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Original article

A new species of *Ornithodoros* (Acari: Argasidae), parasite of *Microlophus* spp. (Reptilia: Tropiduridae) from northern Chile

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ABSTRACT

A new species, *Ornithodoros microlophi* (Acari: Argasidae), belonging to the subgenus *Alectorobius* is described from larvae collected on the lizards *Microlophus atacamensis* (Donoso-Barros, 1966) and *Microlophus quadrivittatus* (Tschudi, 1845) (Squamata: Tropiduridae) in continental and insular localities from northern Chile. Larvae of *O. microlophi* can be distinguished from other Neotropical species of the genus *Ornithodoros* by a combination of the following characters, namely 10 pairs of ventral setae, venter with 6 pairs of sternal setae, dorsal plate pyriform, 19–21 pairs of dorsal setae (typically 20), 13 pairs are dorsolateral and 7 pairs are central, and hypostome with dental formula 4/4 in medial portion and apex pointed. Phylogenetic analysis of 16S rDNA sequences suggests that *O. microlophi* represents an independent lineage within Neotropical species of the Argasidae.

Introduction

The suborder Ixodida is represented in the Neotropical Region by 116 species of hard ticks (Ixodidae) (Guglielmone et al., 2003; Labruna et al., 2005; Barros-Battesti et al., 2007; Nava et al., 2009) and 84 species of soft ticks (Argasidae) (Guglielmone et al., 2003; Labruna and Venzal, 2009; Nava et al., 2010; Dantas-Torres et al., 2012; Venzal et al., 2012a). Particularly in Chile, 20 species of ticks were confirmed to be present in this country, 13 belonging to the family Ixodidae and 7 are grouped in the family Argasidae (González Acuña and Guglielmone, 2005; Marín-Vial et al., 2007; González Acuña et al., 2008; Guglielmone et al., 2010a; Abarca et al., 2012; Venzal et al., 2012b).

At present, the soft ticks distributed in Chile are *Argas keiransi* Estrada-Peña, Venzal and González-Acuña, 2003, *Argas neghmei* Kohls and Hoogstraal, 1961, *Ornithodoros amblus* Chamberlin, 1920, *Ornithodoros spheniscus* Hoogstraal, Wassef, Hays and Keirans, 1985, *Ornithodoros rioplatensis* Venzal, Estrada-Peña and Mangold, 2008, *Ornithodoros* cf. *O. peruvianus* Kohls, Clifford and Jones, 1969, and *Otobius megnini* (Dugés, 1883) (González-Acuña and Guglielmone, 2005; González-Acuña et al., 2008; Venzal et al., 2008, 2012b). With the exception of *O. megnini*, *Ornithodoros* cf. *O. peruvianus* and *O. rioplatensis*, which are parasites of mammals, the remaining species were recorded on birds. But, during a study of ectoparasites associated with small lizards in different localities of Chile, specimens of the genus *Ornithodoros* belonging to a new species were collected. Therefore, the objective of this work was to carry out the formal description of this new species. Additionally, 16S rDNA sequences were used to determine the phylogenetic position of this tick with respect to other Neotropical species of Argasidae.

Materials and methods

The ticks used in this study were larvae of the family Argasidae collected on the Atacamen Pacific Iguana *Microlophus atacamensis* (Donoso-Barros, 1966) (Squamata: Tropiduridae) in Pan de Azúcar National Park (mainland; 26°08′S, 70°39′W; Atacama Region), Pan de Azúcar National Park (island; 26°09′S, 70°41′W; Atacama Region), Santa María Island (23°26′S, 70°35′W; Antofagasta Region), and on the Four-banded Pacific Iguana *Microlophus quadrivittatus* (Tschudi, 1845) in Caleta Vitor (18°45′S, 70°20′W; Arica and Parinacota Region) (Fig. 1). Larvae were cleared in 20% aqueous solution of potassium hydroxide and mounted in Hoyer's medium to create semi-permanent slides for light microscopy and measured using a Nikon Eclipse E200 optical microscope. The measurements (mean, standard deviation, and range) were taken in micrometers. Larval chaetotaxic terminology followed Venzal et al.

