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Two new species of *Ornithodoros* (Ixodida; Argasidae) from the Southern Cone of South America

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Abstract Two new species of the genus *Ornithodoros* were described from larvae collected in Argentina and Chile. *Ornithodoros xerophylus* n. sp. was described from specimens collected on the small rodent *Graomys centralis* in Argentina. The diagnostic characters for this species are a combination of dorsal plate slightly oval with a length of approximately 250 μm, 16 pairs of dorsal setae, hypostome with apex rounded and dental formula 2/2 in most rows, 3/3 apically, and capsule of the Haller's organ oval in shape without reticulations. Larvae of *Ornithodoros lahillei* n. sp. were collected on the reptiles *Philodryas chamissonis* and *Callopistes maculatus* in Chile. The diagnostic characters for *O. lahillei* are a combination of dorsal plate subtriangular with margins corrugated and posterior margin convex, dorsal surface with 14 pairs of setae, absence of postcoxal setae, and hypostome with apex pointed and dental formula 3/3 in anterior third and 2/2 in the middle and basal portion. Phylogenetic analysis of 16S rDNA sequences and a Principal Component Analysis based on morphometric characters provided additional support to the description of *O. lahillei* and *O. xerophylus* as two independent lineages within the genus *Ornithodoros*.

Keywords *Ornithodoros* · New species · Argentina · Chile · Systematics

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Introduction

The tick family Argasidae contains approximately 200 species described around the world (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). In the Neotropical Zoogeographic Region, the integration of morphological and molecular data in systematic studies has resulted in an ongoing increase of the knowledge on specific richness and geographical distribution of Argasidae species. In this sense, and regarding the genus *Ornithodoros,* there are 55 species currently considered to be valid in the Neotropics (Barros-Battesti et al. 2013), and some of them were described in the last 10 years (Venzal et al. 2008, 2012, 2013; Labruna and Venzal 2009; Dantas-Torres et al. 2012; Nava et al. 2013).

Adult ticks of the genus *Ornithodoros* lack morphological characters suitable for specific diagnosis. Therefore, both morphometric and discrete phenotypic characters of larvae and DNA sequences are employed in systematic studies of *Ornithodoros* ticks. In this work two new species of the genus *Ornithodoros* from Argentina and Chile were described based on morphological features of larvae and sequences of the mitochondrial 16S rRNA gene.

Materials and methods

Ticks included in this study were obtained from localities with different ecological characteristics in Argentina and Chile. They were determined as belonging to the genus *Ornithodoros* following Kohls et al. (1965). Seventeen larvae were collected on specimens of *Graomys centralis* (Thomas) (Rodentia: Cricetidae) in Quilino (30°12′S, 64°32′W), Córdoba Province, Argentina. This locality belongs to the Western Chaqueño District located in the Chaco Phytogeographic Province according to the scheme of Cabrera (1976). In Chile, 158 *Ornithodoros* larvae were collected on *Philodryas chamissonis* (Wiegmann) (Serpentes: Colubridae) in Fray Jorge National Park (30°30′S, 71°35′W), and five larvae on *Callopistes maculatus* Gravenhorst (Sauria; Teiidae) in Las Chinchillas National Reserve (31°28′S, 71°03′W). Both sites are located in the Coquimbo Region, northern Chile.

Fifteen slightly engorged larvae of Quilino, 38 larvae of Fray Jorge National Park and five larvae of Las Chinchillas National Reserve, were mounted in Hoyer's medium to create semi-permanent slides for light microscopy. Larvae were 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. Larval chaetotaxic terminology and measures followed Venzal et al. (2008, 2013). Morphometric variables of the ticks included in this study were used to carry out a principal component analysis (PCA) based on Pearson correlation matrix, in order to perform a comparison of the specimens among each other and with other related *Ornithodoros* species from South America.

Representative specimens of the ticks collected in Quilino and Fray Jorge National Park were used for DNA extraction. Sequences of the mitochondrial 16S rRNA gene were obtained following the methodology described by Mangold et al. (1998). Each of the sequences (a ca. 400 bp fragment) 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). Phylogenetic relationships were assessed with the maximum-likelihood (ML) method and the best-fitting substitution model was determined with the Bayesian Information Criterion

by using MEGA 5 (Tamura et al. 2011). GTR model was chosen to create ML trees. Branch support was tested by bootstrap analysis using 1,000 replicates. Sequences of *Argas neghmei* Kohls and Hoogstraal and *Argas monachus* Keirans, Radovsky and Clifford were employed as outgroups. The classification scheme of Argasidae presented by Guglielmone et al. (2010) was followed in this work.

