192 ± 0.004 (0.188–0.198), length of hypostome from apex to inferior toothed portion 0.185 ± 0.003 (0.178–0.190), width in medial basis portion of hypostome 0.049 ± 0.002 (0.046–0.051), width in basis portion of hypostome 0.049 ± 0.001 (0.046–0.051); arises from the basis on a median extension, pointed apically. Dentition formula 3/3 in anterior third, 2/2 towards the base, file 1 with 19–22 denticles (typically 21), file 2 with 18–21 (typically 20) and file 3 with 10–12 denticles (typically 11).

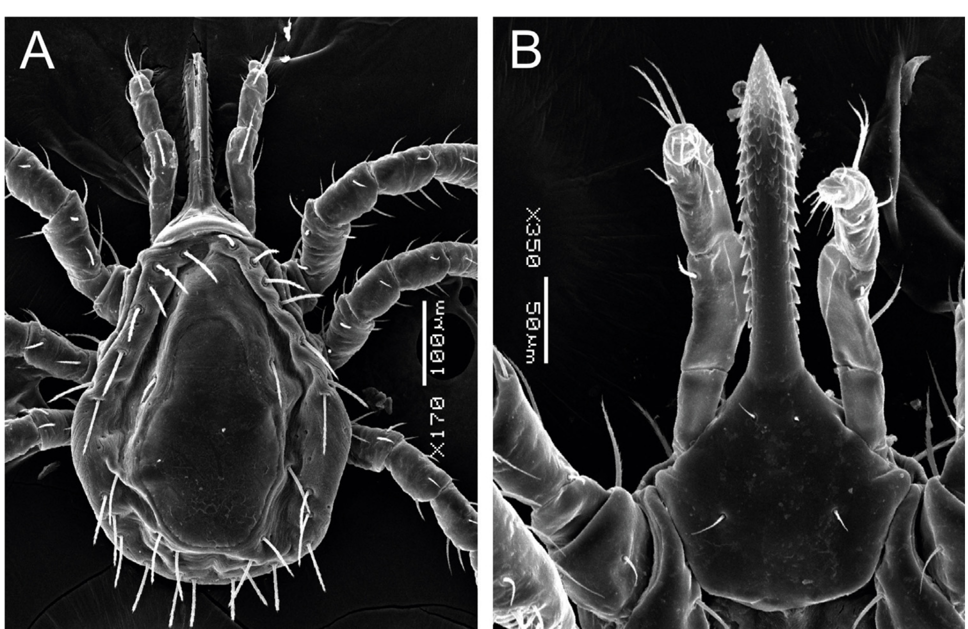

Fig. 3. *Ornithodoros atacamensis* n. sp. scanning electron microscopy of the larva. (A) Dorsal idiosoma. (B) Basis capitulum and hypostome.