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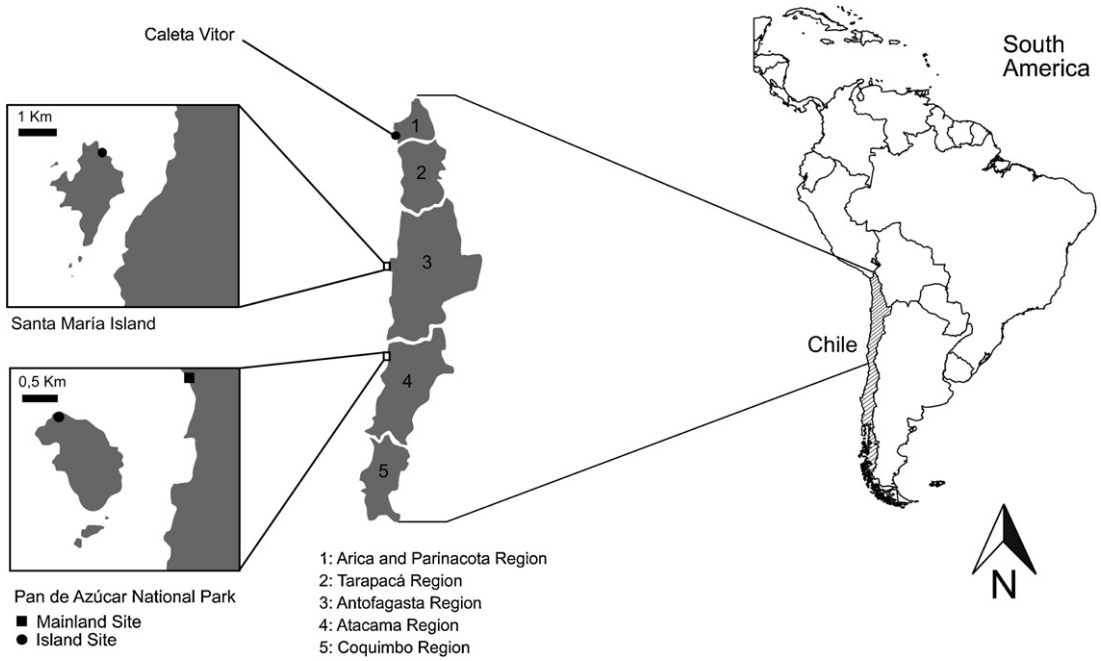

Fig. 1. Map of Chile showing the collection sites of ticks.

(2008) and Labruna et al. (2011). The detail of the material examined is as follows: one larva ex *M. atacamensis* in Pan de Azúcar National Park (mainland), April 1, 2011; 3 larvae ex *M. atacamensis* in the same locality, March 23, 2011; 3 larvae ex *M. atacamensis* in Pan de Azúcar National Park (island), December 17, 2010; 2 larvae ex *M. atacamensis* in Isla Santa María, May 15, 2011; 3 larvae ex *M. quadrivittatus* in Caleta Vitor, June 3, 2010.

Two larvae ex *M. atacamensis* in Pan de Azúcar National Park mainland and island, and one larva ex *M. quadrivittatus* in Caleta Vitor, were used for DNA extraction. A polymerase chain reaction was performed to amplify an approximately 400-bp fragment of the mitochondrial 16S rDNA gene using the primers designed by Mangold et al. (1998). The sequences were compared among each other and with the sequences of the *Ornithodoros* species deposited in GenBank. The alignment was made with the CLUSTAL W program (Thompson et al., 1994). Phylogenetic analysis with Maximum-likelihood (ML) and maximum parsimony (MP) methods were performed with the program Mega 5.0 (Tamura et al., 2011). The ML tree was generated with the GTR model by using a discrete Gamma-distribution (+G). Best fitting substitution models were determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 5. In the MP analysis, the heuristic search procedure was chosen with close neighbor-interchange (CNI) at search level 3, random addition of taxa (10 replicates), and gaps were excluded. Support for the topologies was tested by bootstrapping over 1000 replications. Sequences of *A. neghmei* and *A. keiransi* were considered as outgroups. In this work, we followed the tick classification presented by Guglielmone et al. (2010b).

