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Ornithodoros brasiliensis Aragão (Acari: Argasidae): description of the larva, redescription of male and female, and neotype designation

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Abstract

Ornithodoros brasiliensis is an endemic tick from Brazil and is very aggressive to humans, resulting in pain, fever and intense inflammatory response. After more than 50 years without report, this species was recently found in rural areas of São Francisco de Paula municipality, State of Rio Grande do Sul, southern Brazil, from where it was originally described. Herein, we describe the larva and redescribe the adults of *O. brasiliensis* based on scanning electron microscopy. Since the type was lost we designate the neotype specimen under the number IBSP 10409. In addition, the relationship between *O. brasiliensis* and other species from the Neotropical region that share the morphological characteristics of *Ornithodoros* with dorsal humps on tarsi, and also live under the soil and feed on hosts other than bats, are discussed. Molecular analysis inferred from a portion of the 16S rRNA mitochondrial gene is also provided and it placed *O. brasiliensis* in a cluster supported by a maximal bootstrap value (100%) with *Ornithodoros parkeri*, *Ornithodoros rostratus*, and *Ornithodoros turicata*.

Key words: *Ornithodoros brasiliensis*, argasid ticks, taxonomy, DNA sequence, Brazil

Introduction

Classical argasid tick systematics recognizes five genera, namely *Ornithodoros* Koch, *Antricola* Cooley & Kohls, *Argas* Latreille, *Nothoaspis* Keirans & Clifford, and *Otobius* Banks (Hoogstraal 1985; Guglielmone *et al.* 2010).

Clifford *et al.* (1964) recognized 7 subgenera of *Ornithodoros* as follows: *Ornithodoros* s. str., *Alveonasus* Schulze, *Alectorobius* Pocock, *Pavlovskyella* Pospelova-Shtrom, *Reticulinasus* Schulze, and described *Ornamentum* and *Subparmatus* as new subgenera that were accepted by Hoogstraal (1985). Camicas and Morel (1977) considered *Alectorobius* as a valid genus including the subgenera *Reticulinasus*, *Ornamentum* and *Subparmatus* as well as *Pavlovskyella* (this last genus as a synonym of *Theriodoros*). This classification was maintained by Camicas *et al.* (1998), but Keirans (2009) retained the subgeneric classification proposed by Hoogstraal (1985) who did not recognize *Alectorobius* as a genus.

Klompen and Oliver (1993) recognized three genera in the Ornithodorinae, *Ornithodoros*, *Otobius*, and *Carios*, based on phylogenetic analysis. According to Estrada-Peña *et al.* (2010) the generic divisions proposed by

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Hoogstraal (1985), Klompen and Oliver (1993) and Camicas *et al.* (1998) present taxonomic arrangements characterized by the genera *Ornithodoros*, *Carios* and *Alectorobius*, respectively, that include most of the species of Argasidae. However these generic arrangements are difficult to settle with our current knowledge about this family.

In the current list of valid species names of ticks in the world (Guglielmone *et al*. 2010) the Argasidae consists of 193 species and *Ornithodoros* includes 112 species. According to these authors there is widespread disagreement with reference to the genera and 133 argasid species (most placed into *Carios* or *Alectorobius*).

The genus *Ornithodoros* is currently represented by at least 50 species in the Neotropical Region, from which 13 species occur in Brazil (Dantas-Torres *et al.* 2009, who named 10 of them as belonging to *Carios*).

Morphologically, most of the Brazilian species of *Ornithodoros* share common features (e.g., presence of well-developed cheeks and legs with micromammillate cuticle) with other bat-associated argasid ticks included in the subgenus *Alectorobius*. However, two species, *Ornithodoros brasiliensis* Aragão and *Ornithodoros rostratus* Aragão, share common characters (e.g., presence of tuft of shorter setae laterally on article I of palpi, presence of humps on tarsi and absence of cheeks) with other species included in the subgenera *Ornithodoros* and *Pavlovskyella* (Clifford *et al.* 1964). Both species are known as "ground tick" due to their habits of living buried in sand or soft soil near the main host inhabitations, cellars, stables and even primitive human habitations (Martins *et al.* 2011). They are parasites of mammals, including humans, and birds (Kohls *et al.* 1965). The species *O. brasiliensis* is known just from the State of Rio Grande do Sul (Southern Brazil) where it is endemic. It is closely related to *O. rostratus* that is distributed in four countries of South America.

Although *O. brasiliensis* had not been reported in the last 50 years, it has been recently found in rural areas of São Francisco de Paula municipality, Rio Grande do Sul State, from where it was originally described. The geographical distribution of *O. rostratus* covers Argentina, Bolivia, Paraguay, and Brazil, parasitizing mammals under natural conditions, but also reptiles under laboratory conditions (Venzal *et al.* 2006).

Herein we describe the larva, and redescribe the male and female of *O. brasiliensis* based on scanning electron microscopy. In addition, we provide the mitochondrial 16S rDNA sequence, to further consider the molecular affinities of this species. Because the type of *O. brasiliensis* is not among the surviving Aragão' collection (curator Marinete Amorim, personal communication), we also designate the neotype specimen.