Results

Descriptions

***Ornithodoros xerophylus* n. sp. Venzal, Mangold and Nava** (Figs. 1a–c, 3a)

Larva

Body Idiosoma subcircular. Length including capitulum: 0.906 ± 0.078 (0.813–0.970), and not including capitulum: 0.734 ± 0.067 (0.656–0.800), width: 0.637 ± 0.045 (0.689–0.720). Dorsum: Dorsal plate slightly oval, length 0.243 ± 0.009 (0.232–0.260), width: 0.207 ± 0.015 (0.185–0.225). Dorsal surface with 16 pairs of setae, 7 anterolateral, three central and six posterolateral. Anterolateral setae (Al): Al_1 length 0.073 ± 0.010 (0.061–0.085), Al_2 length 0.070 ± 0.006 (0.061–0.078), Al_3 length 0.071 ± 0.004 (0.066–0.078), Al_4 length 0.070 ± 0.004 (0.061–0.075), Al_5 length 0.079 ± 0.006 (0.073–0.093), Al_6 length 0.081 ± 0.004 (0.075–0.085), Al_7 length 0.087 ± 0.011 (0.075–0.105). Central setae (C): C_1 length 0.075 ± 0.008 (0.061–0.085), C_2 length 0.087 ± 0.009 (0.068–0.097), C_3 length 0.089 ± 0.007 (0.075–0.097). Posterolateral setae (Pl): Pl_1 length 0.091 ± 0.007 (0.078–0.102), Pl_2 length 0.088 ± 0.009 (0.075–0.097), Pl_3 length 0.091 ± 0.007 (0.078–0.097), Pl_4 length 0.092 ± 0.005 (0.085–0.097), Pl_5 length 0.089 ± 0.005 (0.080–0.095), Pl_6 length 0.088 ± 0.005 (0.085–0.097). Venter: Ventral surface with seven pairs of setae plus one pair on anal valves, one posteromedian seta present. Three pairs of sternal setae (St): St_1 length 0.053 ± 0.008 (0.044–0.068), St_2 length 0.053 ± 0.004 (0.049–0.061), St_3 length 0.051 ± 0.005 (0.046–0.061); one pair of postcoxal setae (Pc) length 0.055 ± 0.007 (0.049–0.068); three pairs of circumanal setae (Ca): Ca_1 length 0.046 ± 0.006 (0.036–0.056), Ca_2 length 0.056 ± 0.003 (0.052–0.061), Ca_3 length 0.070 ± 0.004 (0.065–0.078); posteromedian setae (PM) length 0.051 ± 0.008 (0.041–0.061).

Capitulum Basis capituli outline as illustrated in Fig. 1b, posterior margin straight, length from posterior margin of basis capituli to posthypostomal setae: Ph_1 0.096 ± 0.002 (0.092–0.100), length from posterior margin of basis capituli to insertion of hypostome 0.118 ± 0.005 (0.111–0.125), length from posterior margin of basis capituli to apex of hypostome 0.280 ± 0.008 (0.268–0.290), width 0.156 ± 0.010 (0.147–0.175). Two pairs of posthypostomal setae; Ph_1 length 0.012, Ph_2 length 0.034 ± 0.004 (0.027–0.039), distance between Ph_1 setae 0.025 ± 0.004 (0.020–0.030), distance between Ph_2 setae 0.066 ± 0.004 (0.061–0.073). Palpi total length 0.202 ± 0.007 (0.190–0.210), segmental length/width from 1 to 4: (1) 0.047 ± 0.005 (0.039–0.052)/0.036 ± 0.002 (0.032–0.040), (2) 0.062 ± 0.003 (0.058–0.070)/0.037 ± 0.003 (0.035–0.045), (3) 0.060 ± 0.004 (0.053–0.065)/0.031 ± 0.002 (0.030–0.035), (4) 0.030 ± 0.003 (0.027–0.036)/0.019 ± 0.003 (0.015–0.022). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9. Hypostome: length from Ph_1 to apex 0.179 ± 0.005 (0.171–0.185), length from

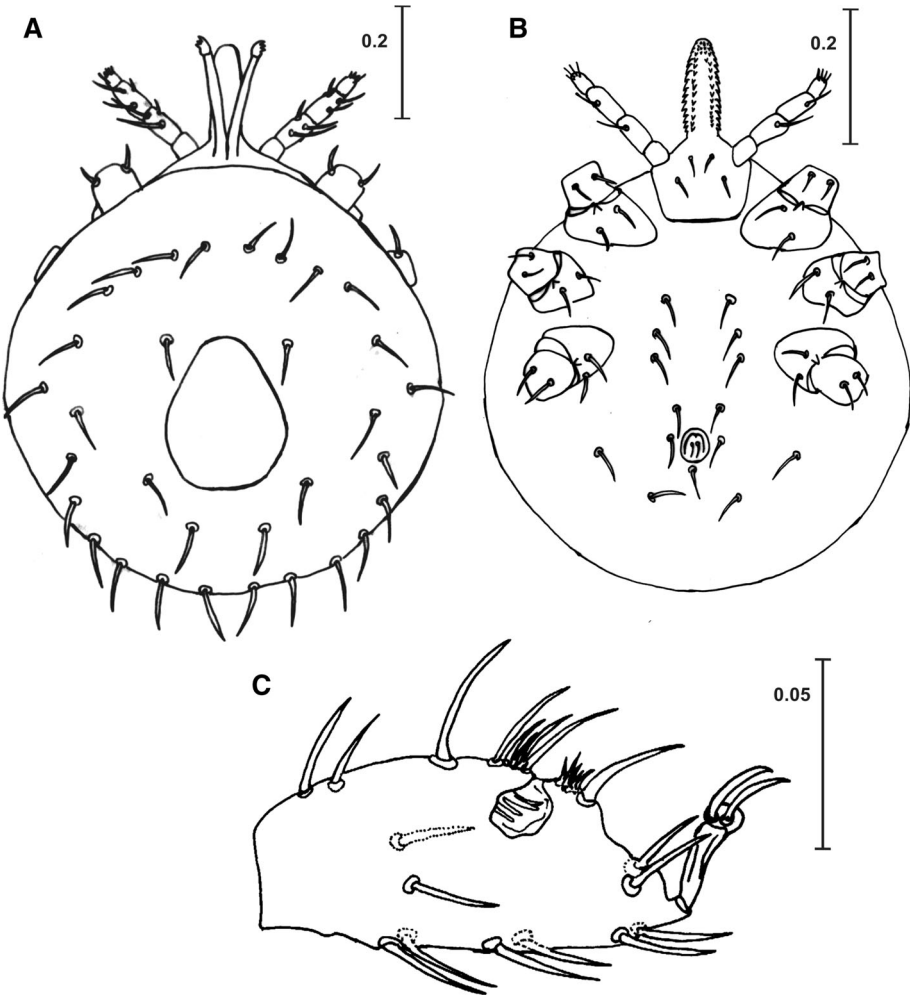

Fig. 1 *Ornithodoros xerophylus* n. sp., drawing of larva. **a** Dorsal. **b** Ventral. **c** Tarsus I. Scale in millimeters

insertion of hypostome in basis capituli to apex 0.156 ± 0.005 (0.149–0.162), width in medial basis portion of hypostome 0.048 ± 0.001 (0.045–0.050), width in basis portion of hypostome 0.047 ± 0.002 (0.045–0.050); apex rounded, dental formula 2/2 in most rows, 3/3 apically, file one with 14–16 denticles, file two with 12–13 denticles (Fig. 3a). Legs: Tarsus I length 0.144 ± 0.010 (0.130–0.160), tarsus I width 0.053 ± 0.005 (0.047–0.061). Setal formula of tarsus I: one pair apical (A), one distomedian (DM), five paracapsular (PC), one posteromedian (PM), one pair basal (B), one pair apicoventral (AV), one pair midventral (MV), one pair basiventral (BV), and 1 pair posterolateral (PL). Capsule of Haller's organ: oval in shape from dorsal view, without reticulations.