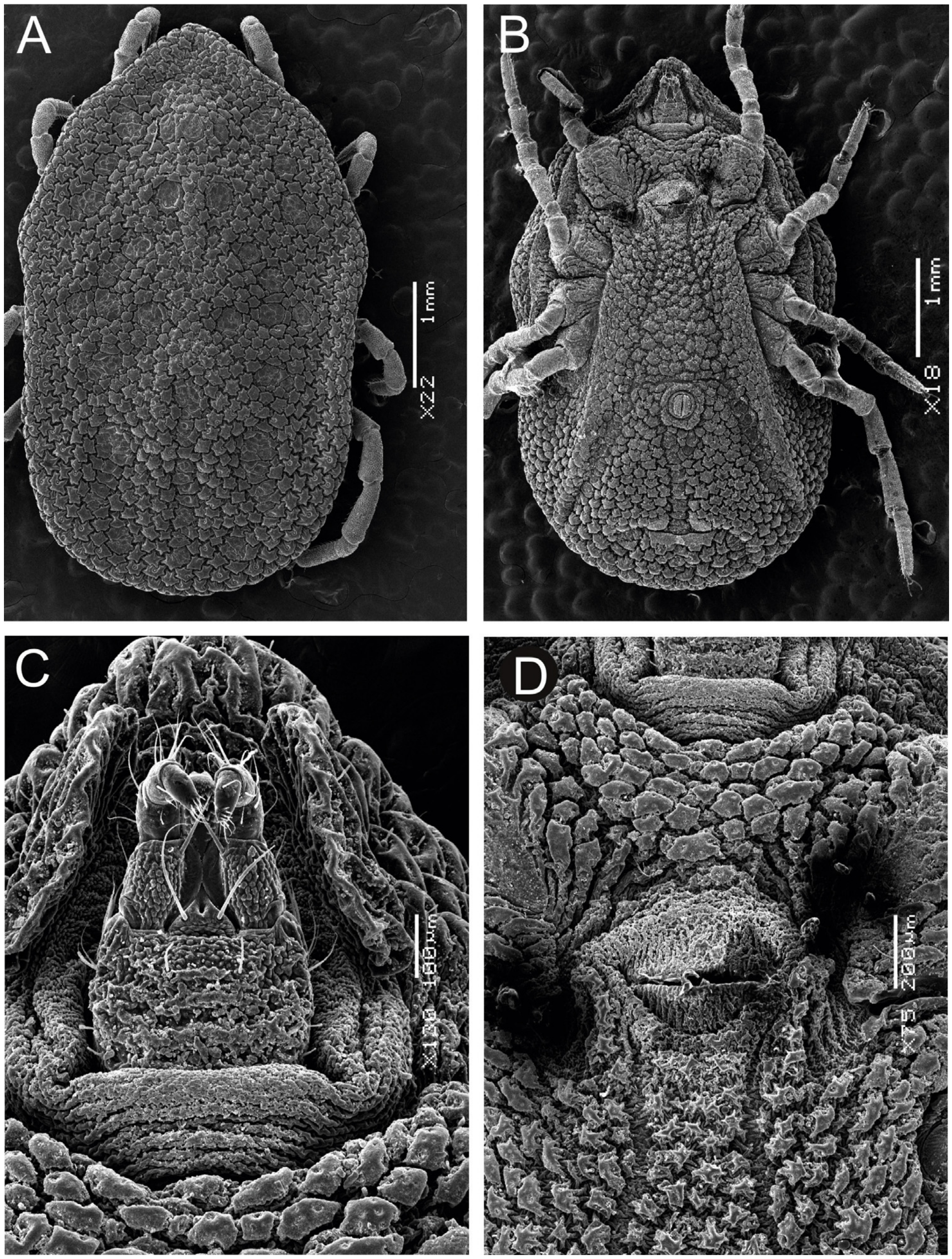

Fig. 4. *Ornithodoros atacamensis* n. sp. scanning electron microscopy of the female. (A) Dorsal idiosoma. (B) Ventral idiosoma. (C) Ventral capitulum. (D) Genital opening.

Legs: Tarsus I length 0.171 ± 0.005 (0.165-0.176), tarsus I width 0.057 ± 0.004 (0.053-0.063). Setal formula of tarsus I: 1 pair apical (A), 1 distomedian (DM), 5 paracapsular (PC), 1 posteromedian (PM), 1 pair basal (B), 1 pair apicoventral (AV), 1 pair midventral (MV), 1 pair basiventral (BV), and 1 pair posterolateral (PL). Capsule of Haller's organ: rounded and without reticulations. Coxa I, II, and III with one ventral spur projecting towards the trochanter. Each coxa provided with two ventral setae.

3.2.2. Female (Fig. 4)

Body: Body flat and oval , 5.9 ± 0.6 (5.3–6.7) long, 3.5 ± 0.3 (3.2–3.9) wide. Posterior margin rounded, lateral margins gently curved along the idiosoma and anteriorly converging to a slightly pointed apex. Dorsum: integument covered by a cell-like configuration of mammillae, slightly larger in marginal regions than in the center of the body. Some mammillae provided of a little seta. Middle of the idiosoma with numerous discs below the level of mammillae arranged in groups, not present at the margins. Lateral suture absent. Venter: Mammillae covering the ventral surface with few discs only present in postcoxal grooves. Genital area located between posterior ends of coxae I, anterior labia larger than posterior. Anus located posteriorly to coxae IV and surrounded with a smooth mammillated anal ring; preanal groove present; median mammillated depression reaching the postanal groove. Spiracular plate small and above coxa IV.

