Ornithodoros peruvianus *Kohls, Clifford & Jones (Ixodoidea: Argasidae) in Chile: a Tentative Diagnosis*

J Venzal, Daniel González-Acuña, A Mangold & A Guglielmone

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SCIENTIFIC NOTE

Ornithodoros peruvianus Kohls, Clifford & Jones (Ixodoidea: Argasidae) in Chile: a Tentative Diagnosis

J Venzal[1], D González-Acuña[2], A Mangold[3], A Guglielmone[3]

[1] Depto de Parasitología Veterinaria, Fac de Veterinaria, Univ de la República, Salto, Uruguay
[2] Facultad de Ciencias Veterinarias, Univ de Concepción, Chillán, Chile
[3] Instituto Nacional de Tecnología Agropecuaria, Rafaela, Santa Fe, Argentina

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Correspondence
Daniel González-Acuña, Facultad de Ciencias Veterinarias, Universidad de Concepción, Casilla 537, Chillán, Chile; danigonz@udec.cl

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Abstract
Three argasid tick larvae were collected on April 2, 2010, from a common vampire bat, *Desmodus rotundus*, captured in the Parque Nacional Pan de Azúcar (26°09′ S, 70°41′ W), Region of Atacama, Chile. The larvae were diagnosed as *Ornithodoros*, and further comparative analysis showed them to be *Ornithodoros peruvianus* Kohls, Clifford & Jones or a species close to it. Phylogenetic analysis based on 16S mitochondrial rRNA gene sequences of *Ornithodoros* species plus four *Argas* species was carried out to clarify the taxonomic position of the larvae. This is the first finding of ticks parasitizing *D. rotundus* in Chile.

Ornithodoros peruvianus Kohls, Clifford & Jones is a Neotropical species known only from six larvae collected from Chiroptera in Peru, three from *Desmodus rotundus*, Chosica, Department of Lima; two from *Glossophaga* sp., Amotape Mountains, Department of Piura; and one from *Molossus obscurus*, Yarinacocha, Department of Loreto. This tick species has been considered by others to belong to *Alectorobius* or *Carios* (Guglielmone *et al* 2010), but we maintain it as a species of *Ornithodoros.*

Herein we report the first record of *O. peruvianus* or a very closely related species from Chile. This is the first record of this tick species after its description and the first record for this host–parasite relationship in Chile.

Three argasid tick larvae, almost fully engorged, were collected by one of us (DGA) on April 2, 2010, from *D. rotundus* captured in the Parque Nacional Pan de Azúcar, (26°09′ S 70°41′ W), Region of Atacama, Chile. Two larvae were mounted in Hoyer's medium to make semi-permanent slides in order to observe and measure 58 morphological characters (when available) following Nava *et al* (2010). The remaining larva was preserved in 100% ethanol and stored at −20°C for molecular analysis. Genomic DNA was extracted following the technique described by Mangold *et al* (1998a). Polymerase chain reactions (PCRs) were performed to amplify nearly 460 bp fragment of the mitochondrial 16S rRNA gene. The PCR protocol, primer statement, and cycling condition were as reported by Mangold *et al* (1998b). The consensus 16S sequence obtained in this study was analysed with 16S sequences of *Ornithodoros* species from GenBank. Sequences were aligned using Clustal W (Thompson *et al* 1994). The sequences from *Argas* genus deposited in GenBank were chosen as outgroups in the analyses. Phylogenetic relationships were performed using a distance-based method, neighbour joining (NJ), with MEGA version 4 (available from www.megasoftware.net) (Tamura *et al* 2007). A neighbour-joining tree was generated using the distance calculated from the Tamura–Nei model (Tamura & Nei 1993). Gaps were excluded from the pairwise comparison to determine the relationship between the specimens from Chile and other Neotropical *Ornithodoros.* Support for the NJ topology was tested by boot-strapping over 1,000 replications (Felsenstein 1985). The 16S sequence from *O. peruvianus* from this study was deposited in the GenBank under the accession number indicated in the NJ tree (Fig 1).

