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Ornithodoros peropteryx (Acari: Argasidae) in Bolivia: an argasid tick with a single nymphal stage

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Abstract By the end of the 1960s, the argasid tick *Ornithodoros peropteryx* was described from larval specimens collected from the bat *Peropteryx macrotis* in Colombia. Since its original description, no additional record of *O. peropteryx* has been reported, and its post-larval stages have remained unknown. During July 2010, 18 larvae were collected from 9 bats (*Centronycteris maximiliani*), resulting in a mean infestation of 2.0 ± 2.2 ticks per bat (range 1–8). These bats were captured in a farm in northeastern Bolivia close to Guaporé River in the border with Brazil. Morphological examinations of the larvae revealed them to represent the species *O. peropteryx*. One engorged larva that was kept alive in the laboratory moulted to a nymph after 9 days. Fourteen days after the larval moulting, the nymph moulted to an adult female without taking any blood meal during the nymphal period. This adult female was used for a morphological description of the female stage of *O. peropteryx*. In addition, the larvae were used for a morphological redescription of this stage. One larva and two legs extirpated from the adult female were submitted to DNA extraction and PCR targeting a fragment of the mitochondrial 16S rDNA gene, which yielded DNA sequences at least 11 % divergent from any available argasid sequence in

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Genbank. We show that *O. peropteryx* ontogeny is characterized by a single, non-feeding, nymphal stage. This condition has never been reported for ticks.

Keywords Argasidae · *Ornithodoros* · Morphology · Bolivia

Introduction

The tick fauna of Bolivia is composed by 35 species, 29 of the Ixodidae family, and only 6 belonging to the Argasidae family (Guglielmone et al. 2003). The argasid species of Bolivia are *Ornithodoros echimys* Kohls et al. 1969, *Ornithodoros hasei* (Schulze 1935), *Ornithodoros kohlsi* Guglielmone and Keirans 2002, *Ornithodoros mimon* Kohls et al. 1969, *Ornithodoros rostratus* Aragao 1911, and *Otobius megnini* (Dugès 1883) (Guglielmone et al. 2003). This contrasting low number of Argasidae species in relation to the number of species of Ixodidae is probably an underestimation due to the lack of taxonomic studies of argasid ticks (Guglielmone et al. 2010).

The argasid tick *Ornithodoros peropteryx* Kohls et al. 1969, was described from 31 larvae collected from the bat *Peropteryx macrotis* (Wagner 1843) (Chiroptera: Emballonuridae) in Colombia (Kohls et al. 1969). Since its original description more than 40 years ago, no additional record of *O. peropteryx* has been reported, and its post-larval stages have remained unknown (Guglielmone et al. 2003). Herein, we report recent findings of *O. peropteryx* from Bolivia, providing data on post-larval stages for the fist time.

Materials and methods

In July 2010, larval ticks were collected from 9 bats of the species *Centronycteris maximiliani* (Fischer 1829) (Chiroptera: Emballonuridae) in a farm in northeastern Bolivia (12°55′S; 62°52′W; elevation: 160 m) close to Guaporé River in the border with Brazil. Three of these ticks were full engorged larvae and were kept alive during transportation to the laboratory, where they were placed in an incubator at 25 °C and 90 % relative humidity. The remaining ticks were placed in vials containing 70 % ethanol, and used for morphological studies. For this purpose, unengorged or partially engorged larvae were mounted in Hoyer's medium to make semi-permanent slides and examined and photographed by light microscopy for morphological and morphometric analyses using an Eclipse E200 optical microscope (Nikon, Tokyo, Japan). Measurements for larvae are in millimetres. Ticks were identified using morphological keys and original species descriptions of *Ornithodoros* spp. (Cooley and Kohls 1944; Kohls et al. 1965, 1969; Jones and Clifford 1972). For the present study, we followed Guglielmone et al. (2010) regarding the current usage of valid tick names.