Results

Description (all measurements in μm)

Ornithodoros microlophi Venzal, Nava and González-Acuña, sp. n. (Figs. 2 and 3)

Larva (n = 12 measured)

Body: Length including capitulum 676, length without capitulum 549, width 470 (measurements of body based on slightly engorged specimen). Dorsum: Dorsal plate pyriform, length 295 ± 10 (284–313), width: 217 ± 12 (196–245). Dorsal surface provided with 19–21 pairs of setae, typically 20, 13–14 dorsolateral setae, typically 13 (7 anterolateral setae and 6 posterolateral setae) and 6–8 central setae, typically 7. Anterolateral setae (Al): Al_1 length 78.5 ± 4 (73–85), Al_2 length 73 ± 4.5 (63–77), Al_3 length 73 ± 4.5 (66–83), Al_4 length 75 ± 4.5 (68–85), Al_5 length 74 ± 4.5 (68–79), Al_6 length 79 ± 5.5 (73–88), Al_7 length 85 ± 4 (79–90). Posterolateral setae (Pl): Pl_1 length 73 ± 2 (71–77), Pl_2 length 73 ± 4.5 (68–83), Pl_3 length 74 ± 4 (68–85), Pl_4 length 72 ± 3 (66–77), Pl_5 length 69 ± 5 (61–83), Pl_6 length 67 ± 4 (63–73). Central setae (C): C_1 length 73 ± 6 (63–83), C_2 length 70 ± 5 (61–77), C_3 length 66 ± 4 (62–73), C_4 length 68 ± 4 (63–75), C_5 length 67 ± 4.5 (61–75), C_6 length 71 ± 4 (61–78), C_7 length 72 ± 3 (66–75).

Venter: 10 pairs of setae plus 1 pair on anal valves and 1 posteromedian seta (PMS) present. Six pairs of sternal setae (St): St_1 length 55 ± 6 (44–63), St_2 length 53 ± 4 (46–61), St_3 length 50 ± 3 (44–53), St_4 length 47 ± 3 (44–53), St_5 length 49 ± 4 (44–56), St_6 length 51 ± 4 (44–61). One pair of postcoxal setae (Pc) length 47 ± 4 (41–53); 3 pairs of circumanal setae (Ca): Ca_1 length 58 ± 5 (51–68), Ca_2 length 61 ± 8 (46–73), Ca_3 length 84 ± 8 (73–95); posteromedian setae (PM) length 53 ± 6 (41–63).

Capitulum: Basis capituli pentagonal, posterior margin straight, length from posterior margin of basis capituli to first pair of posthypostomal setae: Ph_1 145 ± 5.5 (136–156), length from posterior margin of basis capituli to insertion of hypostome 161 ± 5 (153–171), width 198 ± 6 (183–210). Two pairs of posthypostomal setae; Ph_1 length 22 ± 0.5 (22–24), Ph_2 length 28 ± 2 (27–32), distance between Ph_1 setae 27 ± 2 (24–32), distance between Ph_2 setae 80 ± 5 (73–85). Palpi, segmental length/width from I–IV: (I) 55 ± 4 (51–61)/31 ± 2.5 (27–36), (II) 99 ± 4 (97–110)/37 ± 2 (34–41), (III) 96 ± 2.5 (90–100)/34 ± 1 (34–36), (IV) 43 ± 3 (39–49)/22 ± 1 (22–24). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9. Hypostome: Length from Ph_1 to apex 244 ± 7 (232–256), length from insertion of hypostome in basis capituli to apex 226 ± 8 (217–239), width in medial portion