Specimens mounted in Hoyer's were deposited in the tick collection of the Departamento de Parasitología

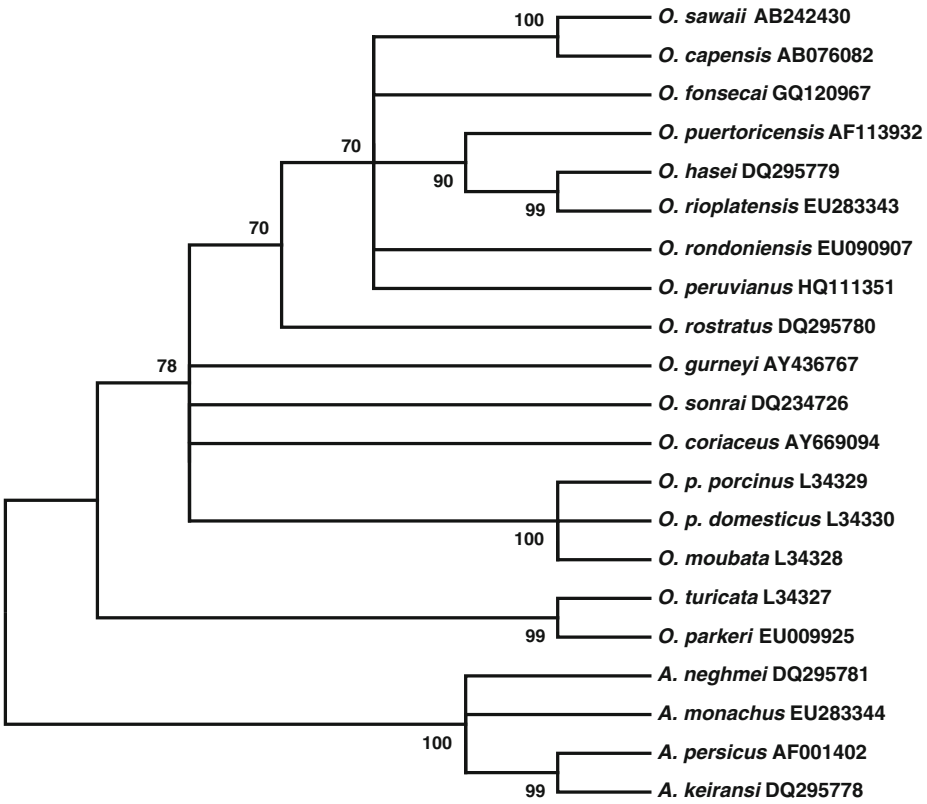

Fig 1 NJ tree performed with the Tamura Nei model based upon 16S sequence of *Ornithodoros peruvianus* obtained in this study and other *Ornithodoros* species retrieved from GenBank. *Argas* species are used as outgroup. Species name and associated GenBank accession numbers are given. *Numbers* represent the percentage of 1,000 bootstrap replicates supporting each branch, and bootstrap values of 70% were used to collapse branches.

Veterinaria, Universidad de la República, Salto, Uruguay, under the accession numbers DPVURU-754 and 755. The specimen used for DNA extraction was deposited in the tick collection (DNA section) of the Estación Experimental Agropecuaria Rafaela, Rafaela, Santa Fe, Argentina.

The three larvae were almost fully engorged. All had damaged hypostomes, an important morphological feature for species identification. Nevertheless, a morphological comparison was attempted. The larvae were provisionally identified as a species close to *O. peruvianus*, *Ornithodoros fonsecai* (Labruna & Venzal) and *Ornithodoros peropteryx* Kohls, Clifford & Jones. Table 1 was constructed for a morphological comparison of the specimens collected with these other species.