Capitulum: Capitulum situated ventrally to the hood in a well-defined camerostome. Cheeks movable, large and elongate, covering palps, hypostome and mouth parts. Basis capituli covered by irregular transverse wrinkles and micro-mammillated, 0.180 ± 0.015 (0.155–0.196) long, 0.232 ± 0.010 (0.218–0.243) wide, with one pair of long posthypostomal setae, one pair of postpalpal setae approximately third of the size of the posthypostomal setae. Palps free, article I micro-mammillated and with a smooth knife-edge flange in its median side. Hypostome rounded apically,

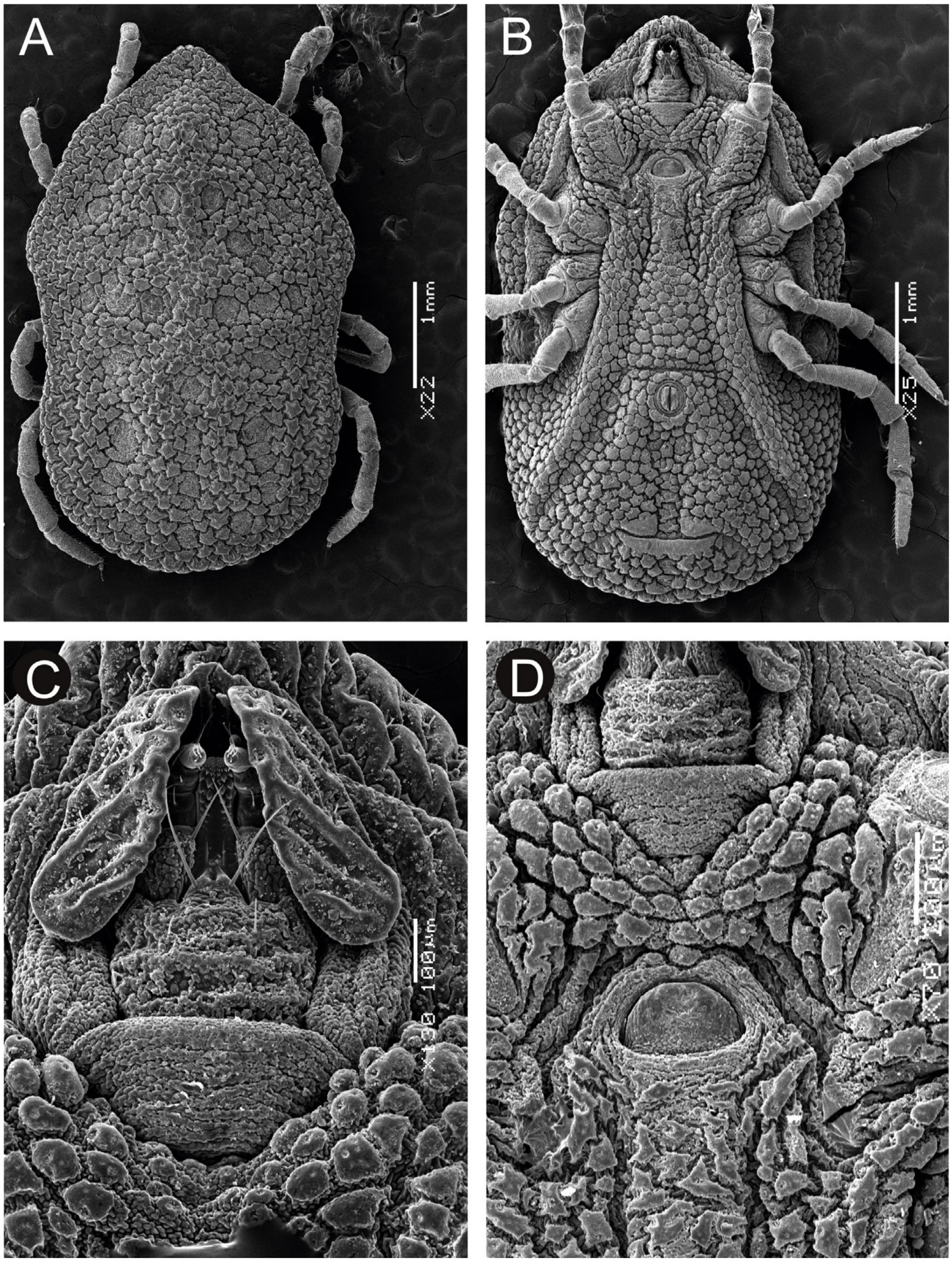

Fig. 5. *Ornithodoros atacamensis* n. sp., scanning electron microscopy of the male. (A) Dorsal idiosoma. (B) Ventral idiosoma. (C) Ventral capitulum. (D) Genital opening.

with dental formula 2/2; small crenulations apically, anterior portion with two denticles in each row and then smaller denticles toward the base. Hood small.