Materials and Methods

Adults of *O. brasiliensis* were obtained from nymphs collected from the ground of a house's basement in São Francisco de Paula municipality (29º 20' 00" S; 48º 30' 21" W), State of Rio Grande do Sul. Adults mated under laboratory conditions and the females laid batches of eggs resulting in a new generation. Larvae emerged 18–20 days after oviposition and some of them were fixed in alcohol. Ten unfed larvae were mounted in Hoyer's medium on slides and examined under a Zeiss MC80DX light microscope for morphological analyses and morphometry. Larval chaetotaxic terminology and measures follow Kohls *et al*. (1965). Five specimens each of larvae, males and females were cleaned according to Keirans *et al.* (1976) and adults were measured under a Leica MZ12 stereomicroscope. All measurements are given in mm, the mean followed by the standard deviation, and range in parentheses. Five larvae, two males and two females were prepared for scanning electron microscopy, as well as the tarsi I–IV of *O. rostratus* collected in July, 2004 on a pig from Nhecolândia municipality (19º14'S, 57º01'W) State of Mato Grosso do Sul (IBSP10461). Part of the micrographs were taken in the Laboratory of Electron Microscopy, Museu de Zoologia da Universidade de São Paulo, using a Zeiss/Leo 440 scanning electron microscope, and other part was taken in the Laboratory of Electron Microscopy, Instituto de Biociências, UNESP Rio Claro (State of São Paulo), using a Hitachi TM3000 scanning electron microscope.

Material of *O. turicata* are deposited at the Acari Collection from Instituto Butantan (IBSP 1061, 2 females from Texas, USA, number 5 from Matheson Collection, 1937) as well as adults of 2 lots of *Ornithodoros rudis* Karsch (IBSP 1062, from Panama; and IBSP 4960, from Paraguay) and 24 lots of *O. rostratus* from different regions of Brazil were also examined.

The specimens of *O. brasiliensis* were processed for DNA extraction by using the DNEasy Tissue kit (Qiagen®), following the manufacturer's recommendations with some modifications according to Desloire *et al.* (2006). For molecular taxonomic studies, a ~400-bp fragment of the 16S rDNA gene was used, as proposed by Mangold *et al.* (1998) using primers: 16S + 1 (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3'); 16S – 1 (5'-CCG GTC TGA ACT CAG ATC AAG T- 3') (Black & Piesman 1994). Fragments of PCR amplified DNA

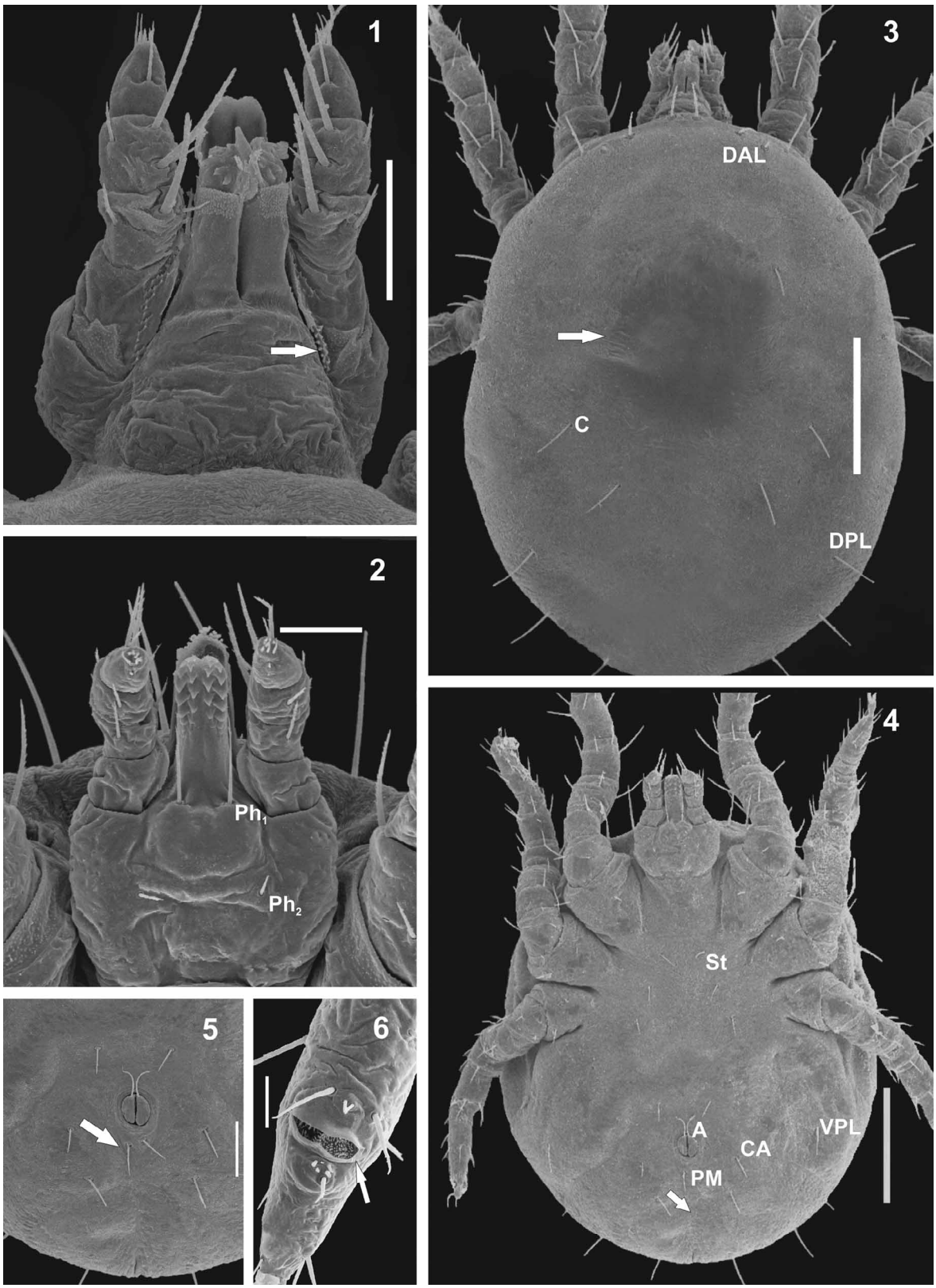
were purified with ExoSAP-IT (USB Corporation) and the products were sequenced using kit Big Dye Terminator (Perkin Elmer).