The specimens collected were different from *O. peropteryx* because the larva of the latter has a reticulate Haller's organ capsule and an elongate dorsal plate, while the Chilean argasids have a non-reticulate Haller's organ and a pyriform dorsal plate. The differences with *O. fonsecai* are more subtle than for *O. peropteryx*; the lengths of the dorsal plate, dorsal anterolateral setae AI_1, dorsal posterolateral setae PI_1, circumanal setae Ca_1, basis capituli, distance Ph_1–Ph_1 and Ph_2–Ph_2 of *O. fonsecai* are considerably shorter than the corresponding values for the larvae collected. Therefore, the larvae collected seem closer to *O. peruvianus*, but they have a slightly shorter dorsal posterolateral setae PI_1 and palpal article I and slightly longer circumanal setae Ca_2, posthypostomal setae Ph_2 and width of the basis capituli. Therefore, we conclude that the species found in Chile is *O. peruvianus* or a closely related species. It is important to note that we had difficulties in correctly measuring the circumanal and posthypostomal setae because some of them were broken. Therefore, these setal measures were of small value for tick identification, and we rely our diagnoses on other morphological features.

The final data set consisted of 21 aligned 16S sequences of 431 bps representing 17 populations of the genus *Ornithodoros* and 4 of *Argas* species as outgroup. The name species of the sequences of *Ornithodoros* and *Argas* species used in the analyses and the associated GenBank accession numbers are given in the NJ tree (Fig 1). The general topology of the phylogenetic tree showed the *Ornithodoros* sequences analysed here form a diverse group in which several more or less supported clades are recognized. The alleged *O. peruvianus* 16S sequences clustered together with other *Ornithodoros* clades but have no strong relationship with any other species of *Ornithodoros* for which the 16S sequences are known. The outgroup, *Argas* species, collapsed into a separate clade supported by 100% of the bootstrap value.

The position of *O. peruvianus* in the neighbour-joining tree (Fig 1) is included loosely in a branch containing species established in the Neotropical region plus *Ornithodoros sawaii* Kitaoka & Suzuki. Additional studies are needed to further understand the phylogenetic position of this tick.

We consider the larvae collected to be most likely *O. peruvianus*. However, the diagnosis of argasid ticks

Table 1 Morphological characteristics of larvae of the specimens collected in this study, *Ornithodoros peruvianus*, *Ornithodoros fonsecai* and *Ornithodoros peropteryx*.

	Specimens collected[h]	*O. peruvianus*	*O. fonsecai*	*O. peropteryx*
Feeding status	Engorged	Slightly engorged	Unengorged	Partly engorged
Body length[a]	ND	1,520 µ	990–1,050 µ	1,008–1,012 µ
Body length[b]	2,112–2,208 µ	ND	600–630 µ	ND
Body width	1,440–1,680 µ	1,300 µ	600–630 µ	520–600 µ
Dorsal plate: form	Pyriform	Pyriform	Pyriform	Elongate
Dorsal plate: length	328–332 µ	320–340 µ	247–250 µ	276–312 µ
Dorsal plate: width	190–200 µ	170–250 µ	167–180 µ	116–140 µ
Dorsal setae: total pairs	14	14	13–14	14
Dorsal setae: dorsolateral pairs	11	11	10–11	11
Dorsal setae: central pairs	3	3	3	3
Dorsal anterolateral setae: Al_1	137–139 µ	132–142 µ	112–127 µ	120–132 µ
Dorsal anterolateral setae: Al_2	144–153 µ	ND	ND	ND
Dorsal anterolateral setae: Al_3	132–151 µ	ND	ND	ND
Dorsal anterolateral setae: Al_4	122–137 µ	ND	ND	ND
Dorsal anterolateral setae: Al_5	120–132 µ	ND	ND	ND
Dorsal anterolateral setae: Al_6	120–127 µ	ND	ND	ND
Dorsal anterolateral setae: Al_7	117–134 µ	ND	ND	ND
Dorsal posterolateral setae: Pl_1	89–96 µ	100 µ	65–77 µ	80–96 µ
Dorsal posterolateral setae: Pl_2	101–103 µ	ND	ND	ND
Dorsal posterolateral setae: Pl_3	89–103 µ	ND	ND	ND
Dorsal posterolateral setae: Pl_4	96–101 µ	ND	ND	ND
Central setae: C_1 length	122–137 µ	ND	ND	ND
Central setae: C_2	91–120 µ	ND	ND	ND
Central setae: C_3	98–122 µ	ND	ND	ND
Ventral setae (pairs): total	7 pairs+1 anal pair+1 PMS	7 pairs+1 anal pair+1 PMS	7 pairs+1 anal pair+1 PMS	7 pairs+1 PMS+1 anal pair
Sternal setae: St_1	77–93 µ	ND	ND	ND
Sternal setae: St_2	86–91 µ	ND	ND	ND
Sternal setae: St_3	93 µ	ND	ND	ND
Circumanal setae: Ca_1	84–91 µ	64–92 µ	45–65 µ	64–68 µ
Circumanal setae: Ca_2	91–96 µ	72–88 µ	90–95 µ	80–88 µ
Circumanal setae: Ca_3	101–108 µ	104–108 µ	100–110 µ	88–96 µ
Postcoxal setae: Pc	53–69 µ	ND	ND	ND
Length of basis capituli[c]	237–240 µ	ND	170–187 µ	ND
Length of basis capituli[d]	304 µ	ND	230–236 µ	ND
Length of basis capituli[e]	ND	510 µ	440–460 µ	450–480 µ
Width of basis capituli	285–313 µ	260 µ	225–250 µ	200–220 µ
Posthypostomal setae Ph_1	ND	12 µ	12–15 µ	20 µ
Posthypostomal setae Ph_2	48–53 µ	32–44 µ	40–45 µ	40–60 µ
Distance Ph_1–Ph_1	29 µ	28 µ	22 µ	20 µ
Distance Ph_2–Ph_2	101–103 µ	100–120 µ	80–87 µ	80–96 µ
Palpal length	332–352 µ	ND	320–350 µ	ND
Length article I	74–79 µ	92–96 µ	75–80 µ	72–88 µ
Length article II	108–120 µ	88–112 µ	95–100 µ[i]	100–108 µ
Length article III	101–110 µ	100–116 µ	95–105 µ[i]	96–104 µ
Length article IV	53 µ	56 µ	42–45 µ	48–56
Setae of palpal article I	0	0	0	0
Setae of palpal article II	4	4	4	4