Among the three live engorged larvae, two died after 3 days in the incubator, whereas the third specimen moulted to a nymph after 9 days. The emerged nymph appeared to be engorged, and retained this appearance during the subsequent days, when the tick was observed daily in the incubator. The tick was kept within a dry plastic vial containing no other tick and no substrate other than a piece of absorbent paper. Fourteen days after the larval moult, the nymph moulted to an adult female without taking any blood meal during the nymphal period. This female tick was kept alive in the incubator for 40 days, when it was finally preserved in 70 % ethanol. When it was alive, we attempted to feed the female by leaving it for about 2 h inside a cotton sleeve glued to the shaved back of a New

Zealand white rabbit, but the tick refused to feed. This adult female was measured in a stereomicroscope; measurements are provided in millimetres.

DNA was extracted from one partially engorged larva by using the Dneasy tissue kit (Qiagen, Chatsworth, California) according to the manufacturer's protocol for isolation of DNA from animal blood. Additionally, DNA was also extracted by using the guanidine isothiocyanate-phenol technique (Sangioni et al. 2005) from two legs that were excised from the single adult female obtained in this study. Extracted DNA samples were subjected to conventional polymerase chain reaction (PCR) targeting a fragment of approximately 460 base pairs of the mitochondrial 16S rDNA (Mangold et al. 1998). PCR products of the expected sizes were purified, and then directly sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems/Perkin Elmer, Foster City, California) with the same primers used in the PCR. Generated sequences were submitted to BLAST analyses (www.ncbi.nlm.nih.gov/blast) to verify closest similarities.

Representative specimens of the ticks collected in the present study have been deposited in the following tick collections: Coleção Nacional de Carrapatos, Faculty of Veterinary Medicine, University of São Paulo, Brazil (CNC), and Department of Veterinary Parasitology, Faculty of Veterinary, Salto, Uruguay (DPVURU).

Results

A total of 18 larvae were collected from the 9 bats, resulting in a mean infestation of 2.0 ± 2.2 ticks per bat (range 1–8). Morphological examination of the larval specimens revealed them to represent the species *O. peropteryx*. Herein, we provide a redescription of the larva of *O. peropteryx* and describe its female stage for the first time.

Redescription of the larva of *Ornithodoros peropteryx* (Fig. 1)

Body

Oval; body measurement based on a slightly engorged specimen. Length (all measurements in mm) including capitulum 0.970, length without capitulum 0.637, width 0.490.

Idiosoma dorsum

Dorsal plate elongate, tapered anteriorly, surface with division type cells (Fig. 1a), length 0.283 ± 0.017 (0.256–0.317), width: 0.123 ± 0.010 (0.110–0.141). Dorsal surface provided with 14 pairs of setae, 11 dorsolateral pairs (7 anterolateral and 4 posterolateral), and 3 central pairs (Fig. 1b). Anterolateral setae (Al): Al_1 length 0.097 ± 0.010 (85–112), Al_2 length 0.091 ± 0.008 (0.078–0.102), Al_3 length 0.085 ± 0.008 (0.073–0.095), Al_4 length 0.079 ± 0.006 (0.068–0.088), Al_5 length 0.073 ± 0.003 (0.068–0.078), Al_6 length 0.075 ± 0.007 (0.068–0.088), Al_7 length 0.071 ± 0.003 (0.066–0.073). Central setae (C): C_1 length 0.088 ± 0.009 (0.073–0.102), C_2 length 0.082 ± 0.004 (0.078–0.088), C_3 length 0.078 ± 0.003 (0.075–0.083). Posterolateral setae (Pl): Pl_1 length 0.074 ± 0.003 (0.070–0.078), Pl_2 length 0.074 ± 0.001 (0.073–0.075), Pl_3 length 0.069 ± 0.005 (0.066–0.078), Pl_4 length 0.066 ± 0.005 (0.058–0.070).