The mitochondrial 16S rDNA sequence (426bp) of *O. brasiliensis* (GU198363) was aligned with sequences previously determined for other argasid species available in Genbank, *Argas reflexus* Fabricius (AF001401), *Argas monachus* Keirans, Radovsky & Clifford (EU283344), *Argas neghmei* Kohls & Hoogstraal (DQ295781), *Argas monolakensis* Schwan, Corwin & Brown (L34305), *Ornithodoros turicata* (L34327*)*, *Ornithodoros parkeri* Cooley (EU009925), *Ornithodoros gurneyi* Warburton (AY436767), *Ornithodoros coriaceus* Koch (AY668970), *O. rostratus* (DQ295780), *Ornithodoros porcinus* Walton (L34329), *Ornithodoros moubata* (Murray) (L34328), *Carios fonsecai* Labruna & Venzal (GQ120967), *Carios mimon* (Kohls, Clifford & Jones) (GU198362), *Carios rondoniensis* Labruna, Terassini, Camargo, Brandao, Ribeiro & Estrada-Pena (EU090907), *Antricola guglielmonei* Estrada-Pena, Barros-Battesti & Venzal (EU090905) and *Antricola mexicanus* Hoffmann (L34323). Phylogenetic analyses were inferred by maximum parsimony (MP) and Bayesian (B) methods. Maximum parsimony (MP) trees were inferred using PAUP* v. 4.0b10 (Swofford 2002) and Bayesian (B) inferences were carried out with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) using GTR model. The first 100,000 trees from 1,000,000 generations were discarded as burn in, and posterior probability values were calculated on the consensus of last tree samples. *Ixodes holocyclus* (AB051844) was used as an outgroup.

Results

Description of Larva (Figures 1–6)

Gnathosoma - basis capituli, length from palpal apex to posterior margin 0.313±0.014 (0.295–0.333) by 0.274± 0.013 (0.255–0.293) wide (Figure 1). *Palpi* length from apices to basis of article I 0.217±0.008 (0.202–0.230) long by 0.062± 0.002 (0.058–0.066) wide (at the level of article II). Length of palpal articles I–IV: 0.061±0.003 (0.057–0.065), 0.050±0.004 (0.046–0.057), 0.056±0.004 (0.050–0.061), and 0.057±0.003 (0.055–0.062), respectively. Short spines present on dorsal surface of palpal article I. Number of setae on palpal articles I–IV is 0, 4, 5, and 9, respectively. Ventral basis rectangular (Figure 2); two pairs of posthypostomal setae, Ph_1 setae very long reaching the middle of hypostome, Ph_1 setae length 0.069±0.001 (0.053–0.105), Ph_2 setae length 0.028±0.004 (0.022–0.034). Distance between Ph_1 setae 0.048±0.011 (0.032–0.064) and between Ph_2 setae 0.100±0.005 (0.094–0.110). *Hypostome* arises directly from the basis capituli, not from a median extension, slightly notched apically, 0.121±0.007 (0.110–0.129) long (from Ph_1 to the apex) by 0.063±0.005 (0.050–0.068) wide. Denticles present only on the anterior half, in 2/2 arrangement, internal and external files with 7 and 8 denticles, respectively, crenulations absent. *Idiosoma* outline oval rounded anteriorly 1.003 ±0.054 (0.935–1.073) long by 0.962±0.094 (0.867–1.154) wide, excluding capitulum. *Dorsal plate* almost rounded slightly longer than wide, 0.197±0.017 (0.171–0.219) long by 0.122±0.006 (0.113–0.131) wide (Figure 3). *Dorsum* with 13–14 (typically 13) pairs of setae thin, 10–11 (typically 10) dorsolateral pairs (7 anterolateral pairs and 3 posterolateral pairs), and 3 central pairs: C (central) setae, C1, C2, C3, length 0.084±0.016 (0.046–0.105), 0.088±0.024 (0.047–0.119) and, 0.090±0.026 (0.035–0.126), respectively; DAL (dorsal anterolateral) setae, DAL1-DAL7, length 0.122±0.035 (0.041–0.150), 0.125±0.021 (0.085–0.153), 0.097±0.014 (0.060–0.110), 0.110±0.024 (0.050–0.132), 0.096±0.016 (0.053–0.112), 0.098±0.017 (0.051–0.111) and, 0.088±0.015 (0.049–0.099), respectively; DPL (dorsal posterolateral) setae, length DPL1-DPL3, 0.107±0.017 (0.063–0.125), 0.115±0.022 (0.056–0.130), and 0.113±0.022 (0.062–0.133), respectively. *Venter* with 8 pairs of setae plus 1 posteromedian seta: 3 sternal (St), length St1-St3, 0.048±0.007 (0.038–0.062), 0.045±0.009 (0.027–0.060), and 0.046±0.009 (0.026–0.055), respectively; 3 circumanal (CA), length CA1–CA3, 0.084±0.016 (0.046–0.106), 0.088±0.024 (0.047–0.119), and 0.090±0.026 (0.035–0.126), respectively; 1 ventral posterolateral (VPL) length 0.065±0.011 (0.044–0.083); and 1 anal (A) length 0.040±0.003 (0.032–0.045); plus 1 posteromedian seta (PM) length 0.056±0.004 (0.052–0.067) (Figure 4). One specimen with 2 PM (Figure 5). Mild transverse postanal groove as shown (Figure 4). *Legs.* Tarsus I 0.301±0.023 (0.266–0.339) long by 0.080±0.008 (0.068–0.094) wide. Setal formula: 1 pair A (anterior), DM (distomedian) absent, 5 PC (paracapsular), 1 PM (posteromedian), 1 pair B (basal), 1 pair AV (apicoventral), 1 pair MV (midiventral), and 1 pair PL (posterolateral). Capsule of Haller's organ open, numerous halleral spinose setae present (Figure 6).