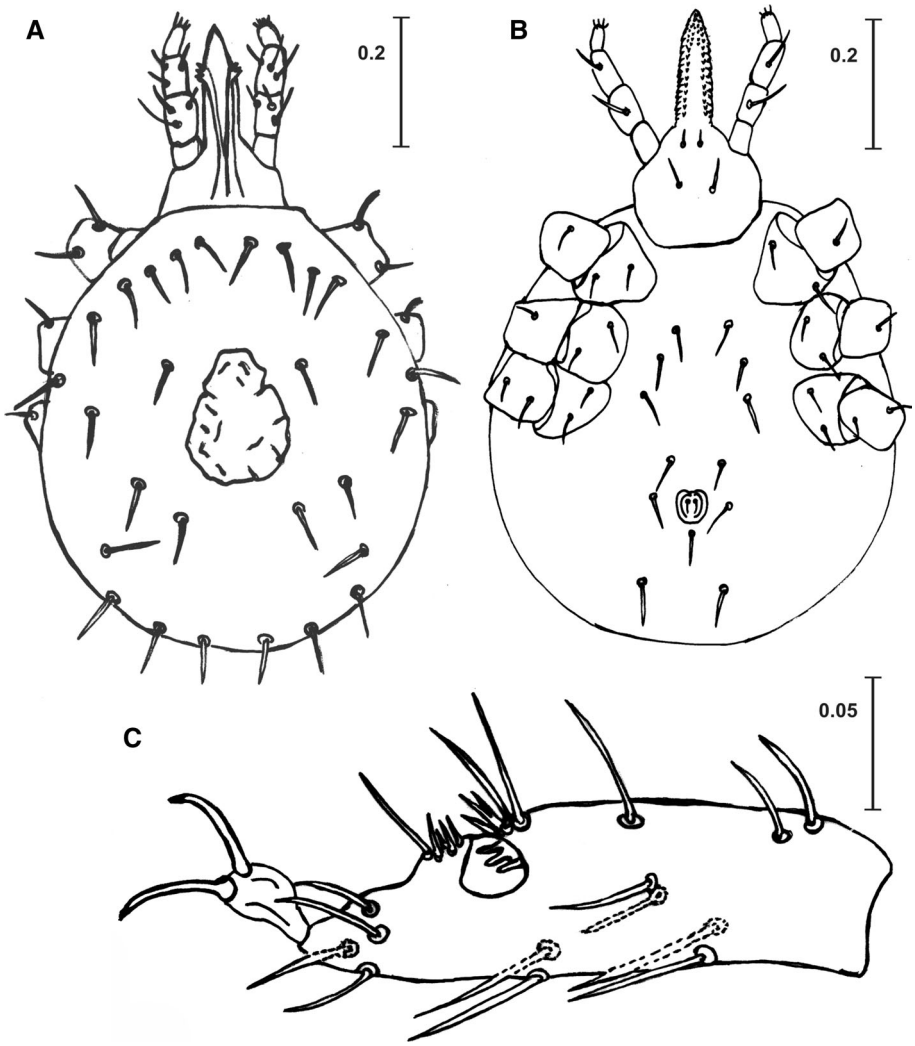

Fig. 2 *Ornithodoros lahillei* n. sp., drawing of larva. **a** Dorsal. **b** Ventral. **c** Tarsus I. Scale in millimeters

Type host *Graomys centralis* (Thomas) (Rodentia: Cricetidae).

Type locality Quilino (30°12′S, 64°32′W), Córdoba Province, Argentina.

Type specimens Holotype larva mounted in slide, host: *G. centralis*, Quilino, Córdoba Province, Argentina, coll. A.J. Mangold, S. Nava, J.M. Venzal and M. Mastropaolo, March 22, 2011, deposited in the US National Tick Collection (USNTC), Georgia Southern University, Statesboro, USA (USNMENT 00862999).

Paratypes Nine larvae mounted in slide, same host and locality, coll. S. Nava, June 18, 2006, deposited in the tick collection of INTA Rafaela, Santa Fe, Argentina (INTA 2249). Four larvae mounted in slide, same host, locality, collectors and data of INTA paratypes, deposited in the tick collection of the Departamento de Parasitología Veterinaria

(DPVURU), Facultad de Veterinaria, Universidad de la República, CENUR Noroeste, Salto, Uruguay (DPVURU 875). One larvae mounted in slide, same host, locality, collectors and data for holotype, deposited in DPVURU (DPVURU 876).

Etymology The specific epithet "xerophylus" refers to the arid environment where the type specimens were collected.