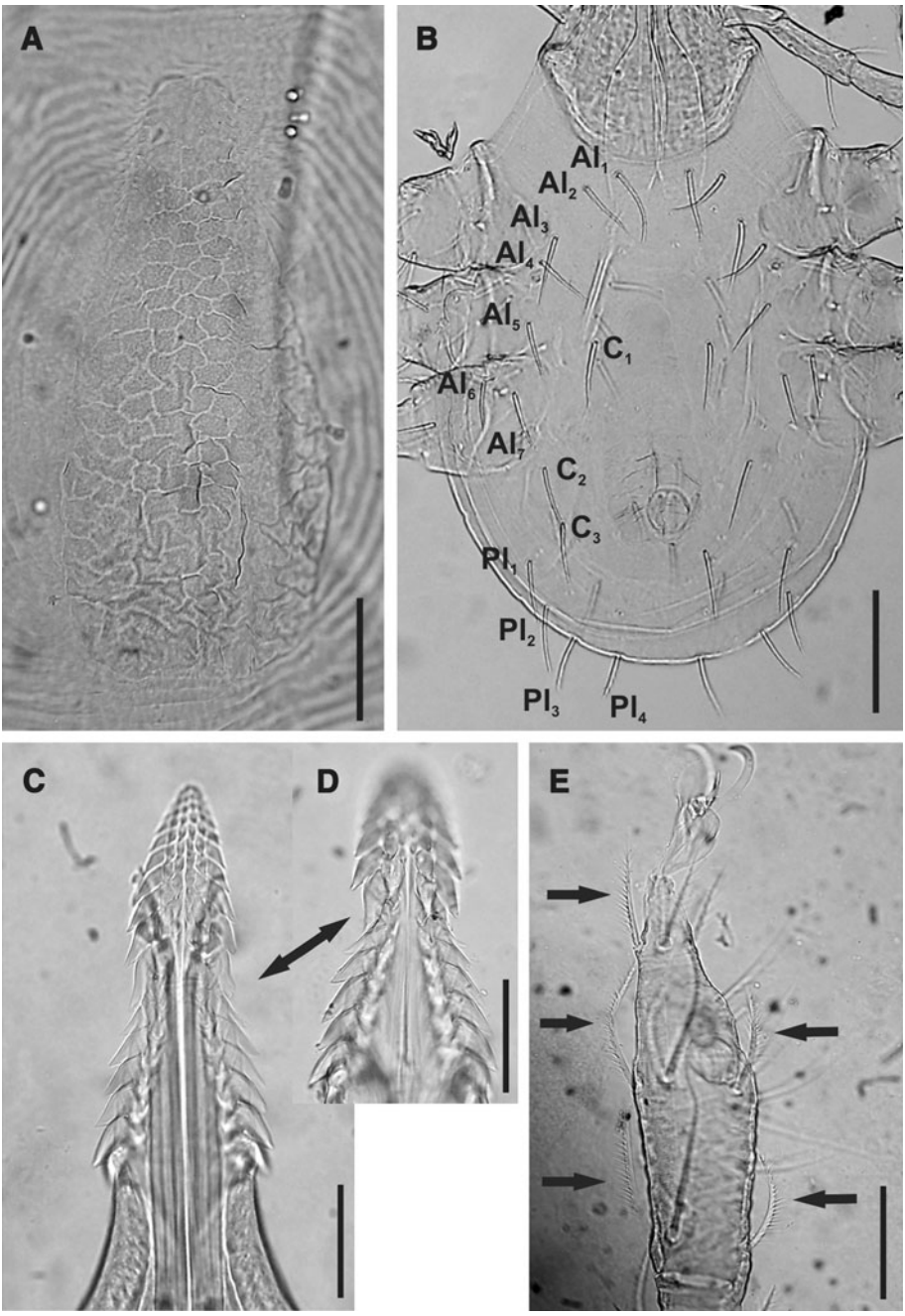

Fig. 1 *Ornithodoros peropteryx* larva. **a** Dorsal plate; bar: 0.070 mm. **b** Dorsal setae composed by 14 pairs: 7 anterolateral (*Al*), 3 central setae (*C*), and 4 posterolateral (*Pl*); bar: 0.120 mm. **c** and **d** Hypostome of two different larvae; *arrow* indicates the midlength narrow with teeth projected posteriorily; bars: 0.080 and 0.050 mm respectively. **e** Tarsus 1 with fringed setae (*arrows*); bar: 0.070 mm