FIGURES 1–6. Scanning electron microscopy of *Ornithodoros brasiliensis* larva. 1. Capitulum dorsal view, presence of short spines palpal article I (arrowed). 2. Capitulum ventral view. 3. Idiosoma dorsal view, dorsal plate (arrowed). 4. Idiosoma ventral view, mild transverse postanal groove present (arrowed). 5. Opisthosoma ventral view, presence of two posteromedian setae in one specimen (arrowed). 6. Capsule of Haller's organ open, numerous halleral spinose setae present (arrowed). *Abbreviations*: A, anal setae; C, dorsal central setae; CA, circumanal setae, DAL, dorsal anterolateral setae; DPL, dorsal posterolateral setae; Ph, post hypostomal setae; PM, posteromedian setae; ST (sternal setae), VPL, ventral posterolateral setae. *Scale bars*: 1, 90µm; 2, 60µm; 3–4, 300µm; 5, 40µm; 6, 30µm.

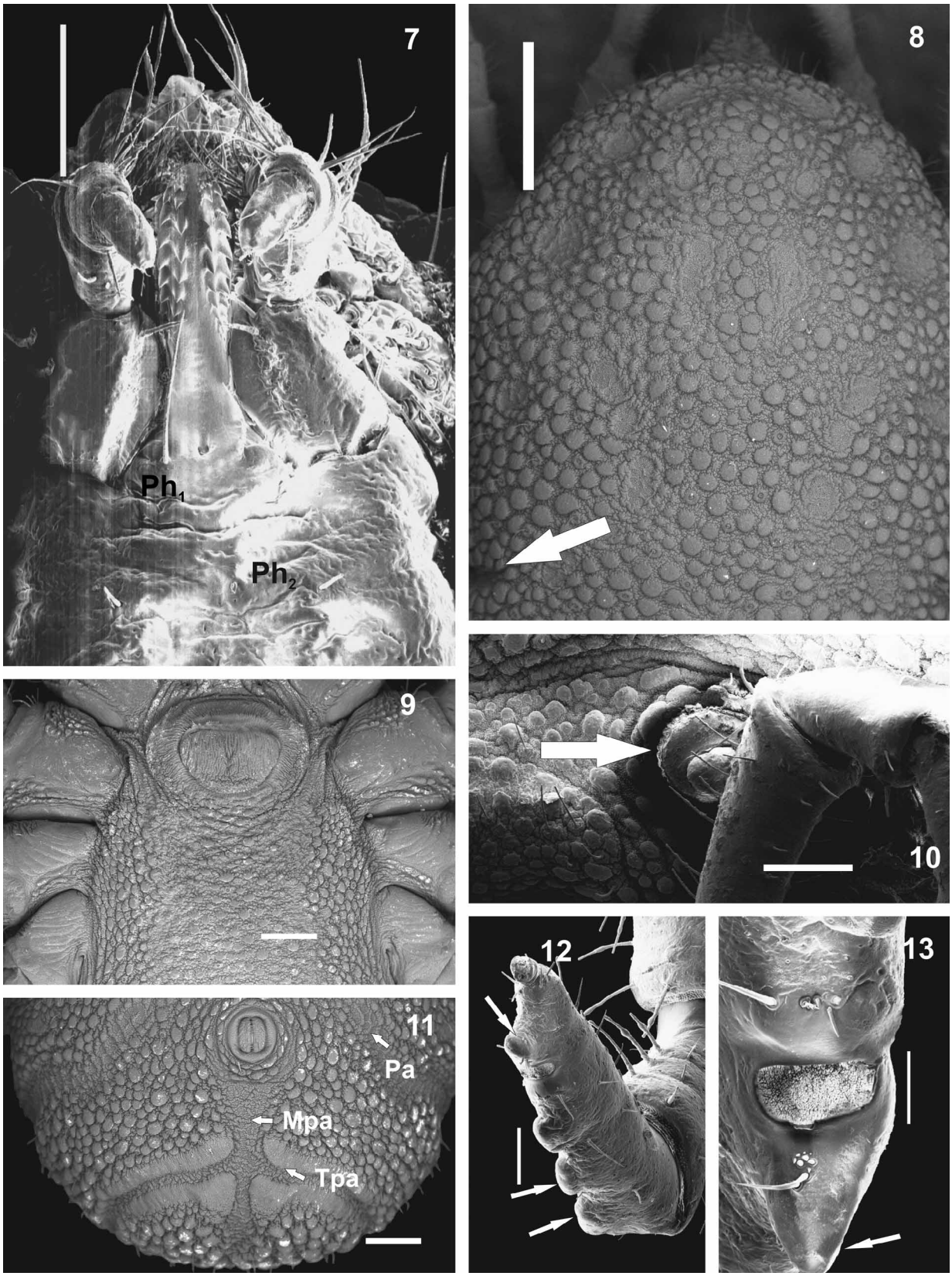
Redescription of Adults (Figures 7–20, 26–27)