Legs: long, entirely micro-mammillated with exception for coxae I–IV, which are partially mammillated (coxa IV almost unmammillated). Coxae I and II separated by a mammillated integument portion; coxae II to IV contiguous. Tarsi narrow without humps and protuberances, provided with stout bifid claws. Pulvilli present.

3.2.3. Male *(Fig. 5)*

Similar to the female except in the genital area. Body 5.0 ± 0.2 (4.6–5.4) long, 3.0 ± 0.2 (2.8–3.3) wide. Basis capituli 0.177 ± 0.012 (0.162–0.204) long, 0.215 ± 0.008 (0.205–0.235) wide.

Type host: *Liolaemus bisignatus* (Philippi 1860) (Squamata: Liolaemidae).

Type locality: Pan de Azúcar National Park (26°17′S; 70°38′W), Atacama Region, Chile.

Type specimens: Holotype larva mounted in a slide, allotype larva and two paratype larvae mounted in separated slides, host: *L. bisignatus*, Pan de Azúcar National Park, Atacama Region, Chile, coll. S. Muñoz-Leal and D. González-Acuña, December 8, 2014, deposited in USNTC, Georgia Southern University, Statesboro, USA (USNMENT 00862222, 00862223, 00862890, 00862891, respectively).

Paratypes: Ten larvae in 70% ethanol, one female and one male, same locality, collectors and data of the holotype, deposited in USNTC (USNMENT 00862892, 00862893). Ten larvae in 70% ethanol, same host, locality, collectors and data for the holotype deposited in INTA, Rafaela, Argentina (INTA 2306). Ten larvae in 70% ethanol, same host, locality, collectors and data of the holotype, deposited in the tick collection of the Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile (CLZCh-