Idiosoma venter

Ventral surface provided with 7 pairs of setae plus 1 pair on anal valves, 1 posteromedian seta present. Three pairs of sternal setae (St): St_1 length 0.075 ± 0.006 (0.068–0.085), St_2 length 0.068 ± 0.004 (0.061–0.073), St_3 length 0.069 ± 0.004 (0.066–0.078); one pair of postcoxal setae (Pc) length 0.055 ± 0.005 (0.049–0.061); three pairs of circumanal setae (Ca): Ca_1 length 0.061 ± 0.006 (0.054–0.071), Ca_2 length 0.064 ± 0.004 (0.058–0.068), Ca_3 length 0.074 ± 0.001 (0.073–0.075); posteromedian setae length 0.066 ± 0.008 (0.054–0.073).

Capitulum

Basis capituli, length from posterior margin of basis capituli to posthypostomal (Ph) setae Ph_1 0.209 ± 0.011 (0.197–0.227), length from posterior margin of basis capituli to insertion of hypostome 0.268 ± 0.014 (0.251–0.293), length from posterior margin of basis capituli to apex of hypostome 0.441 ± 0.018 (0.421–0.470), width 0.244 ± 0.005 (0.239–0.256). Posterior margin broadly rounded. Two pairs of posthypostomal setae; Ph_1 length 0.019 ± 0.002 (0.017–0.022), Ph_2 length 0.049, distance between Ph_1 setae 0.021 ± 0.002 (0.019–0.024), distance between Ph_2 setae 0.074 ± 0.003 (0.071–0.080). Palpi total length 0.309 ± 0.017 (0.284–0.333), segmental length/width from I to IV: (I) 0.068 ± 0.006 (0.061–0.075)/0.032 ± 0.001 (0.032–0.034), (II) 0.099 ± 0.003 (0.095–0.105)/0.035 ± 0.002 (0.034–0.039), (III) 0.092 ± 0.009 (0.078–0.097)/0.033 ± 0.002 (0.032–0.036), (IV) 0.046 ± 0.004 (0.041–0.054)/0.022 ± 0.001 (0.022–0.024). Setal number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9. Hypostome: arises from a large median extension, and narrows near midlength where denticles are projected posteriorly (Fig. 1c, d), instead of the typical postero-lateral projection of the remaining teeth. This unusual feature was clearly seen on the 10 examined specimens. Length from Ph_1 to apex 0.238 ± 0.013 (0.219–0.254), length from inferior toothed portion to apex 0.180 ± 0.014 (0.166–0.195), width in basis toothed portion of hypostome 0.084 ± 0.005 (0.078–0.088); apex blunt. Dentition formula: 2/2 apical and 3/3 in the first third, 2/2 in median and basis portion. File 1 with 15–17 denticles, file 2 with 14–16 denticles, and file 3 with 6–9 denticles.

Legs

Presence of fringed setae in coxae and several leg segments (Fig. 1e). Tarsus I length 0.250 ± 0.007 (0.239–0.261), tarsus I width 0.069 ± 0.003 (0.063–0.073). Setal formula of tarsus I: 1 pair apical (A) fringed, 1 distomedian (DM) fringed, 5 paracapsular (PC), 1 posteromedian (PM), 1 pair basal (B) fringed, 1 pair apicoventral (AV) fringed, 1 pair midventral (MV) fringed, 1 pair basiventral (BV) fringed, and 1 pair posterolateral (PL) fringed. Reticulations present in capsule of Haller's organ.

Redescription based on 10 larvae collected on *C. maximiliani* bats from Departamento del Beni, Provincia de Iténez, northeastern Bolivia (12°55′S; 62°52′W; elevation: 160 m) on 28 July 2010 (DPVURU-782–786; CNC-2301).