Female (Figures 7–13, 26–27): *Gnathosoma* ventral basis slightly wider than longer, with irregular transverse wrinkles; length since basis to apex of hypostome 0.688±0.012 (0.683–0.695) by 0.925±0.010 (0.920–0.930) wide; palpi rounded laterally with tufts of long setae present on articles 2 and 3, tuft of shorter setae laterally on article I, average length of palpal articles 1–4: 0.322, 0.241, 0.168, and 0.161, respectively. Hypostome short and small rounded apically, 0.585±0.010 (0.583–0.590) long (from Ph_1 to apex) by 0.146 ± 0.015 (0.140–0.155) wide, dental formula 2/2, with 3/3 near to apex. Denticles arranged from the median to apex, external and internal rows with 9, 8 and 1 denticles, respectively; corona with small and numerous denticles. With 2 pairs of posthypostomal setae, Ph_1 0.307±0.005 (0.305–0.310) long, reaching about three-fourths length of hypostome, Ph_2 0.005±0.001 (0.005–0.006) long (Figure 7). Distance between setae of Ph_1 0.145±0.015 (0.140–0.155), between setae of Ph_2 0.380±0.005 (0.375–0.380). Hood reduced, camerostome well-developed, cheeks absent. *Idiosoma* outline oval, rounded anteriorly, broadest at level of coxa IV. Length from anterior to posterior body margin 7.475±0.380 (7.040–7.760), breadth 4.643±0.350 (4.242–4.880); submarginal and dorsoventral grooves distinct; mammillae large, close but not crowded, with small numerous hairs uniformly distributed and with many setae present on anterior and lateral margins; discs present (Figure 8). *Idiosoma ventral* with mammillae as dorsally, larger on the posterior margin, undefinitive on the supracoxal folds. Genital aperture at the level of coxae II, posterior margin rounded (Figure 9). Preanal (PA), transverse postanal (Tpa) and medium postanal (Mpa) grooves present (Figure 11); the later crossing the transverse terminating at the posterior border. Coxal folds extending from coxae I to coxae IV. Four pairs of bulging lateral structures resembling large mammillae on supracoxal folds between legs I–IV, visible in both optical and scanning microscopy (Figures 18, 19). Spiracular plates rounded, macula prominent (Figure 10), located laterally between the coxae III–IV, length 0.275±0.010 (0.265–0.285), breadth 0.265±0.010 (0.260–0.270). *Legs:* mammillae absent on all tarsi, proximal dorsal humps and claws present; tarsus I with 4 dorsal humps, 1 pointed (distal), anterior to Haller's organ (Figure 12) and 3 rounded (proximal), posterior (Figure 11), length 0.947±0.046 (0.925–1.000); tarsi II, III also present proximal and distal humps dorsally, smaller than those from tarsus I (Figure 26). Tarsus IV with 1 distal and 1 proximal strong dorsal humps (Figure 27), length 1.165±0.065 (1.160–1.220). Haller's organ with large capsule opening transversely with numerous halleral setae, and a group with 8 small plus 2 bigger prehalleral setae (Figure 12).