Table 1 (continued).

	Specimens collected[h]	*O. peruvianus*	*O. fonsecai*	*O. peropteryx*
Setae of palpal article III	5	5	5	5
Setae of palpal article IV	9	9	9	9
Hypostome: length[f]	ND	300 µ	260–280 µ	260 µ
Hypostome: length[g]	ND	ND	217–230 µ	200–204 µ
Hypostome: width	77 µ	84 µ	65–75 µ	ND
Apex	ND	Blunt	Pointed	Pointed
Apical dental formula	ND	3/3	3/3	3/3
Median dental formula	ND	2/2	2/2	2/2
Basal dental formula	ND	2/2	2/2	2/2
Denticles in hypostomal row 1	ND	18	17–18	17–18
Denticles in hypostomal row 2	ND	17	15–17	17
Denticles in hypostomal row 3	ND	13	9–10	8–10
Tarsus I: length	285 µ	272–300 µ	285–300 µ	280–290 µ
Tarsus I: width	76 µ	ND	66–76 µ	ND
Haller's organ capsule	Not reticulate	Not reticulate	Not reticulate	Reticulate
Description	This study	Kohls *et al* (1969)	Labruna and Venzal (2009)	Kohls *et al* (1969)

ND not determined.

[a] Including capitulum.

[b] Not including capitulum.

[c] Length of basis capituli to Ph1.

[d] Length of basis capituli to insertion of hypostome.

[e] Length of basis capituli to final hypostome.

[f] Measured to point to Ph_1.

[g] Measured to point of inferior toothed portion.

[h] Based on two larvae without part of hypostome.

[i] Corrections of measures in Labruna and Venzal (2009).

is usually more difficult than the diagnosis of Ixodidae species (Estrada-Peña *et al* 2010), and in the specimens collected, the hypostome was damaged, making categoric identification particularly difficult. Therefore, this diagnosis has to be considered tentative because not all morphological characters fit those mentioned in the original description of *O. peruvianus.* However, these problems do not diminish the validity of the present finding, which constitutes the first detection of a tick on *D. rotundus* from Chile.

More detailed studies are needed to understand the ecology of argasid ticks. Until now, several Neotropical *Ornithodoros* species, including *O. peruvianus*, are known only for the larval stage.

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