Description of the female of *Ornithodoros peropteryx* (Fig. 2)

Dorsal

Body outline oval, slightly pointed anteriorly, sides nearly paralel (Fig. 2a). Length from pointed anterior end to posterior body margin 3.665, breadth 2.284. Sub-marginal grooves

discrete, fused anteriorly. Integument composed of very small, shallow mammillae bearing short hairs (Fig. 2d). Discrete discs, in depressed areas over the medium area and within the marginal grooves.

Ventral

Mammillae as dorsally, but indefinite on the supracoxal folds. Genital opening at the level of coxa II, width 0.347, length 0.162, posterior labium broadly U-shaped (Fig. 2c). Transverse postanal, and medium postanal grooves present, the later terminating at the transverse postanal groove; coxal folds extending from genital labium to near the level of

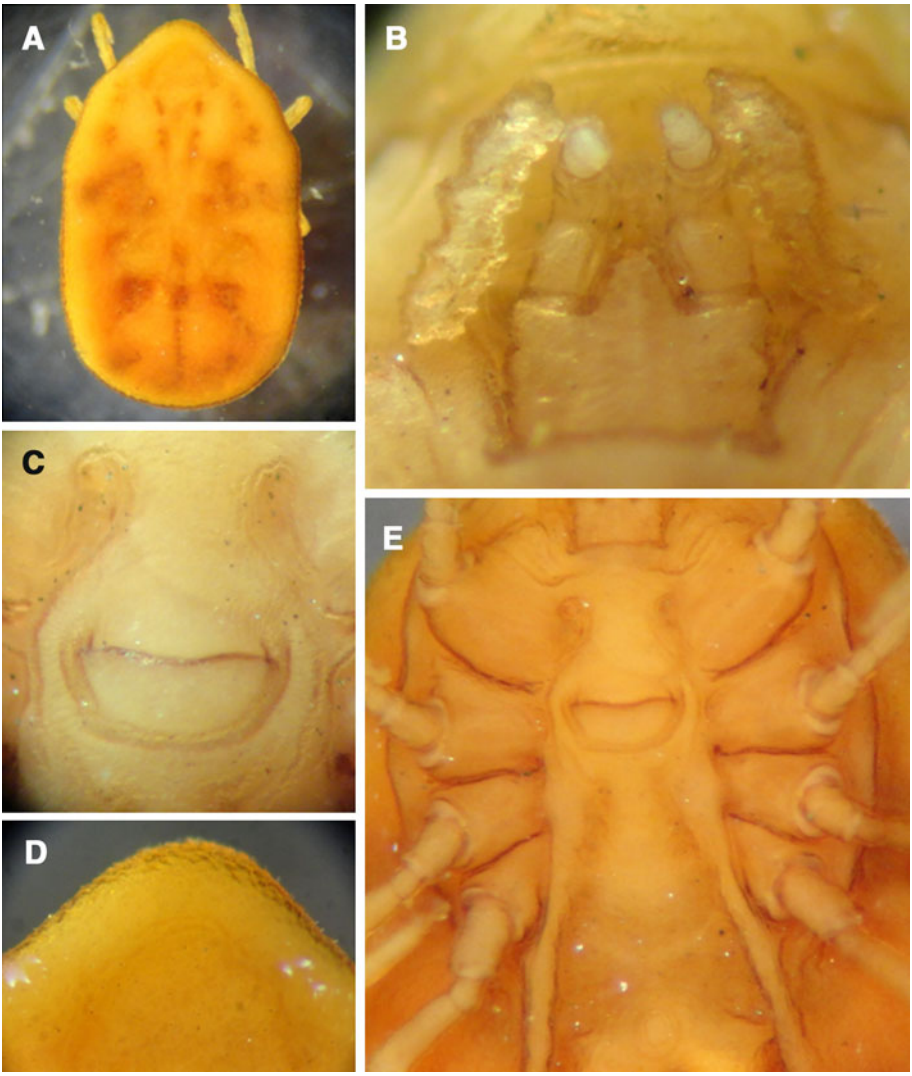

Fig. 2 *Ornithodoros peropteryx* female. **a** Dorsal view. **b** Gnatosoma. **c** Genital opening. **d** Detail on the posterior dorsal integument. **e** Coxae I–IV

the anus, where they diverge to the postero-lateral angles. Anal plate elliptical; length 0.143, width 0.113. Spiracular plate above coxa IV; length 0.097, width 0.104. Distinct cheeks; length 0.341. Width 0.107. Camerostome discrete.