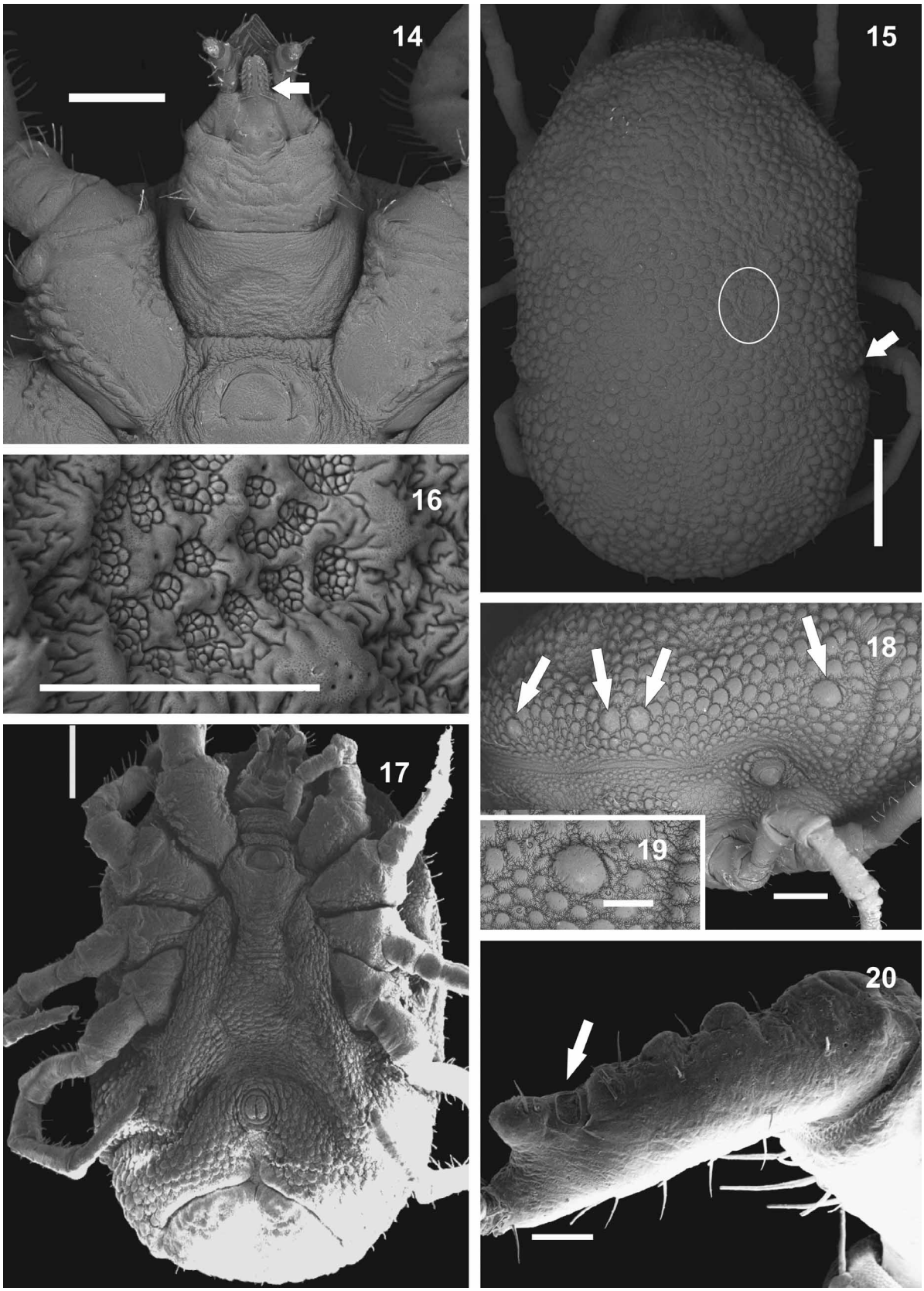
Male (Figures 14–21, 26–27): *Gnathosoma* smaller than female, length since basis to apex of hypostome 0.320±0.002 (0.312–0.315) by 0.540±0.010 (0.545–0.550) wide (Figure 14). *Idiosoma* outline oval, rounded anteriorly, broadest at level of coxae II (where are placed the second and third pairs of bulging lateral structures) and IV close to the dorsoventral groove (Figures 15, 18); length from anterior to posterior body margin 5.755±0.350 (5.466–6.125), breadth 3.500±0.250 (3.300–3.780); integument with small numerous mammillae and setae, discs present (Figures 15, 16). Ventral grooves similar to the female as well as the 4 pairs of bulging lateral structures on supracoxal folds (Figure 18, 19). Spiracular plates rounded as in female, prominent located laterally between the coxae III–IV (Figure 18), length 0.264±0.010 (0.260–0.270), breadth 0.255±0.010 (0.250–0.260). *Legs:* tarsus I with 4 dorsal humps, 3 posterior and 1 anterior to Haller's organ, length 0.695±0.020 (0.675–0.725) (Figure 20). Tarsus IV similar to the female (Figure 25), length 0.945±0.020 (0.925–0.955); tarsi II, III also present proximal and distal rounded humps and 3 smaller humps between them (Figure 26). Tarsus IV with proximal and distal humps smaller than the other tarsi (Figure 27). Haller's organ as in female (Figure 20).

For comparison, the tarsi I–IV of *O. rostratus* (Figures 21–25) and the tarsi III and IV of *O. brasiliensis* (Figures 26–27) were illustrated.

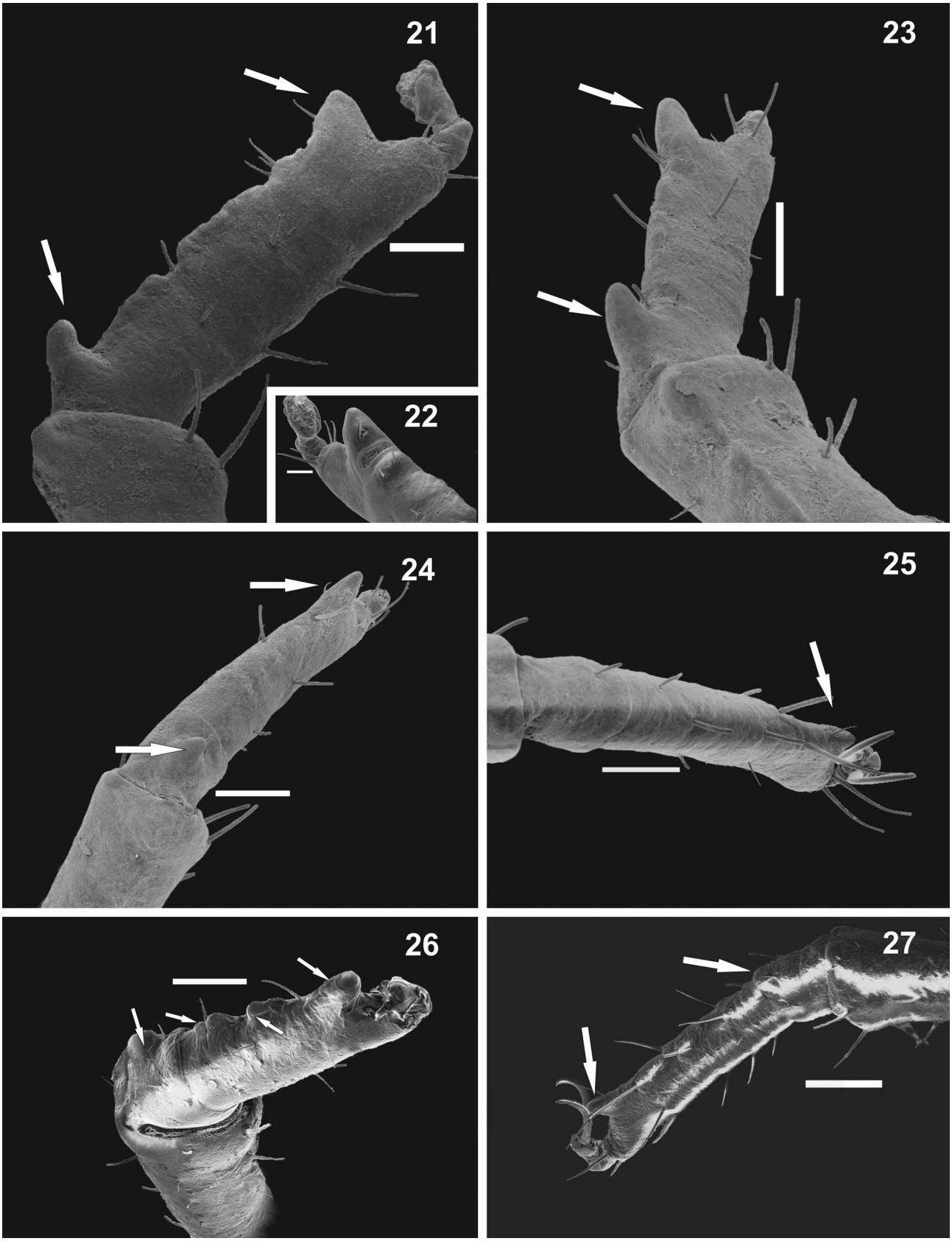
Through the phylogenetic relationships based on a partial sequence of the mitochondrial 16S rDNA gene (Figure 28), *O. brasiliensis* grouped with *O. rostratus* within a branch strongly supported (100% of bootstrap) that also contained the sequences of *O. parkeri*, *O. coriaceus*, *O. gurneyi* and *O. turicata*, but the sequence divergence between these species varied from 16 to 22%. The sequence divergence between *O. brasiliensis* and *O. rostratus* (Acession Number DQ295780) was 15%.