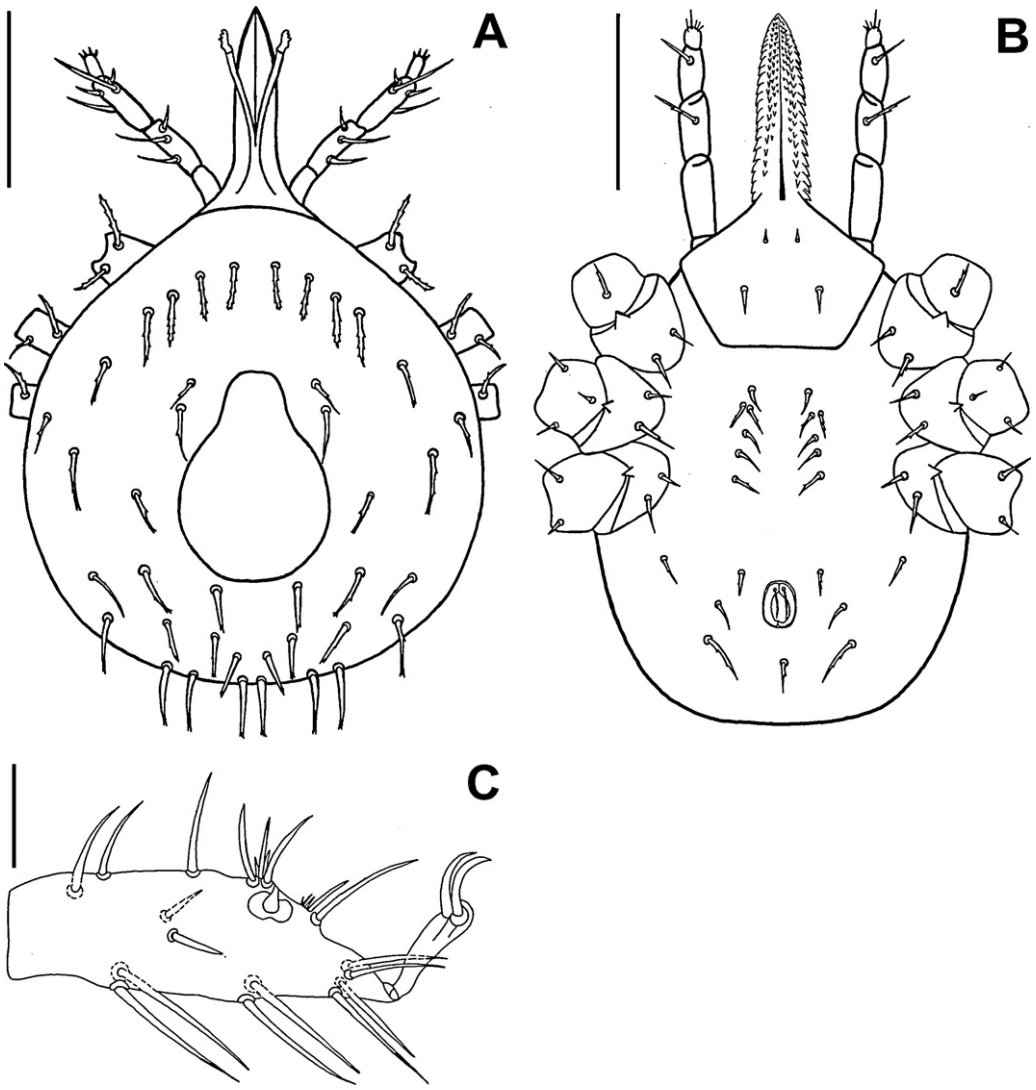

Fig. 2. *Ornithodoros microlophi* sp. nov., drawing of larva. (A) Dorsal (bar: 250 μm). (B) Ventral (bar: 225 μm). (C) Tarsus I (bar: 50 μm).

of hypostome 60 ± 5 (53–68), width in basis portion of hypostome 60 ± 5 (51–68), apex pointed. Dentition formula 2/2–3/3 apical, 4/4 (3/3 in some specimen) median, and 2/2 basal.

File 1 with 24–26 (typically 24–25) denticles, file 2 with 22–25 denticles (typically 23), file 3 with 16–18 (typically 17) denticles, file 4 with 1–12 denticles, variable.

