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Immature argasid ticks: diagnosis and keys for Neotropical region

Carrapatos argasídeos imaturos: diagnoses e chaves para a região Neotropical

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Abstract

Many argasid tick species are known only through their larval descriptions, in which the chaetotaxy, together with other external morphological characteristics, has been used to separate genera and species. However, the illustrations of these features are based on optical microscopy alone and many of these features are not clearly defined. Because of the difficulties in determining the larval and nymph stages of some genera, we have prepared illustrated keys for the immature stages of argasids, including an up-to-date list of the known species of the Neotropical region. We have also included an illustrated key for larvae of the *Ornithodoros* species from Brazil, based on scanning electron microscopy.

Keywords: Argasidae, Ornithodoros, immature, identification, key, Brazil.

Resumo

Muitos carrapatos argasídeos são conhecidos somente por descrições larvais, nas quais a quetotaxia associada a outros caracteres morfológicos tem sido usada para separar gêneros e espécies. No entanto, as ilustrações sobre esses caracteres são baseadas somente em microscopia óptica e muitos deles não estão claramente definidos. Devido às dificuldades em determinar estágios larvais e ninfais de alguns gêneros, elaboramos chaves ilustradas para os estágios imaturos de argasídeos, incluindo uma lista atualizada de espécies conhecidas da região Neotropical. Incluímos também uma chave ilustrada para larvas das espécies de *Ornithodoros* do Brasil baseada em microscopia eletrônica de varredura.

Palavras-chave: Argasidae, Ornithodoros, imaturos, identificação, chave, Brasil.

Introduction

The argasid fauna comprises around 200 known species in the world (NAVA et al., 2009; GUGLIELMONE et al., 2010; DANTAS-TORRES et al., 2012; VENZAL et al., 2013a). Of these, 87 are recognized in the Neotropical region, distributed into five genera: *Antricola* (17 species), *Argas* (12 species), *Ornithodoros* (55 species), *Nothoaspis* (2 species), and *Otobius* (1 species). In Brazil, 21 species of Argasidae are currently known (DANTAS-

*Corresponding author: Darci Moraes Barros-Battesti Laboratório Especial de Coleções Zoológicas, Instituto Butantan, Av. Vital Brasil, 1500, CEP 05503-900, São Paulo, SP, Brasil e-mail: darci.battesti@butantan.gov.br TORRES et al., 2012), as follows: 16 of *Ornithodoros*, 3 of *Antricola*, 1 of *Argas*, 1 of *Nothoaspis*. There is no current record of *Otobius* in Brazil, but this genus is represented by two species around the world; in the Neotropics, only *Otobius megnini* (Dugès, 1883) has been recorded.

The adult and nymphal stages of some argasid species are morphologically very similar, especially within the genus *Ornithodoros*, which makes it problematic to critically assess distribution and species relationships based on previous contributions. Most descriptions of nymphal stages are poor in details, lacking figures or illustrations of the instars, which

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hinders morphological differentiation (ESTRADA-PEÑA et al., 2010). In the absence of DNA studies on these species, only larval morphological features have been adequately defined for specific determination (VENZAL et al., 2008).

Because of the difficulties in determining the larvae and nymphs of some genera and species, we have prepared illustrated keys for these immature stages based on optical and scanning electron microcopy, in order to help in identifying the generic taxa of Argasidae in the Neotropics. Here, we also present a current list of all argasid species in the Neotropical region, including a key to the genera of immature stages in this region and also a key to larvae of *Ornithodoros* species in Brazil.

Materials and Methods

The specimens illustrated in this study were cleaned by means of ultrasound (40 kHz), using distilled water and commercial detergent in the proportions 8:2. The cleaning process was completed in three separate stages: 3 minutes in water + detergent, 2 minutes in distilled water, and an additional 2 minutes in distilled water. Micrographs were made by using a Zeiss LEO 440 digital scanning microscope. The plates were made using CorelDraw X 5, version 2010. The figures were prepared Photoshop CS 6, version 2012.

The diagnosis and keys for immature ticks of Argasidae in the Neotropics were based on Cooley and Kohls (1944), Kohls and Clifford (1964), Kohls et al. (1965, 1969, 1970), Clifford et al. (1964), Keirans et al. (1977), Klompen (1992), Klompen and Oliver (1993), Venzal et al. (2008), Labruna et al. (2008, 2011), Labruna and Venzal (2009), Nava et al. (2010, 2013), Barros-Battesti et al. (2011, 2012), Dantas-Torres et al. (2012) and Venzal et al. (2013a).