FIGURES 7–13. Scanning electron microscopy of *Ornithodoros brasiliensis* female. 7. Capitulum. 8. Idiosoma dorsal view, dorsoventral groove distinct (arrowed). 9. Idiosoma ventral view. 10. Idiosoma lateral view, spiracular plate (arrowed). 11. Preanal, transverse postanal and medium postanal grooves (arrowed). 12. Tarsus I, with a pointed dorsal hump, anterior to Haller's organ, and 3 posterior rounded (arrowed). 13. Hallers' organ, with large capsule and distal hump (arrowed). *Abbreviations*: PA, preanal groove; Ph, post hypostomal setae; Tpa, transverse postanal groove; Mpa, medium postanal groove. *Scale bars*: 7–10–12, 300µm; 8–9–11, 500µm; 13, 60µm.



FIGURES 14–20. Scanning electron microscopy of *Ornithodoros brasiliensis* male. 14. Capitulum, hypostome smaller than the female (arrowed). 15. Idiosoma dorsal view, the circle corresponds a disc of the tegument, dorsoventral groove distinct (arrowed). 16. A disc of the tegument increased 500x. 17. Idiosoma ventral view. 18. Four pairs of bulging lateral structures on supracoxal folds (arrowed). 19. Bulging lateral structure in focus. 20. Tarsus I, rounded and prominent dorsal humps present, large capsule of Haller's organ with numerous halleral setae (arrowed). *Scale bars*: 14, 500µm; 15, 1000µm; 16, 200µm; 17, 600µm; 18, 500µm; 19, 250µm; 20, 60µm.



FIGURES 21–27. Tarsi of adults of *Ornithodoros rostratus* (Figures 21–25) and *Ornithodoros brasiliensis* (Figures 26–27). 21. Tarsus I of *O. rostratus*, the distal and proximal dorsal humps (arrowed). 22. Capsule of Haller's organ of *O. rostratus.* 23–25. Tarsi II–IV of *O. rostratus*, distal and proximal humps present on figures 23–24 (arrowed), and the proximal hump on tarsus IV is absent on figure 25. 26. Tarsus III of *O. brasiliensis*, rounded dorsal humps present (arrowed). 27. Tarsus IV of *O. brasiliensis*, proximal and dorsal humps present. (arrowed) *Scale bars*: 21, 90µm; 22, 40µm; 23–25, 120µm; 26–27, 200µm.

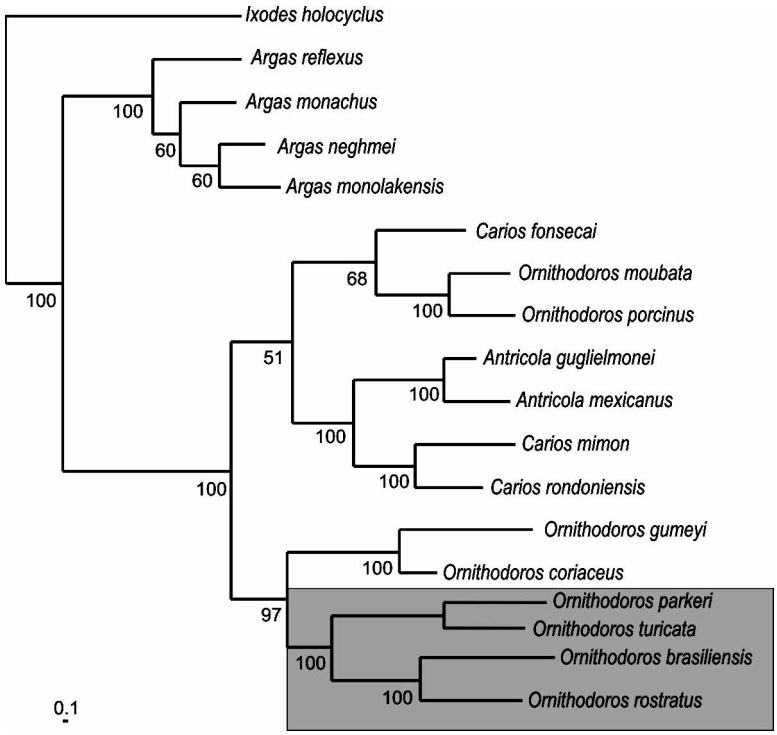


FIGURE 28. Phylogenetic tree based on 16 Argasidae ticks and *Ixodes holocyclus* (outgroup). The alignment was made with 631 characters (including gaps) of the 16S rRNA mitochondrial gene used in the analysis. The Bayesian support (posterior probabilities) values are derived from 500 replicates. The branch length scale is the number of substitutions per site. The species with similar morphology are in a grey box.