***Ornithodoros lahillei* n. sp. Venzal, González-Acuña and Nava** (Figs. 2a–c, 3b)

Larva

Body Idiosoma oval. Length including capitulum 1.023 ± 0.078 (0.931–1.166), and not including capitulum 0.774 ± 0.078 (0.666–0.931), width 0.653 ± 0.056 (0.568–0.745). Dorsum: Dorsal plate subtriangular, surface smooth in some portions with margins corrugated and posterior margin convex, length 0.207 ± 0.008 (0.195–0.219), width 0.146 ± 0.010 (0.134–0.166). Dorsal surface with 14 pairs of setae, seven anterolateral, three central and four posterolateral. Anterolateral setae (Al): Al_1 length 0.075 ± 0.003 (0.068–0.078), Al_2 length 0.073 ± 0.004 (0.066–0.080), Al_3 length 0.070 ± 0.003 (0.066–0.075), Al_4 length 0.068 ± 0.004 (0.058–0.073), Al_5 length 0.070 ± 0.003 (0.063–0.073), Al_6 length 0.066 ± 0.003 (0.061–0.071), Al_7 length 0.069 ± 0.006

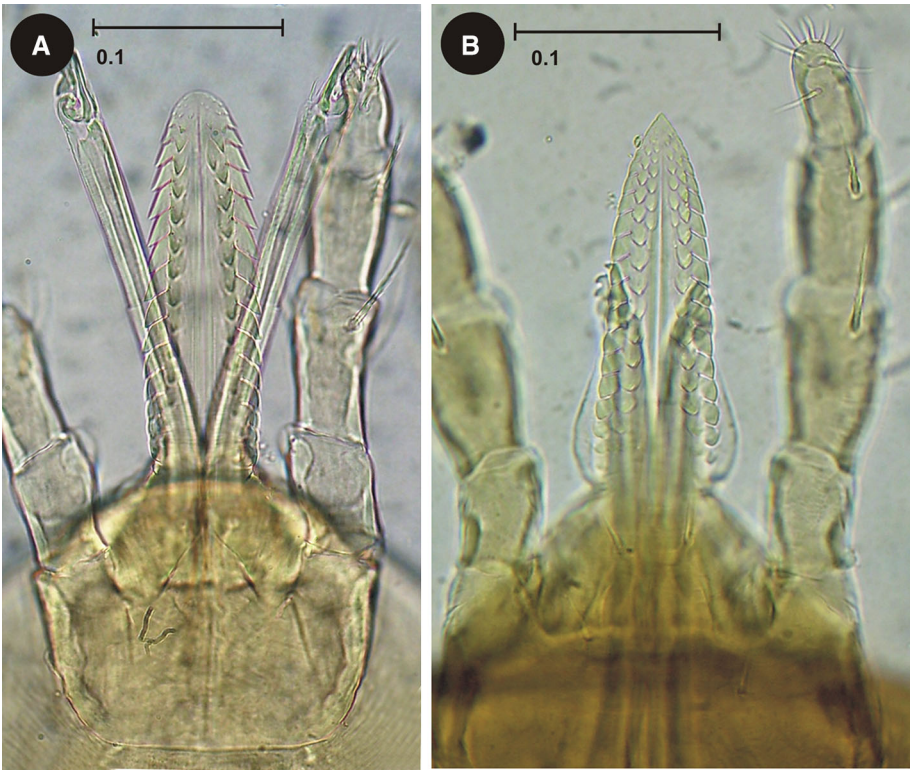

Fig. 3 *Ornithodoros xerophylus* n. sp. **a** Hypostome. *Ornithodoros lahillei* n. sp. **b** Hypostome. Scale in millimeters

(0.063–0.085). Central setae (C): C_1 length 0.068 ± 0.003 (0.063–0.073), C_2 length 0.068 ± 0.005 (0.063–0.075), C_3 length 0.073 ± 0.004 (0.066–0.078). Posterolateral setae (Pl): Pl_1 length 0.077 ± 0.005 (0.066–0.085), Pl_2 length 0.077 ± 0.007 (0.066–0.085), Pl_3 length 0.079 ± 0.007 (0.066–0.085), Pl_4 length 0.074 ± 0.006 (0.061–0.080). Venter: Ventral surface with six pairs of setae plus one pair on anal valves, one posteromedian seta present. Three pairs of sternal setae (St): St_1 length 0.041 ± 0.003 (0.036–0.046), St_2 length 0.038 ± 0.004 (0.034–0.049), St_3 length 0.038 ± 0.002 (0.036–0.041); three pairs of circumanal setae (Ca): Ca_1 length 0.030 ± 0.003 (0.027–0.034), Ca_2 length 0.048 ± 0.003 (0.044–0.053), Ca_3 length 0.059 ± 0.002 (0.053–0.061); posteromedian setae (PM) length 0.049 ± 0.007 (0.036–0.061), postcoxal setae absent.

Capitulum Basis capituli outline as illustrated in Fig. 2b, posterior margin broadly rounded, length from posterior margin of basis capituli to posthypostomal setae: Ph_1 0.138 ± 0.006 (0.132–0.146), length from posterior margin of basis capituli to insertion of hypostome 0.165 ± 0.005 (0.156–0.171), length from posterior margin of basis capituli to apex of hypostome 0.336 ± 0.014 (0.313–0.362), width 0.210 ± 0.012 (0.185–0.232). Two pairs of posthypostomal setae; Ph_1 length 0.023 ± 0.001 (0.022–0.024), Ph_2 length 0.029 ± 0.001 (0.028–0.030), distance between Ph_1 setae 0.031 ± 0.005 (0.022–0.039), distance between Ph_2 setae 0.072 ± 0.003 (0.068–0.078). Palpi total length 0.231 ± 0.005 (0.222–0.239), segmental length/width from 1 to 4: (1) 0.054 ± 0.005 (0.049–0.061)/0.036 ± 0.003 (0.032–0.044), (2) 0.073 ± 0.003 (0.068–0.078)/0.041 ± 0.004 (0.037–0.049), (3) 0.067 ± 0.005 (0.053–0.073)/0.038 ± 0.003 (0.034–0.044), (4) 0.050 ± 0.006 (0.044–0.066)/0.025 ± 0.004 (0.022–0.036). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9. Hypostome: length from Ph_1 to apex 0.196 ± 0.005 (0.188–0.205), length from insertion of hypostome in basis capituli to apex 0.169 ± 0.004 (0.166–0.178), width in medial basis portion of hypostome 0.040 ± 0.002 (0.037–0.044), width in basis portion of hypostome 0.048 ± 0.003 (0.044–0.053); apex pointed, dental formula 3/3 in anterior third, then 2/2 to base, file 1 with 16–18 denticles, file 2 with 14–17 denticles, file 3 with 5–7 denticles, corona of fine denticles apically. Legs: Tarsus I length 0.195 ± 0.008 (0.183–0.207), tarsus I width 0.067 ± 0.003 (0.061–0.073). Setal formula of tarsus I: one pair apical (A), one distomedian (DM), five paracapsular (PC), one posteromedian (PM), one pair basal (B), one pair apicoventral (AV), one pair midventral (MV), one pair basiventral (BV), and one pair posterolateral (PL). Capsule of Haller's organ: oval in shape from dorsal view, without reticulations.