Capitulum

Basis capituli 0.315 wide, 0.128 long. Palpi well developed. Hypostome very short, devoid of teeth, rounded apically, not reaching palpal article 2 (Fig. 2b); length 0.086.

Legs

All coxae contiguous, decreasing in size from I to IV, without spurs (Fig. 2e). Coxa I 0.402 long, coxa II 0.324 long, coxa III 0.251 long, coxa IV 0.237 long. Tarsus I 0.429 long, 0.103 wide; tarsus II 0.311 long, 0.104 wide; tarsus III 0.374 long, 0.099 wide; tarsus IV 0.405 long, 0.106 wide.

Description based on one unfed female specimen reared in laboratory from an engorged larva collected from a bat *C. maximiliani* from Departamento del Beni, Provincia de Iténez, northeastern Bolivia (12°55′S; 62°52′W; elevation: 160 m) on 28 July 2010 (CNC-2301).

Molecular analysis

The DNA sequence generated from the larva (427-bp) was identical to the sequence obtained from the adult female; these sequences were deposited in GenBank under the accession numbers JX888714 and KC493651. By Blast analysis, the sequence of *O. peropteryx* was most similar (88–89 % similarity) to *Antricola mexicanus* (L34323), *Antricola guglielmonei* (L34323), *Carios capensis* (JQ824326), *Nothoaspis amazoniensis* (HM047065), and *Ornithodoros sawaii* (AB242430).

Discussion

The larval specimens collected in the present study were classified as *O. peropteryx* because their external morphology was compatible with the description provided by Kohls et al. (1969) for this species. However, it was also compatible with *Ornithodoros knoxjonesi* Jones and Clifford 1972, which was described three years later, based on larvae collected on the bat *Balantiopteryx plicata* (Chiroptera: Emballonuridae) in Nicaragua (Jones and Clifford 1972). Table 1 shows a comparative analyses of the external characters of *O. peropteryx* from Colombia, *O. knoxjonesi* from Nicaragua (data from their original descriptions), and *O. peropteryx* from Bolivia (present study). It can be seen that most of the characters are similar, especially those with greater taxonomic value such as dorsal plate shape and dimensions, number of idiosomal setae, and hypostomal dental formula. Variations in some of the remaining characters are compatible with intraspecific variations between geographically distinct populations.

Jones and Clifford (1972) stated that one main difference between *O. peropteryx* and *O. knoxjonesi* is the unusual feature of the hypostome (narrows in the midlength) in the later species. However, these authors clearly demonstrated that they were not sure if this feature was really valid or if it was a result of some damage to the hypostome. In the earlier description of *O. peropteryx,* Kohls et al. (1969) did not mention this hypostome midlength

Table 1 Comparative morphological characteristics and measurements (in mm) for the larvae of *Ornithodoros peropteryx* (data from Kohls et al. 1969), *O. knoxjonesi* (data from Jones and Clifford 1972), and *O. peropteryx* from Bolivia (reported in the present study)