Legs: Tarsus I length 198 ± 7 (183–207), tarsus I width 62 ± 3 (58–68). 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 without reticulations.

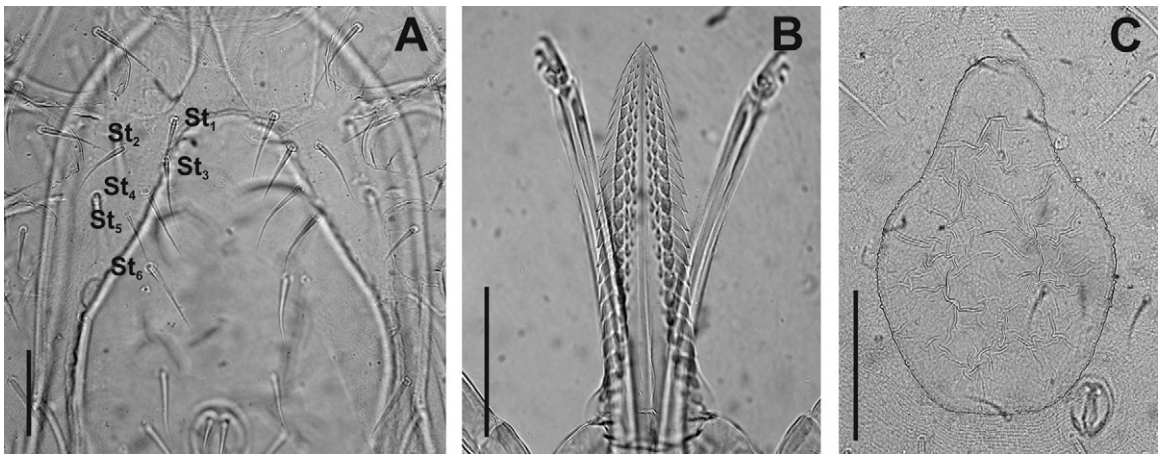

Fig. 3. *Ornithodoros microlophi* sp. nov., optical micrograph of the larva. (A) Sternal setae: St_1 to St_6 (bar: 60 μm). (B) Hypostome (bar: 100 μm). (C) Dorsal plate (bar: 100 μm).