The larval terminology for *Antricola*, *Argas*, and *Nothoaspis* followed Kohls et al. (1965, 1969, 1970) and Nava et al. (2010). Nymphs were determined by means of the original descriptions as well as from specimens obtained from colonies maintained at the parasitology laboratory of the Butantan Institute. The key for larval species of *Ornithodoros* in Brazil was based on Kohls et al. (1965) with modifications proposed by Venzal et al. (2008) and Labruna and Venzal (2009).

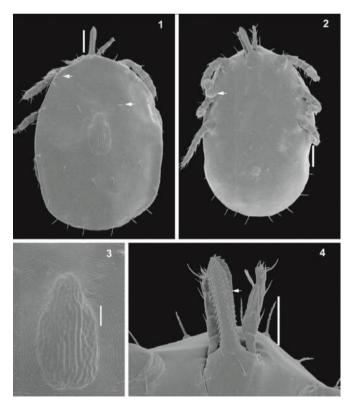
Results and Discussion

The main morphological characteristics of adults and nymphs of the family Argasidae are as follows: tegument granulated, mammillated, coriaceous or tuberculated; dorsal scutum absent. The spiracular plates are small and they are localized lateroposteriorly, between coxae III and IV. There is a pair of coxal glands in a ventral position that open between coxae I and II. All palpal articles are free, and article IV is not inserted in a depression in article III. Eyes, if present, are located laterally, close to the supracoxal folders. Pulvilli absent or rudimentary in nymphs and adults; however, they can be well developed in *Antricola* larvae. Sexual dimorphism is generally slight, based mainly on the shape of the genital aperture (VENZAL et al., 2006).

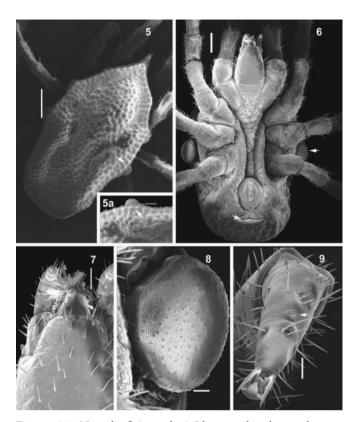
Larvae present a vestigial scutum and the capitulum is placed at the terminal position, while in nymphs and adults the capitulum is inserted in the ventral idiosoma; the camerostome is more accentuated in post-larval stages. Few species present eyes. The generic diagnosis for larvae and nymphs of argasid and lists of species in the Neotropical region are shown below. Species present in Brazil are in bold.

Generic diagnosis

Antricola – Larvae (Figures 1-4): dorsal surface with 14 pairs of setae, typically 14 (11 dorsolateral, 3 central dorsal); dorsal plate, large and elongated with lateral margins parallel, narrowing anteriorly; eyes absent; ventral surface with 11 pairs of setae (3 sternal setae, 3 postcoxal setae, 4 circumanal + 1 on valves), and 1 posteromedial seta; 2 pairs of long post hypostomal setae, hypostome pointed, dentition 3/3 in anterior three-fourths, then 2/2 posteriorly to basis; palpi with 18 setae, number of setae on palpal article 1-4, respectively 0, 4, 5 and 9; pulvilli large, claws absent (except in A. marginatus); dorsal hump absent; Haller's organ with a rounded capsule, open only in a small central portion. Nymphs (Figures 5-9): outline suboval, pointed anteriorly; idiosoma covered by tubercles, most of them bearing short setae, some single, others in groups; hypostome short, broad and rounded apically, with small denticles on anterior and lateral margins; cheeks absent; spiracular plate oval, relatively large, expanded and dorsally visible in some specimens, with numerous minute



Figures 1-4. Larva of *Antricola*. 1. Idiosoma dorsal view, showing dorsolateral and central setae (arrow). 2. Idiosoma ventral view, showing pulvilli enlarged (arrow). 3. Dorsal plate. 4. Gnathosoma ventral view, showing hypostome 3/3 (arrow). *Scale bars*: 1-2, 90 μ m; 3, 30 μ m; 4, 60 μ m.