Type host *Philodryas chamissonis* (Wiegmann, 1834) (Serpentes: Colubridae)

Type locality Fray Jorge National Park (30°30′S, 71°35′W), Coquimbo Region, Chile.

Type specimens Holotype larva, allotype larva and 2 paratype larvae mounted in same slide, host: *P. chamissonis*, Fray Jorge National Park, Coquimbo Region, Chile, coll. D. González-Acuña and S. Muñoz-Leal., December 8, 2010, deposited in USNTC, Georgia Southern University, Statesboro, USA (USNMENT 00862995, 00862296, 00862297).

Paratypes Forty larvae in 70 % ethanol, same host, locality, collectors and data for the holotype deposited in USNTC (USNMENT 00862998). Twenty larvae in 70 % ethanol, same host, locality, collectors and data for the holotype deposited in INTA (INTA 2250). Thirty-seven larvae in 70 % ethanol, same host, locality, collectors and data for the holotype deposited in the tick collection of the Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile (FVCH-Ix-049). Thirty-four larvae mounted in slide and 20 larvae in 70 % ethanol, same host, locality, collectors and data for the

holotype deposited in DPVURU (DPVURU 873). Five larvae mounted in slide, host: *Callopistes maculatus*, Las Chinchillas National Reserve (31°28′S, 71°03′W), Coquimbo Region, Chile, coll. D. González-Acuña and S. Muñoz-Leal, December 4, 2010, deposited in DPVURU (DPVURU 874).

Etymology The species is named for Fernand Lahille, in recognition of his contribution to the study of South American ticks.

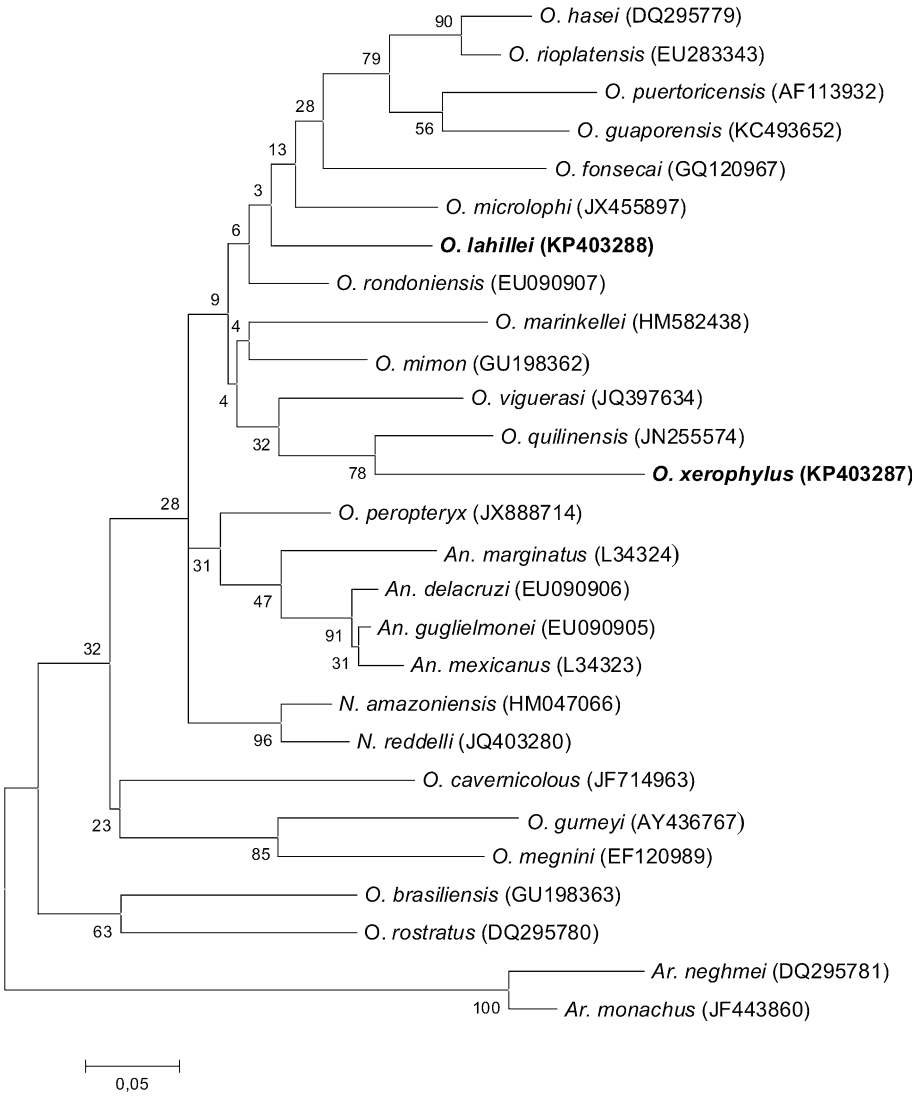

Fig. 4 Maximum-likelihood tree based on 16S rDNA partial sequences. *Numbers* represent bootstrap support generated from 1,000 replications. GenBank accession numbers are indicated in *brackets*. An., *Antricola*; O., *Ornithodoros*; N., *Nothoaspis*; Ot., *Otobius*; Ar., *Argas*