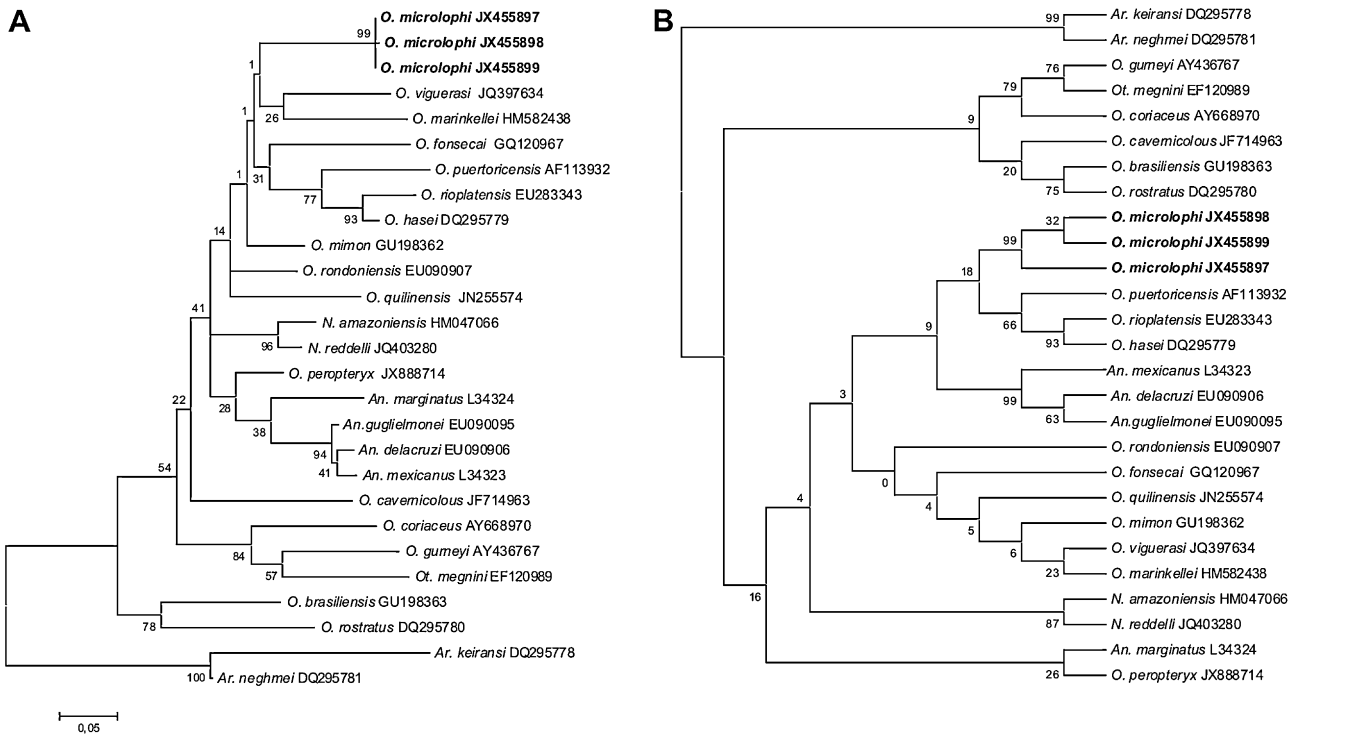

Fig. 4. Maximum-likelihood (A) and maximum parsimony (B) trees based on 16S rDNA partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are also indicated. *An.*, *Antricola*; *O.*, *Ornithodoros*; *N.*, *Nothoaspis*; *Ot.*, *Otobius*; *Ar.*, *Argas*.

Taxonomic summary

Type host
Microlophus atacamensis (Donoso-Barros, 1966).

Type locality
Pan de Azúcar National Park (mainland) (26°08′S, 70°39′W), Atacama Region, Chile.

Type specimens

Holotype: larva mounted in slide, host: *M. atacamensis*, Pan de Azúcar National Park (mainland) (26°08′S, 70°39′W), Atacama Region, coll. D. González-Acuña and S. Muñoz-Leal, May 1, 2010, deposited in the U.S. National Tick Collection (USNTC), Georgia Southern University, Statesboro, U.S.A. (USNMENT00714492).

Paratypes: 7 larvae in 70% ethanol, same host, locality, and collectors, March 25, 2011 (USNMENT00714449). Seven larvae: 3 in slide and 4 in 70% ethanol, same host, locality, and collectors, March 25, 2011, deposited in the tick collection of the Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Regional Norte, Salto, Uruguay (DPVURU 788). Two larvae in slide, host: *M. quadrivittatus*, Caleta Vitor (18°45′S, 70°20′W), Arica and Parinacota Region, Chile, same collectors, June 3, 2011 (DPVURU 789); other 2 larvae in slide same data (DPVURU 790). Six larvae in slide, host: *M. atacamensis*, Pan de Azúcar National Park (island) (26°09′S, 70°41′W), Atacama Region, Chile, same collectors, December 17, 2010, deposited in the tick collection of the Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile (FVCH-Ix-63); 3 larvae: one in slide and 2 in 70% ethanol, same data (FVCH-Ix-64); 5 larvae: one in slide and 4 in 70% ethanol, same data (FVCH-Ix-65); 8 larvae: 6 in slide and 2 in 70% ethanol, same data (FVCH-Ix-66). Four larvae in slide, host: *M. atacamensis*, Santa María Island (23°26′S, 70°35′W), Antofagasta Region, Chile, same collectors, May 15, 2011 (FVCH-Ix-67). Three larvae: one in slide and 2 in 70% ethanol, host: *M. atacamensis*, Pan de Azúcar National Park (island), same collectors, December 17, 2010, deposited in the tick collection of INTA Rafaela, Santa Fe, Argentina (INTA 2192).

Etymology

The specific epithet '*microlophi*' refers to the host of the type specimens, lizards of the genus *Microlophus*.