Figures 5-9. Nymph of *Antricola.* 5. Idiosoma dorsal view, showing lateral tubercles elongated (arrow). 5a. Lateral tubercles in detail (arrow). 6. Idiosoma ventral view, showing post-anal groove weakly produced and a wide prominent tubercle posterior to the transverse post-anal groove, without setae (arrow). 7. Capitulum, showing hypostome slightly longer than wide with a few small denticles (arrow). 8. Spiracular plate. 9. Tarsus I, showing Haller's organ with a rounded capsule open only in a small central portion (arrow). *Scale bars*: 5, 600 μ m; 5a, 200 μ m; 6, 300 μ m; 7, 80 μ m; 8, 40 μ m; 9, 60 μ m.

pores; dorsal humps on tarsus I absent; claws present; Haller's organ similar to the larvae.

List of species (Neotropics, N = 17; Brazil, N = 3): A. armasi De La Cruz and Estrada-Peña, 1995; A. centralis De La Cruz and Estrada-Peña, 1995; A. cernyi De La Cruz, 1978; A. coprophilus (McIntosh, 1935); A. delacruzi Estrada-Peña, Barros-Battesti and Venzal, 2004; A. granasi De La Cruz, 1973; A. guglielmonei Estrada-Peña, Barros-Battesti and Venzal, 2004; A. habanensis De La Cruz, 1976; A. hummelincki De La Cruz and Estrada-Peña, 1995; A. inexpectata Estrada-Peña, Barros-Battesti and Venzal, 2004; A. marginatus (Banks, 1910); A. martelorum De La Cruz, 1978; A. mexicanus Hoffmann, 1958; A. naomiae De La Cruz, 1978; A. occidentalis De La Cruz, 1978; A. siboneyi De La Cruz and Estrada-Peña, 1995; and A. silvai Cerný, 1967.

Comments: The diagnosis for larvae of *Antricola* was based on Clifford et al. (1964), Kohls et al. (1965) and De La Cruz (1976). The nymphal diagnosis followed De La Cruz (1976). There are at least 2-3 nymphal instars (ESTRADA-PEŃA et al., 2008), although the exact number of instars for this genus is unknown.

Argas – Larvae (Figures 10-14): dorsal surface with around 25-30 pairs of setae (14-16 DL; 12-13 C), dorsal plate oval and elongated; ventral surface with less than 7 pairs of setae + 1 pair on valves; posteromedial seta present or absent; 2 pairs of short post-hypostomal setae; hypostome rounded at apex, dentition 2/2 at basis to 3/3 at apex. Nymphs (Figures 15-18): outline oval, discs present, distributed more or less symmetrically dorsally; idiosoma mammillated, flattened dorsoventrally, with suture and lateral margin demarcating the dorsal and ventral surfaces; Haller's organ with transversely slit-like aperture, placed slightly laterally.

List of species (Neotropics, N = 12; Brazil N = 1): *A. cucumerinus* Neumann, 1901; *A. dalei* Clifford, Keirans, Hoogstraal and Corwin, 1976; *A. dulus* Keirans, Clifford and Capriles, 1971; *A. keiransi* Estrada-Peña, Venzal and González-Acuña, 2003; *A. magnus* Neumann, 1896; *A. miniatus* Koch, 1844; *A. monachus* Keirans, Radovsky and Clifford, 1973; *A. moreli* Keirans, Hoogstraal and Clifford, 1979; *A. neghmei* Kohls and Hoogstraal, 1961; *A. persicus* (Oken, 1818); *A. radiatus* Railliet, 1893; and *A. transversus* Banks, 1902.

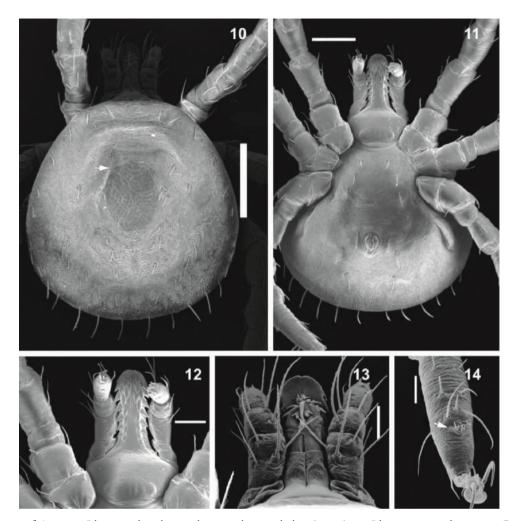
Comments: Most species are known from all stages. Larval and nymphal diagnoses were based on Kohls et al. (1970). This genus presents 2-4 nymphal instars, but usually 3; the main factor affecting the number of instars is the volume of ingested blood (SANTOS et al., 2010).