	O. peropteryx (Colombia)	*O. knoxjonesi* (Nicaragua)	*O. peropteryx* (Bolivia)
Dorsal plate:	Elongate	Elongate	Elongate
Dorsal plate: length	0.276–0.312	0.272	0.256–0.317
Dorsal plate: width	0.116–0.140	0.140	0.110–0.141
Dorsal setae (pairs): total	14	14–15	14
Dorsal setae (pairs): dorsolateral	11	11 a 12	11
Dorsal setae (pairs): central	3	3	3
Dorsal anterolateral setae: length	0.120–0.132	0.058–0.060	0.085–0.112
Dorsal posterolateral setae: length	0.080–0.096	0.035–0.040	0.058–0.071
Ventral setae (pairs): total	7 pairs + 1 anal pair + 1 PMS	8 pairs + 1 PMS	7 pairs + 1 anal pair + 1 PMS
Circumanal setae: Ca_1 length	0.064–0.068	0.048	0.054–0.071
Circumanal setae: Ca_2 length	0.080–0.088	0.044	0.058–0.068
Circumanal setae: Ca_3 length	0.088–0.096	0.048	0.073–0.075
Length of basis capituli (3)	0.450–0.480	0.421	0.421–0.470
Width of basis capituli	0.200–0.220	0.188	0.239–0.256
Posthypostomal setae: Ph_1 length	0.020	0.012	0.017–0.022
Posthypostomal setae: Ph_2 length	0.040–0.060	0.020	0.049
Distance of Ph_1	0.020	0.020	0.019–0.024
Distance of Ph_2	0.080–0.096	0.068–0.080	0.071–0.080
Hypostome: length (4)	0.260	ND	0.219–0.254
Hypostome: length (5)	0.200–0.204	0.168	0.166–0.195
Hypostome: width	ND	0.073	0.078–0.088
Apex	Pointed	ND	Blunt
Dental formula: first third	"3/3"	"3/3"	"3/3"
Dental formula: middle portion	"2/2"	"2/2"	"2/2"
Dental formula: basis	"2/2"	"2/2"	"2/2"
Denticles in hypostomal row 1	17–18	20	15–17
Denticles in hypostomal row 2	17	19	14–16
Denticles in hypostomal row 3	8–10	5–6	6–9

PMS posteromedian seta, *ND* not determined

narrow; however it can be clearly seen in the larval drawing provided by the authors that there is a slight narrow at the level of hypostomal midlength. Because these authors had only two specimens with entire hypostome, we hypothesize that they did not consider midlength narrow with teeth projected posteriorily as a valid character; they rather thought it to be a damage, as suggested by Jones and Clifford (1972) for *O. knoxjonesi*; therefore they did not included this "damage" on their drawing. Unfortunately, the only two type specimens of *O. peropteryx* with intact hypostome are currently lost (L. Beati, personal communication), and all the type specimens available for us had broken hypostome, precluding confirmation of this suspicion. Anyhow, at this moment we prefer to consider the specimens of Bolivia as *O. peropteryx,* and suggest that *O. knoxjonesi* could be a junior

synonym of *O. peropteryx*. Indeed, this conservative decision is less problematic than creating a new species for the specimens collected in Bolivia. To reinforce this decision, it is noteworthy that all these specimens (*O. peropteryx* from Colombia and Bolivia, and *O. knoxjonesi* from Nicaragua) were collected from bats belonging to the same subfamily (Emballonurinae), indicating that these ticks are also ecologically similar.

Noteworthy, in the dichotomic key proposed by Jones and Clifford (1972) for morphological identification of New World *Ornithodoros* larvae, these authors separated *O. knoxjonesi* from *O. peropteryx* by the number of setal pairs on ventral idiosoma, considered to be 7 in the former, and 8 in the later. However, Kohls et al. (1969) described the *O. peropteryx* larva with 7 ventral pairs plus 1 anal pair (total 8 pairs), while Jones and Clifford (1972) described *O. knoxjonesi* with 8 ventral pairs (including the anal pair). These 8 pairs are clearly seen in the drawings of the two species provided by these authors. Therefore, the taxonomic key reported by Jones and Clifford (1972) is not valid to separate the two species, reinforcing our suspicion that they are conspecifc.