Table 1 Morphometric (average and range in millimeters) variables of larvae of *Ornithodoros xerophylus* (n=9), *Ornithodoros quilinensis* (n=10), *Ornithodoros microlophi* (n=12) and *Ornithodoros lahillei* (n=10) utilized in the principal component analysis (PCA)

	O. xerophylus	*O. quilinensis*	*O. microlophi*	*O. lahillei*
Dorsal plate: length	0.243 (0.232–0.260)	0.200 (0.172–0.221)	0.295 (0.284–0.313)	0.207 (0.195–0.219)
Dorsal plate: width	0.207 (0.185–0.225)	0.152 (0.141–0.168)	0.217 (0.196–0.245)	0.146 (0.134–0.166)
Dorsal setae (pairs): total	16	14	20 (19–21)	14
Dorsal setae (pairs): dorsolateral	13	11	13	11
Dorsal setae (pairs): central	3	3	7 (6–8)	3
Central setae 1: length	0.075 (0.061–0.085)	0.051 (0.048–0.055)	0.073 (0.063–0.083)	0.068 (0.063–0.073)
Central setae 2: length	0.087 (0.068–0.097)	0.073 (0.067–0.079)	0.070 (0.061–0.077)	0.068 (0.063–0.075)
Central setae 3: length	0.089 (0.075–0.097	0.071 (0.062–0.077)	0.066 (0.062–0.073)	0.073 (0.066–0.078)
Ventral setae (pairs): total	8	8	11	7
Sternal setae 1: length	0.053 (0.044–0.068)	0.046 (0.041–0.050)	0.055 (0.044–0.063)	0.041 (0.036–0.046)
Sternal setae 2: length	0.053 (0.049–0.061)	0.045 (0.041–0.048)	0.053 (0.046–0.061)	0.038 (0.034–0.049)
Sternal setae 3: length	0.051 (0.046–0.061)	0.044 (0.038–0.050)	0.050 (0.044–0.053)	0.038 (0.036–0.041)
Circumanal setae 1: length	0.046 (0.036–0.056)	0.040 (0.036–0.043)	0.058 (0.051–0.068)	0.030 (0.027–0.034)
Circumanal setae 2: length	0.056 (0.052–0.061)	0.040 (0.036–0.045)	0.061 (0.046–0.073)	0.048 (0.044–0.053)
Circumanal setae 3: length	0.070 (0.065–0.078)	0.050 (0.038–0.057)	0.084 (0.073–0.095)	0.059 (0.053–0.061)
Posteromedian setae: length	0.051 (0.041–0.061)	0.042 (0.036–0.050)	0.053 (0.041–0.063)	0.049 (0.036–0.061)
Postcoxal setae: length	0.055 (0.049–0.068)	0.038 (0.031–0.043)	0.047 (0.041–0.053)	0
Length of basis capituli[a]	0.096 (0.092–0.100)	0.091 (0.086–0.096)	0.145 (0.136–0.156)	0.138 (0.132–0.146)
Length of basis capituli[b]	0.118 (0.111–0.125)	0.104 (0.098–0.113)	0.161 (0.153–0.171)	0.165 (0.156–0.171)
Width of basis capituli	0.156 (0.147–0.175)	0.151 (0.144–0.156)	0.198 (0.183–0.210)	0.210 (0.185–0.232)
Distance of posthypostomal setae 1	0.025 (0.020–0.030)	0.016 (0.014–0.019)	0.027 (0.024–0.032)	0.031 (0.022–0.039)
Distance of posthypostomal setae 2	0.066 (0.061–0.073)	0.074 (0.065–0.086)	0.080 (0.073–0.085)	0.072 (0.068–0.078)
Palpal length	0.202 (0.190–0.210)	0.143 (0.132–0.151)	0.285 (0.264–0.313)	0.231 (0.222–0.239)
Length article I	0.047 (0.039–0.052)	0.037 (0.036–0.041)	0.055 (0.051–0.061)	0.054 (0.049–0.061)
Length article II	0.062 (0.058–0.070)	0.043 (0.038–0.048)	0.099 (0.097–0.110)	0.073 (0.068–0.078)

Table 1 continued

	O. xerophylus	*O. quilinensis*	*O. microlophi*	*O. lahillei*
Length article III	0.060 (0.053–0.065)	0.043 (0.038–0.048)	0.096 (0.090–0.100)	0.067 (0.053–0.073)
Length article IV	0.030 (0.027–0.036)	0.024 (0.019–0.029)	0.043 (0.039–0.049)	0.050 (0.044–0.066)
Width article I	0.036 (0.032–0.040)	0.034 (0.031–0.038)	0.031 (0.027–0.036)	0.036 (0.032–0.044)
Width article II	0.037 (0.035–0.045)	0.035 (0.033–0.038)	0.037 (0.034–0.041)	0.041 (0.037–0.049)
Width article III	0.031 (0.030–0.035)	0.031 (0.029–0.033)	0.034 (0.034–0.036)	0.038 (0.034–0.044)
Width article IV	0.019 (0.015–0.022)	0.020 (0.019–0.021)	0.023 (0.022–0.024)	0.025 (0.022–0.036)
Hypostome length[c]	0.179 (0.171–0.185)	0.133 (0.120–0.141)	0.244 (0.232–0.256)	0.196 (0.188–0.205)
Hypostome length[d]	0.156 (0.149–0.162)	0.118 (0.110–0.122)	0.226 (0.217–0.239)	0.169 (0.166–0.178)
Hypostome width in median portion	0.048 (0.045–0.050)	0.043 (0.041–0.048)	0.060 (0.053–0.068)	0.040 (0.037–0.044)
Hypostome width in basis portion	0.047 (0.045–0.050)	0.036 (0.033–0.041)	0.060 (0.051–0.068)	0.048 (0.044–0.053)
Apical dental formula	3/3	2/2	2/2–3/3	3/3
Median dental formula	2/2	2/2	3/3–4/4	2/2
Denticles in hypostomal row 1	14–16	13–15	24–26	16–18
Denticles in hypostomal row 2	12–13	11–14	22–25	14–17
Denticles in hypostomal row 3	2–3	0	16–18	5–7
Tarsus I: length	0.144 (0.130–0.160)	0.129 (0.127–0.134)	0.198 (0.183–0.207)	0.195 (0.183–0.207)
Tarsus I: width	0.053 (0.047–0.061)	0.053 (48–57)	0.062 (58–68)	0.067 (0.061–0.073)