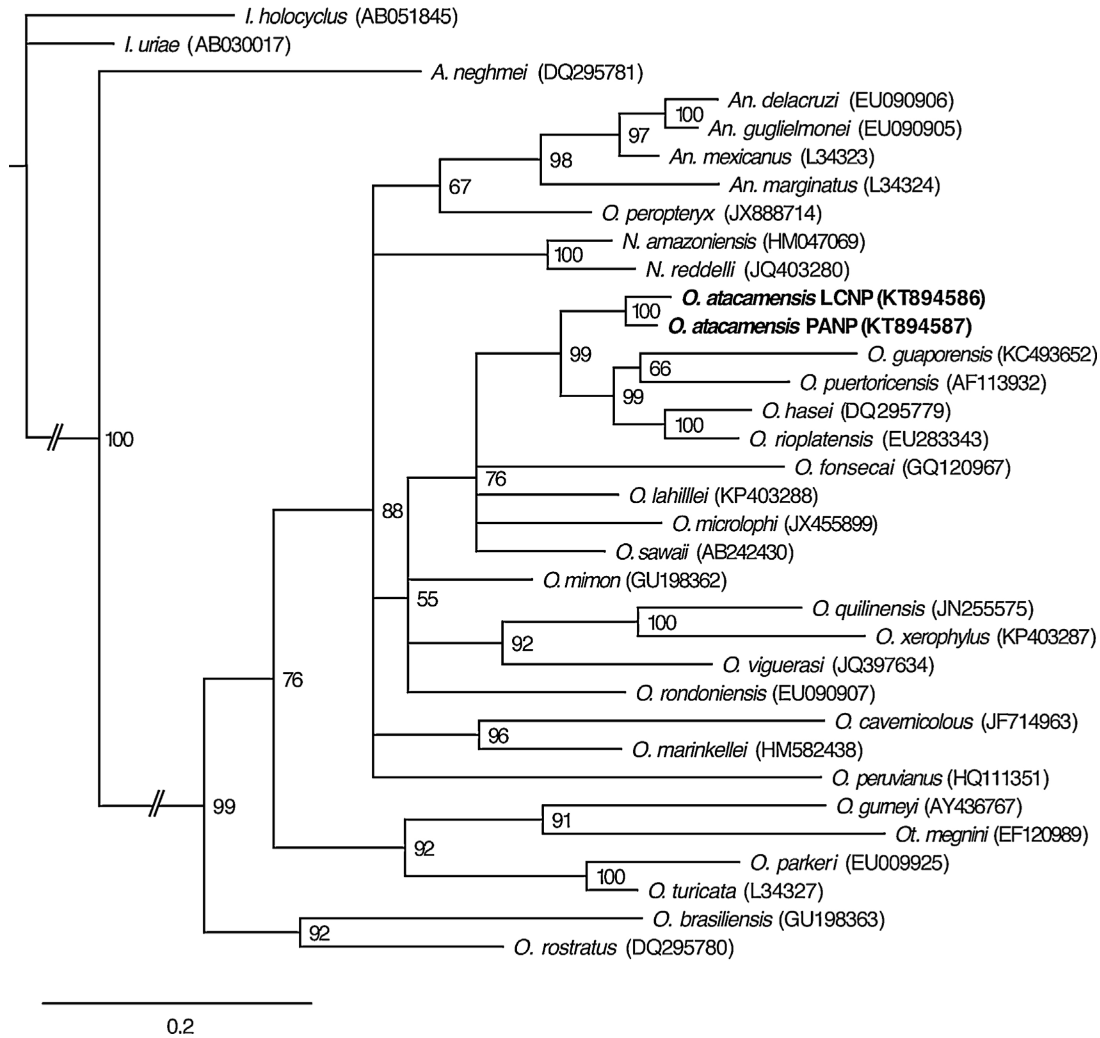

Fig. 6. Bayesian tree inferred from mitochondrial 16S rDNA partial sequences. Numbers represent the Bayesian Posterior Probability (%). Abbreviations: *An.*, *Antricola*; *Ar.*, *Argas*; *N., Nothoaspis*; *O., Ornithodoros*; *Ot., Otobius*. GenBank accession numbers of each sequence are indicated in parenthesis. *Ornithodoros atacamensis* is indicated in bold.

UdeC 67-1–67-10). Ten larvae mounted in slides, two females and one male in 70% ethanol, same host, locality, collectors and data of the holotype, deposited in DPVURU, Uruguay (DPVURU—878). Three larvae mounted in slides, one female and three males in 70% ethanol, same host, locality, collectors and data of the holotype, deposited in the "Coleção Nacional de Carrapatos" (CNC), University of São Paulo, São Paulo, Brazil (CNC-3256,—3262). Two larvae mounted in slides, host: *Callopistes maculatus* Gravenhorst 1838 (Squamata: Teiidae). Las Chinchillas National Reserve (31°280S, 71°030W), Coquimbo Region, Chile, coll. S. Muñoz-Leal and D. González-Acuña, December 12, 2010, deposited in DPVURU, Uruguay (DPVURU – 878).

Etymology: the species is named for the type locality, which is situated in the core of Atacama Desert.

3.3. Analysis of mitochondrial 16S rDNA sequences and PCA

DNA from the female and larvae from PANP yielded identical 16S rDNA sequences (GenBank KT894587). On the other hand, the sequence of the larva collected in LCNP (GenBank KT894586) was 95.7% (408/426-bp) identical to the PANP sequence. The Bayesian tree inferred from mitochondrial 16S rDNA sequences of some Neotropical Argasidae are presented in Fig. 6. The Both haplotypes of *O. atacamensis* formed a single clade (bootstrap 100%) grouping within a larger clade (99% bootstrap) composed by *O. puertoricensis, O. guaporensis, O. rioplatensis,* and *O. hasei.* Pairwise differences of these species with *O. atacamensis* from PANP and LCNP sequences were respectively as follow: 10.3/12.3% with *O. rioplatensis,* 13.8/14.3% with *O. hasei*, 12.4/14.2% with *O. puertoricensis,* and 15.3/17.3% with *O. guaporensis*. For the rest of the *Ornithodoros* spp. included in the phylogenetic analysis the difference was higher than 12.4%.

The results of the PCA for the morphological characters showed a clear difference between *O. atacamensis* and the remaining species included in the analysis (Fig. 7). The first principal component, which in this case explains the 52.24% of the total variance, is almost fully loaded with the following characters: central setae, circumanal setae and hypostome length. On the other hand the second component explains a 27.82% of the total variance, and heavier characters are represented by dorsal plate width and length, postcoxal setae and hypostome width.