Neotype designation

The neotype was designated under the number IBSP10409 (female, molted in laboratory from nymph collected at São Francisco de Paula, Rio Grande do Sul, 08/VIII/2008, leg J.R. Martins). The measurements of this specimen are:

Gnathosoma ventral basis length since basis to Ph_1 0.680 by 0.970 wide; length of palpal articles 1–4: 0.300, 0.238, 0.165, and 0.159, respectively. Hypostome 0.430 long by 0.150 wide. *Idiosoma* length 6.600 by 4.020 wide. Tarsus I length 0.900; tarsus IV length 1.100.

Discussion

The morphological similarities and common life cycle history show some distinct groups in *Ornithodoros*, even though the taxonomic and molecular situation of the genus *Ornithodoros* is not clear yet and subgenera have not been considered in the lists of valid species (Horak *et al.* 2002; Guglielmone *et al.* 2003, 2010).

In the Neotropical region, *O. brasiliensis*, *Ornithodoros furcosus* Neumann, *Ornithodoros nicollei* Mooser, and *O. rostratus* share characters such as the absence of cheeks, presence of dorsal humps (protuberances) on tarsi in adults, and hypostomal formula 2/2 on larvae, among others (Clifford *et al.* 1964). Although adults of *O. brasiliensis* present the same morphological characteristics, the larvae molt to first instar nymphs without feeding, in contrast to the other three species that need a rapid blood meal in each stage.

Species relationships

The larva of *O. brasiliensis* is bigger than any other known larva of Ornithodorinae. Despite of its enormous size, the length of the hypostome and palpi of *O. brasiliensis* is smaller than those of *O. rostratus* and *O. nicollei*. In *O. brasiliensis* the dorsal plate is large, almost rounded and smooth, and the cuticle is so thin that the dorsal plate becomes transparent and hard to see when submitted to clarification methods. The dorsal plates of *O. nicollei* and *O. rostratus* are smaller and have an anterior concavity. On the other hand the short spines on the dorsal surface of palpal article I in *O. brasiliensis* are also present in the larvae of *O. nicollei* and *O. rostratus*. According to Clifford *et al.* (1964), this character could be observed in the larva of *O. turicata* but the dorsal plate in this species is absent, as it is in *O. parkeri* (Kohls *et al.* 1965).

Regarding the larval chaetotaxy, *O. brasiliensis* resembles *O. rostratus* but the length of setae Ph_1 and the distance between Ph_1–Ph_1 is twice that compared with *O. brasiliensis*. Setae Ph_2 is shorter but the distance between them is bigger than in *O. rostratus*. On the other hand the length of DAL and DPL is similar in both species but in *O. rostratus* these setae are thicker. The length of CA_1–CA_3 is slightly bigger in *O. brasiliensis*. The length of dorsal and ventral setae of *O. nicollei* is similar to those of *O. rostratus*. The setal formula of tarsus I is similar in these species but the length is three times bigger in *O. brasiliensis*.

Aragão and Fonseca (1961) presented a key for adult species of *Ornithodoros* from Brazil. According to these authors, the main morphological character used to separate the species *O. brasiliensis* from *O. rostratus* was the presence and absence of dorsal humps on tarsus IV in *O. brasiliensis* and *O. rostratus*, respectively. In fact, both species have dorsal humps in all tarsi but the number and shape of the humps are different. In *O. brasiliensis* the dorsal humps are rounded (Figures 12, 20, 26, 27) while in *O. rostratus* they are bigger and more pointed (Figures 21–25). Tarsus IV in *O. brasiliensis* have distal and proximal humps, but these are small (Figure 27), whereas in *O. rostratus* the proximal humps are absent (Figure 25). On the other hand, tarsus IV in *O. turicata* has only a distal hump, similar to *O. rostratus* and in *O. parkeri* it is absent (Cooley & Kohls 1944).

Although *O. parkeri* and *O. turicata* are Nearctic species (Kohls *et al.* 1965; Guglielmone *et al.* 2003), they are morphologically and molecularly close to *O. brasiliensis* and *O. rostratus*. These last three species have four pairs of bulging structures present laterally in the supracoxal folds. These structures are located at the level of leg I (a pair), two pairs at the level of leg III and one pair at the level of leg IV (Figures 18, 19). They were also observed in nymphs from the instar II of *O. brasiliensis*.

Molecular analysis

The DNA sequence is closely related to those of *O. brasiliensis*, *O. parkeri*, *O. rostratus* and *O. turicata* (Figure 28). Nevertheless, the lack of molecular studies with the full diversity of argasid ticks hinders the understanding of phylogenetic relationships among the Argasidae. Recent studies have shown that the genus *Ornithodoros* is paraphyletic (Labruna *et al.* 2008; Nava *et al.* 2009; Barros-Battesti *et al.* 2010). The joint study of morphological and molecular characters and the inclusion of new species in these studies are necessary for real discussion of the phylogenetic position of genus *Ornithodoros* as well as other genera within the Argasidae.

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