[a] Length of basis capituli to Ph1

[b] Length of basis capituli to insertion of hypostome

[c] Measured to point of insertion of hypostome in basis capituli

[d] Measured to point to inferior toothed portion

Analysis of 16S rDNA sequences and PCA

Ornithodoros xerophylus n. sp. was found to be phylogenetically related to *Ornithodoros quilinensis* Venzal, Nava and Mangold (Fig. 4), which is not unexpected because they are morphologically similar and exhibit a sympatric distribution in the north of Córdoba Province, Argentina. Percent sequence divergence between *O. xerophylus* n. sp. and *O. quilinensis* was 13.1. A close association between *O. lahillei* n. sp. and the remaining *Ornithodoros* species included in the analysis was not found (Fig. 4). Genetic divergence between *O. lahillei* n. sp. and the other Neotropical species of argasid ticks was always higher than 10 %.

PCA was conducted with 42 morphometric variables of the larvae of *O. xerophylus* n. sp., *O. quilinensis*, *O. lahillei* n. sp. and *Ornithodoros microlophi* Venzal, Nava and González-Acuña (Table 1). PCA also showed a clear differentiation between all four *Ornithodoros* sample species analyzed (Fig. 5).

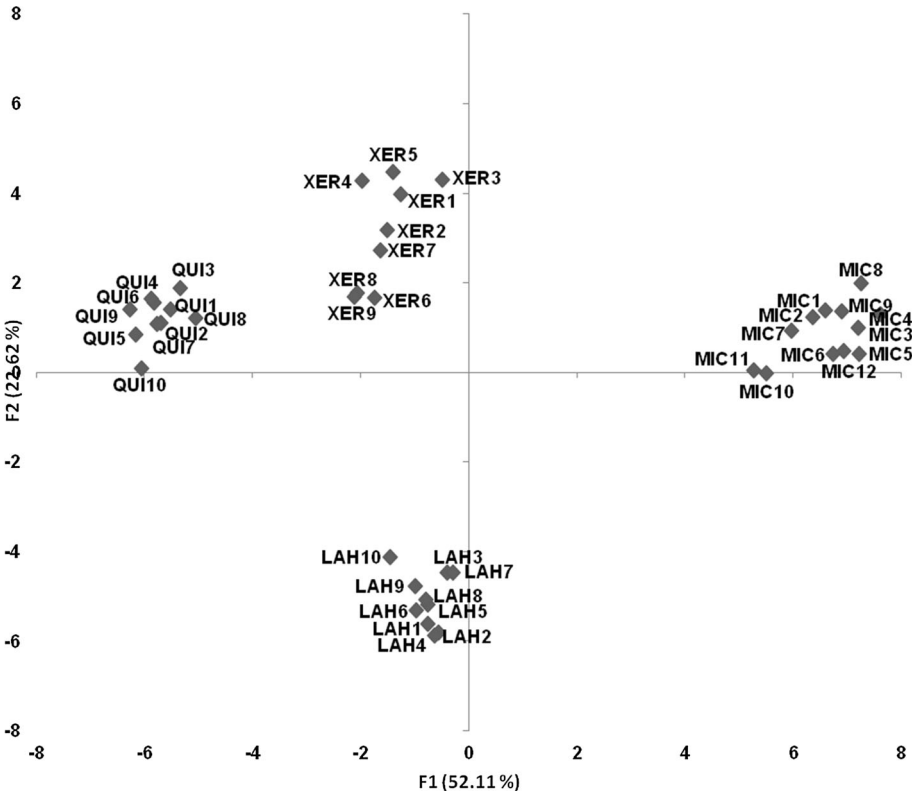

Fig. 5 Principal components analysis of the body and setal measurements of the larvae of *Ornithodoros xerophylus* (XER1–XER9), *Ornithodoros quilinensis* (QUI1-QUI10), *Ornithodoros lahillei* (LAH1-LAH10) and *Ornithodoros microlophi* (MIC1-MIC12), using the features detailed in Table 1 Each point constitutes the position of each measured specimen on the reduced space

Discussion

Larvae of *O. xerophylus* n. sp. are diagnosed by a combination of dorsal plate slightly oval with a length of approximately 250 μm, 16 pairs of dorsal setae, hypostome with apex rounded and dental formula 2/2 in most rows and 3/3 only apically, and capsule of the Haller's organ oval in shape without reticulations. Morphologically, *O. xerophylus* n. sp. is similar to *O. quilinensis*, but these species can be differentiated among each other by the number of dorsal setae (16 pairs in *O. xerophylus* n. sp., 14 pairs in *O. quilinensis*), length of dorsal plate (higher in *O. xerophylus* n. sp. than in *O. quilinensis*) and shape of capsule of the Haller's organ (oval in *O. xerophylus* n. sp., irregular in *O. quilinensis*). *Ornithodoros xerophylus* n. sp. also has morphological similitude with other *Ornithodoros* species associated to small rodents and marsupials as *Ornithodoros casebeeri* Jones and Clifford, *Ornithodoros chironectes* Jones and Clifford, *Ornithodoros echimys* Kohls, Clifford and Jones and *Ornithodoros tuttlei* Jones and Clifford. However, the larva of *O. xerophylus* n. sp. can be separated from those of *O. casebeeri, O. chironectes, O. echimys* and *O. tuttlei* by the dental formula in most rows of hypostome (2/2 in *O. xerophylus* n. sp., 3/3 in *O. casebeeri* and *O. chironectes,* 4/4 in *O. echimys* and 5/5 in *O. tuttlei*). Additionally, *O. casebeeri* and *O. echimys* have 17–18 and 14 pairs of dorsal setae, respectively, but *O. xerophylus* n. sp has a dorsal surface with 16 pairs of setae.