4. Discussion

4.1. Species relationships

Larvae of *O. atacamensis* are phenotypically closely related to Neotropical ticks of the *O. talaje* species group: *O. puertoricensis*, *O. rioplatensis*, *O. talaje* s. s., *O. guaporensis* and *O. hasei* (Venzal et al., 2008; Nava et al., 2013). However, larvae of *O. atacamensis* and *O.*

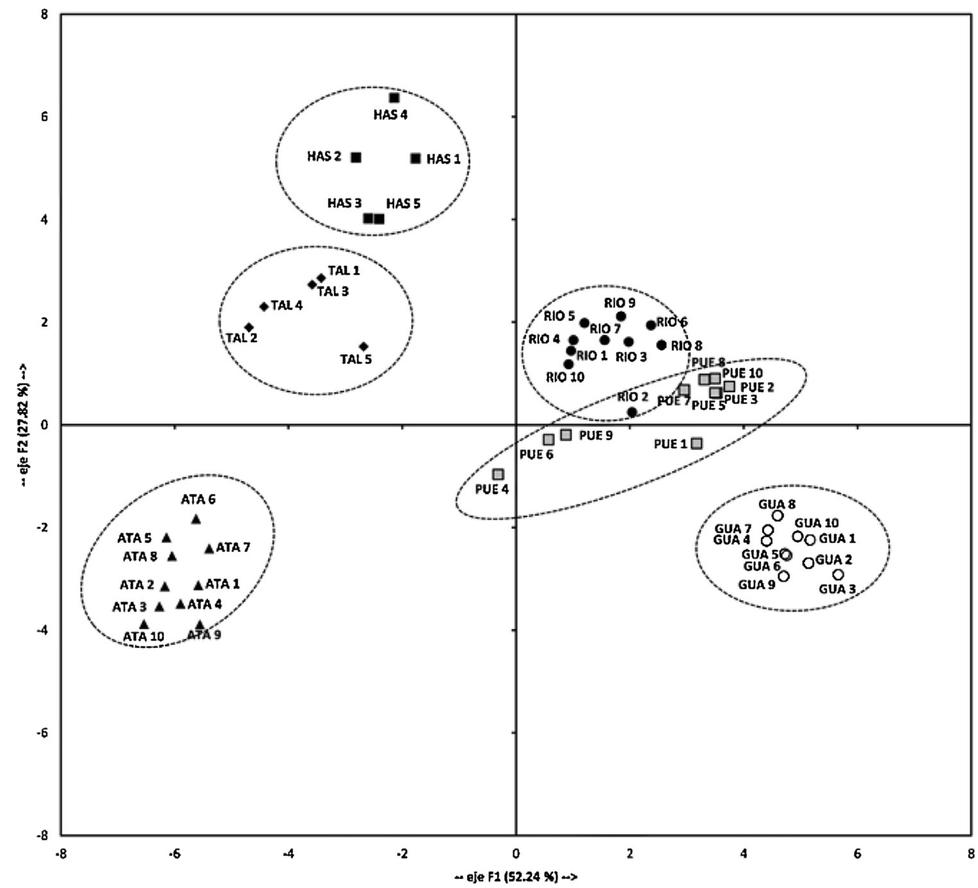

Fig. 7. Principal components analysis using 41 morphological characters of *O. atacamensis* (ATA 1–10), *O. guaporensis* (GUA 1–10), *O. talaje* (TAL 1–5), *O. rioplatensis* (RIO 1–10), *O. puertoricensis* (PUE 1–10) and *O. hasei* (HAS 1–5) using the features detailed in Table 1. Each point constitutes the position of each measured specimen on the reduced space.

talaje s. s. can be separated from *O. guaporensis*, *O. rioplatensis*, *O. puertoricensis* and *O. hasei* by the total number of dorsal setae: 17 in *O. atacamensis* and *O. talaje*, 18 in *O. puertoricensis*, 19 in *O. hasei,* and 20 in *O. guaporensis* and *O. rioplatensis*. Larvae of *O. atacamensis* can be differentiated from *O. talaje* s. s. by the three following characters: body size of the unengorged larvae of *O. atacamensis* (0.761–0.834 mm long and 0.438–0.499 mm wide) larger and wider than *O. talaje* s. s. (0.438–0.499 mm long and 0.325–0.395 mm wide); dorsal plate longer in *O. atacamensis* (0.238–0.318 mm), and for the presence of five pairs of central setae on the dorsal idiosoma in *O. atacamensis* rather than four in *O. talaje* s. s. These morphological and morphometrical differences were supported by the PCA analysis.

Ornithodoros atacamensis should also be compared with *Ornithodoros microlophi* Venzal et al., 2013 and *Ornithodoros lahillei* Venzal et al., 2015, two soft ticks associated with reptiles (at least larval stages) occurring in the same localities but not in the same hosts from which *O. atacamensis* larvae were collected (Venzal et al., 2013, 2015). Larvae of *O. microlophi* can be easily differentiated from *O. atacamensis* by the presence of six pairs of sternal setae, and from larvae of *O. lahillei* by the absence of the postcoxal setal pair.