Discussion

Species relationship

The morphological characteristics of *O. microlophi* indicate that this species belongs to the subgenus *Alectorobius* according to the definition of Clifford et al. (1964). The principal difference is the presence of 10 pairs of ventral setae in *O. microlophi*, whilst the remaining species of *Alectorobius* have 8 or 9 pairs of ventral setae. Other Neotropical species of the family Argasidae with 10 pairs of ventral setae are members of a different genus, *Nothoaspis reddelli* Keirans and Clifford, 1975, and *Nothoaspis amazoniensis* Nava, Venzal and Labruna, 2010, but the setae arrangement in these 2 taxa is different from that of *O. microlophi* (Nava et al., 2010; this work). *O. microlophi* has 10 pair of ventral setae, and 6 of them are sternal, and this is a diagnostic character for *O. microlophi*.

There are additional morphological differences between *O. microlophi* and other species of *Ornithodoros* that are parasites of lizards such as *Ornithodoros darwini* Kohls, Clifford and Hoogstraal, 1969, *Ornithodoros galapagensis* Kohls, Clifford and Hoogstraal, 1969, and *Ornithodoros cyclurae* De la Cruz, 1984. The number of dorsal setae and the shape of hypostome (rounded in *O. darwini* and *O. galapagensis*, pointed in *O. microlophi*) allow separating these species. The hosts of *O. darwini* and *O. galapagensis* are *Amblyrhynchus cristatus* Bell, 1825, *Conolophus* sp. Fitzinger, 1843, and *Conolophus subcristatus* Gray, 1831 (Squamata: Iguanidae) in Galapagos Archipelago, and *O. galapagensis* was also found in this locality on *Microlophus albemarlensis* (Baur, 1890) (Kohls et al., 1969; Keirans et al., 1980). The principal differences between *O. microlophi* and *O. cyclurae* are the number of dorsal setae and the hypostomal dentition (de la Cruz, 1984; this work).

Ornithodoros rioplatensis is a species belonging to the '*Ornithodoros talaje* group' recorded parasitizing the lizards *Liolaemus chillanensis* Müller and Hellmich, 1932, and *Phymaturus*

flagellifer (Bell, 1843) (Squamata: Tropiduridae) in Chile (Venzal et al., 2008). Hypostome dentition and the number of ventral setae are characters that clearly distinguish *O. microlophi* larvae from those of *O. rioplatensis* (Venzal et al., 2008; this work).

O. microlophi is the second species of *Ornithodoros* recorded on lizards of the genus *Microlophus* [*O. galapagensis* was previously collected on *M. albemarlensis* in the Galapagos Archipelago (Keirans et al., 1980)]. The genus *Microlophus* contains 9 species endemic to the Galapagos Archipelago and 12 species mostly confined to a linear strip of 5000 km along rain-shadowed western coastal deserts of South America (Benavides et al., 2007). Therefore, a more widely geographical distribution is expected for *O. microlophi*.

Molecular analysis

The 16S rDNA sequences obtained from the 2 larvae collected on *M. atacamensis* in Pan de Azúcar National Park mainland (sequence I) and island (sequence II) are similar, differing only in 0.26% (GenBank accession numbers JX455897 and JX455898). The genetic difference between these 2 sequences (I and II) and the sequence obtained from the larva collected in Caleta Vitor (sequence III) (GenBank accession number JX455899) is 2.1% (I–III) and 2.3% (II–III), respectively.

The distance between the 2 sites of the Pan de Azúcar National in relation to Caleta Vitor is approximately 800 km.

In the phylogenetic analysis performed with 16S rDNA sequences (Fig. 4a and b), *O. microlophi* was not related with the remaining taxa. The topologies of the 2 phylogenetic trees (ML and MP) allows concluding that *O. microlophi* is an independent lineage, at least considering the Neotropical species of the genus *Ornithodoros* included in the analysis. Thus, the evidence obtained from 16S rDNA sequences supports the conclusion reached with the comparative analysis of the morphological diagnostic characters of *O. microlophi*.

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