Larvae of *O. lahillei* n. sp. are easily distinguished from all other larvae of the *Ornithodoros* species described in the Neotropics by a combination of dorsal plate subtriangular with margins corrugated and posterior margin convex, dorsal surface with 14 pairs of setae, absence of postcoxal setae, and hypostome with apex pointed and dental formula 3/3 in anterior third and 2/2 in the middle and basal portion. *Ornithodoros darwini* Kohls et al. is the most similar species to *O. lahillei* n. sp. in terms of morphology. They share a similar patter of ventral setae, dorsal plate subtriangular with posterior margin convex and the absence of postcoxal setae. However, *O. lahillei* n. sp. has 14 pairs of dorsal setae and hypostome with dental formula 3/3 in anterior third and 2/2 in the middle and basal portion, while *O. darwini* has 46–48 pairs of dorsal setae and dentition 4/4 at tip, 3/3 beyond midlength and then 2/2 to near the base (see Kohls et al. 1969). Similarities exist in the chaetotaxy and hypostome of *O. lahillei* n. sp. with the larva of the genus *Antricola*, but they differ for the absence of post-coxal setae and pulvillo.

Phylogenetic analysis of 16S rDNA sequences (Fig. 4) showed that *O. xerophylus* n. sp. and *O. lahillei* n. sp. represent two independent lineages within the genus *Ornithodoros* in the Neotropical Region, supporting in that way the morphological diagnosis performed with both quantitative and discrete characters of larvae. There are many species of *Ornithodoros* for which DNA sequences were not yet obtained. Additional 16S rDNA sequences and other molecular markers are needed to assess with more accuracy than is currently possible the phylognetic relationships among Neotropical species of the genus *Ornithodoros*.

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References

Barros-Battesti DM, Ramirez DG, Landulfo GA, Faccini JLH, Dantas-Torres F, Labruna MB, Venzal JM, Onofrio VC (2013) Immature argasid ticks: diagnosis and keys for Neotropical region. Ver Bras Parasitol Vet 22:443–456

Cabrera AL (1976) Enciclopedia argentina de agricultura y jardinería. Fascículo 1. Regiones fitogeográficas argentinas. Tomo III, 2nd edn. ACME, Buenos Argentina

Dantas-Torres F, Venzal JM, Bernardi LFO, Ferreira RL, Onofrio VC, Marcili A, Bermudez S, Ribeiro A, Barros-Battesti DM, Labruna MB (2012) Description of a new species of bat-associated argasid tick (Acari: Argasidae) from Brazil. J Parasitol 98:36–45

Guglielmone AA, Robbins RG, Apanaskevich DA, Petney TA, Estrada-Peña A, Horak IG, Shao R, Barker SC (2010) The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid names. Zootaxa 2528:1–28

Hall TA (1999) BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Heath ACG (2012) A new species of soft tick (Ixodoidea: Argasidae) from the New Zealand lesser short-tailed bat, *Mystacina tuberculata* Gray. Tuhinga 23:29–37

Kohls GM, Sonenshine DE, Clifford CM (1965) The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). II. Identification of the larvae of the western hemisphere and descriptions of three new species. Ann Entomol Soc Am 58:331–364

Kohls GM, Clifford CM, Hoogstraal H (1969) Two new species of *Ornithodoros* from the Galápagos Islands (Acarina: Argasidae). J Med Entomol 6:75–78

Labruna MB, Venzal JM (2009) *Carios fonsecai* sp. nov. (Acari, Argasidae), a bat tick from the central-western region of Brazil. Act Parasitol 54:355–363

Mangold AJ, Bargues MD, Mas-Coma S (1998) Mitochondrial 16S rRNA sequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among metastriata (Acari: Ixodidae). Parasitol Res 84:478–484

Nava S, Venzal JM, Terassini FA, Mangold AJ, Camargo LMA, Labruna MB (2010) Description of a new argasid tick (Acari: Ixodida) from bat caves Brazilian Amazon. J Parasitol 96:1089–1101

Nava S, Venzal JM, Terassini FA, Mangold AJ, Camargo LMA, Casás G, Labruna MB (2013) *Ornithodoros guaporensis* (Acari, Ixodida: Argasidae), a new tick species from the Guaporé River Basin in the Bolivian Amazon. Zootaxa 3666:579–590

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Thompson JD, Higgins D, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Venzal JM, Estrada-Peña A, Mangold AJ, González-Acuña D, Guglielmone AA (2008) The *Ornithodoros* (*Alectorobius*) *talaje* species group (Acari: Ixodida: Argasidae): description of *Ornithodoros* (*Alectorobius*) *rioplatensis* n.sp. from southern South America. J Med Entomol 45:832–840

Venzal JM, Nava S, Mangold AJ, Mastropaolo M, Casás G, Guglielmone AA (2012) *Ornithodoros quilinensis* sp. nov. (Acari, Argasidae), a new tick species from the Chacoan region in Argentina. Act Parasitol 57:329–336

Venzal JM, Nava S, González-Acuña D, Mangold AJ, Muñoz Leal S, Lado P, Guglielmone AA (2013) A new species of *Ornithodoros* (Acari: Argasidae), parasite of *Microlophus* spp. (Reptilia: Tropiduridae) from northern Chile. Ticks Tick Borne Dis 4:128–132

Vial L, Camicas JL (2009) Description of a new soft tick species of the genus *Ornithodoros* Koch, 1844 (Acari: Argasidae) from Oman. Fauna Arab 24:135–143