Camicas et al. (1998) considered *O. knoxjonesi* a synonym of *Ornithodoros dyeri*, a species considered to be valid by Jones and Clifford (1972). However, *O. dyeri* can be easily separated from *O. knoxjonesi* by the number of ventral setae on the larval idiosoma; 9 pairs in *O. dyeri* (Jones and Clifford 1972). Moreover, *O. dyeri* differ from the ticks described in the present study through adult morphology, and because more than one nymphal stage was reported for *O. dyeri* (Cooley and Kohls 1940). Post larval stages of *O. knoxjonesi* are not known (Guglielmone et al. 2003).

As shown in Table 1; Kohls et al. (1969) considered the hypostome apex of Colombian *O. peropteryx* to be pointed, whereas we consider it to be blunt in Bolivian *O. peropteryx*. This feature was not mentioned by Jones and Clifford (1972) for *O. knoxjonesi.* We are aware that this character has a strong subjective background, and should be evaluated with caution.

Based on a single engorged larva of *O. peropteryx*, we report an argasid tick that reached the adulthood after gone through a single nymphal stage. This finding contrasts to what has been known for almost all ticks belonging to the Argasidae family, since one typical character that differs Argasidae from Ixodidae ticks is that the former go through 2 or more nymphal stages in their ontogeny, while the later go through a single nymphal stage (Balashov 1972; Sonenshine 1991). Until the present study, the only known exceptions were the argasid species belonging to the genus *Otobius,* which were observed in some occasions to reach adulthood after a single nymphal stage (Bacha 1957; Wanchinga and Barker 1986). Since biological data remain totally unknown for most of the argasid species, especially in the Neotropical region, it is possible that more argasid ticks are also characterized by a single nymphal stage.

Although we did not have the nymphal stage of *O. peropteryx* for a formal morphological description, we observed it under a stereomicroscope while it was alive, before moulting to adult. It is noteworthy that this nymph had no cheeks, and its integument was entirely micromammilated; these features can still be seen in the nymphal exuvia (Fig. 3) that was preserved in 70 % ethanol. As shown in Fig. 3, the engorged larvae, the nymphal stage (exuvia), and the adult female of *O. peropteryx* have nearly the same size. Herein we showed that the nymphal stage of *O. peropteryx* was able to moult to adult without feeding. Because the adult female has a reduced, toothless hypostome, it is possible that this female is able to reproduce without taking a blood meal (autogeny). In this case, *O. peropteryx* could be a tick species that would complete its life cycle upon a single blood meal, namely the larval feeding. The large size of the engorged larva seems to be a crucial adaptative feature for the completion of such a possible peculiar life cycle.

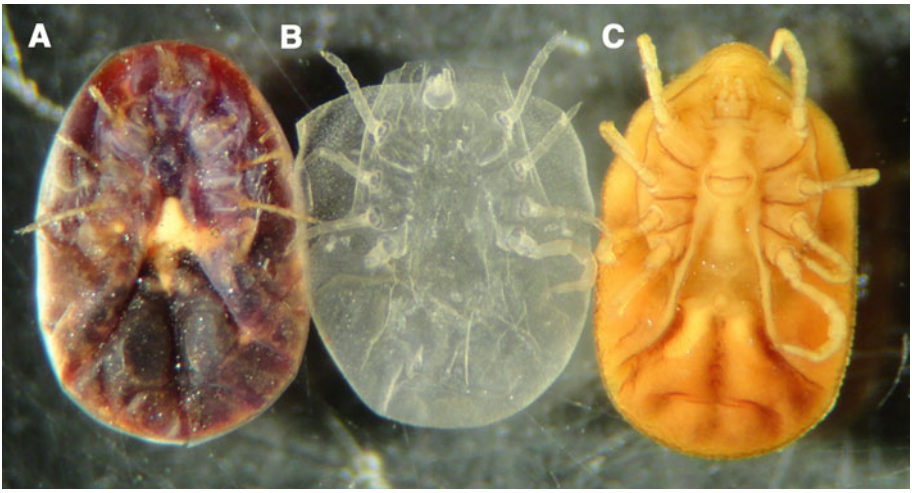

Fig. 3 Ventral view of *Ornithodoros peropteryx* engorged larva (**a**), nymphal exuvia (**b**), and adult female (**c**)

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