Adults of *O. atacamensis* are phenotypically similar to mature stages of other representatives of the *O. talaje* species group, and according to Venzal et al. (2008), their morphological characters are not suitable to separate the species of this complex. However, by the comparison of unengorged female specimens, *O. atacamensis* can be clearly differentiated, since it lacks thick lateral margins anteriorly converging in a sharped angular apex, a morphological feature that can be notably recognized in other representatives of the group such as *O. guaporensis*, *O. puertoricensis* and *O. rioplatensis* (Endris et al., 1989; Venzal et al., 2008; Nava et al., 2013) (Fig. 8). Instead of these characteristics, the idiosoma of *O. atacamensis* presents thin lateral margins slightly raised and converging in a slightly pointed apex. Adults of *O. talaje* s. s. are similar to *O. atacamensis*, as both ticks share an oval body, lacking of an abrupt pointed apex in the anterior portion. However, *O. talaje* s. s. has an idiosoma with a flattened posterior margin (Cooley and Kohls, 1994), which, in contrast, is rounded in *O. atacamensis*. On the other hand, *O. hasei* has also been recognized as another Neotropical representative of the *O. talaje* species group (Nava et al., 2013) and in this way it also should be compared with *O. atacamensis*. The description of mature stages of *O. hasei* was presented by Matheson (1935), including drawings of the hypostome, palpi, tarsi, chelicerae and a sole picture of an adult of unspecified sex. Although the original description is explicative, it is difficult to associate the described morphological characters, particularly those of the dorsal and ventral idiosoma with the available photograph of the *O. hasei* adult. Consequently, making a reliable comparison of these characters with *O. atacamensis* is hardly possible. However, a difference between the two species can be easily observed based on morphometric data: adults of *O. hasei* are smaller (3.4 mm long—2 mm wide) than *O. atacamensis* mature stages (female: 5.3–6.7 mm long, 3.2–3.9 mm wide; male: 4.6–5.4 mm long, 2.8–3.3 wide).

In order to gain a better understanding of the morphological relationships within *the O. talaje* species group, future studies should consider the redescription of *O. hasei* and *O. talaje* s. s. adults.

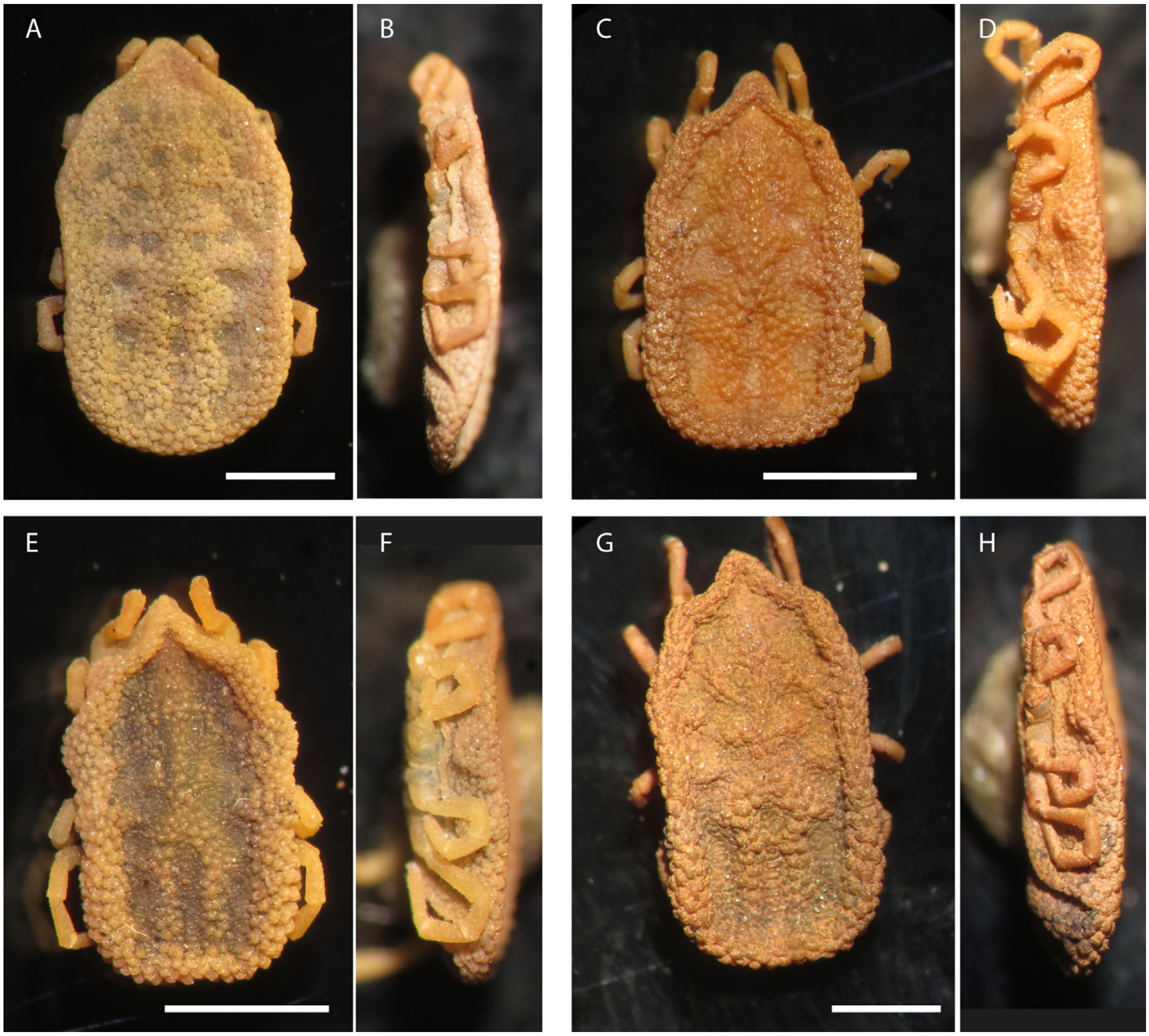

Fig. 8. Dorsal and lateral views of adult females of *Ornithodoros atacamensis* (A and B), *O. guaporensis* (C and D), *O. puertoricensis* (E and F) and *O. rioplatensis* (G and H). Scale bar of all the images is equivalent to 2 mm.

4.2. Phylogenetic analyses

Morphological similarities of *O. atacamensis* with the *O. talaje* species group were also corroborated by phylogenetic analyses, in which the mitochondrial 16S rDNA sequence of *O. atacamensis* grouped basal to a clade composed by corresponding sequences of the *O. talaje* species group, with the exception of *O. talaje* s. s., for which no sequence of 16S rDNA is available.

Larvae of *O. atacamensis* from PANP and LCNP are morphologically identical; however, their mitochondrial 16S rRNA sequences were 4.3% divergent. Both tick populations (PANP and LCNP) are separated by a large area of the coastal Atacama Desert (≈215 km), and considering that their hosts (small reptiles) have low vagility (Fox and Shipman, 2003), the encounter between them is likely impossible to occur. In this sense, the lack of gene flow between isolated populations of *O. atacamensis* might be leading to the creation of unique haplotypes of mitochondrial 16S rRNA gene in each population. Although with the evidence of the present study this is an *a priori* conclusion, analyses performed in other congeneric ticks, inhabiting similar dry environments, have shown a high genetic variability in mitochondrial genes. This is the case of populations of *Ornithodoros sonrai* species group in desert areas of Western Africa, in which mitochondrial 16S rRNA gene haplotypes can reach up to 15% of genetic divergence (Vial et al., 2006). Considering the ecologic scenario where it occurs, the morphological similarities and observed genetic divergence between its populations, *O. atacamensis* it is likely to be a complex of sibling species. However, studies including the analysis of more genes and specimens from other localities, such as LCNR, are needed to prove this hypothesis. Until more evidence does not exist, the two analysed tick populations, PANP and LCNP, should be considered as one species.

4.3. Biological observations

The larva of *O. atacamensis* was collected in three different arid environments of northern Chile (PANP, LCNP and LCNR), always in association with reptiles. Other vertebrates, such as rodents and birds, were also captured and examined for ticks in these same three localities and none of them was parasitized by *O. atacamensis*. Therefore, we suggest that at least larval stages of this new species of soft tick might be mainly associated with reptiles. Adults of *O. atacamensis* were also found in reptile-frequented rock crevices in PNPA, which reinforce our proposition about host specificity. In addition, in the laboratory we were unable to feed immature stages on rabbits, mice, guinea pigs and chickens. Finally, if we consider the geographic distribution of the reptiles parasitized with *O. atacamensis*, then this soft tick could be occurring within a larger latitudinal range, towards the north and also to the south of the localities included in this study.

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