Nothoaspis - Larva: Dorsal plate with isosceles triangle shape occupying entire length of the dorsum of unfed specimens; dorsal surface with 12-13 pairs of setae; hypostome with apex pointed, dental formula 2/2 with 20 denticles in each row, corona absent. Nymphs: Idiosoma twice longer than wide, anteriorly more abruptly narrowing than posteriorly; false shield (nothoaspis) covered by cells (irregular in shape and size) occupying the anterocentral area of dorsum, most of them at least with 1 seta; setae short, except for posterior margin of idiosoma, where setae are larger. Ventral surface with integument also covered by cells (irregular in shape and size), except for a narrow area located between coxae I and III; anus subcircular, lateral to coxa IV, valves each with 1 pair of setae; spiracular plate small, similar to that of male. Basis capituli subrectangular in outline, with 1 pair of post-hypostomal setae and at least 7 pairs of sublateral setae, bordered posteriorly by integumental fold; postpalpal setae absent; hood large, broadly rounded, not entirely covering capitulum, cheliceral blades, palpal articles II-IV visible dorsally; ventrally, article I forms elongate flaps protecting the pointed hypostome, dental formula 4/4 apically, 5/5 at base.

List of species (Neotropics, N = 2; Brazil, N = 1): *N. reddelli* Clifford and Keirans, 1975; and *N. amazoniensis* Nava, Venzal and Labruna, 2010.

Comments: The diagnoses of larvae and nymphs were based on the original descriptions. Two nymphal instars: the first one does not feed and has reduced hypostome (NAVA et al., 2010).

Ornithodoros – Larvae (Figures 19-28): dorsal surface of idiosoma with 13-14 pairs of setae (with some exceptions*), dorsal plate absent in few species, but present in the majority, varying in shape, from triangular to piriform (bat-associated group) to elongated subrectangular with anterior extremity narrowed; ventrally with 7-8 pairs + 1 on valves, and 1 unpaired seta posteromedially (which may be absent). Basis capituli with lateral angles slightly

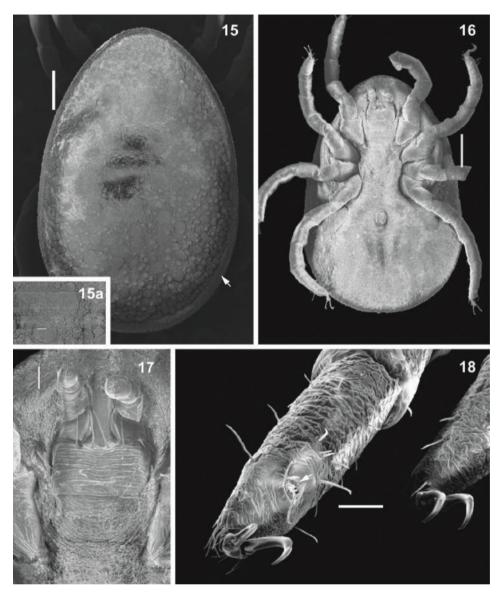


Figures 10-14. Larva of *Argas.* 10. Idiosoma dorsal view, showing elongated plate (arrow). 11. Idiosoma ventral view. 12. Gnathosoma ventral view, showing dentition 4/4 close to the apex. 13. Gnathosoma dorsal view. 14. Tarsus I, showing Haller's organ with small capsule aperture transversely slit-like (arrow). *Scale bars*: 10, 200 µm; 11, 120 µm; 12, 60 µm; 13, 40 µm; 14, 140 µm.

rounded, lateral auriculae present or absent, hypostome with apex rounded or pointed, dental formula: 5/5 to 2/2 at apex, 4/4 to 2/2 in medial portion and 2/2 at basis; Haller's organ with capsule aperture transversely slit-like, large, occupying all of dorsum with many small setae, or small occupying part of the dorsum. Nymphs (Figures 29-32): outline oval, slightly pointed anteriorly, idiosoma covered by tile-like mammillae; presence of 4 pairs of bulging lateral structures resembling large mammillae on supracoxal folds between legs I-IV (soil-living group) or absent (bat-associated group), hypostome rounded on apex; humps present (only in the soil-living group) or absent (bat-associated group), Haller's organ similar to the larvae.

List of species (Neotropics, N = 55; Brazil, N = 16): *O. amblus* Chamberlin, 1920; *O. aragaoi* Fonseca, 1960; *O. azteci* Matheson, 1935; *O. brasiliensis* Aragão, 1923; *O. brodyi* Matheson, 1935; *O. capensis* Neumann 1901; *O. casebeeri* Jones and Clifford, 1972; *O. cavernicolous* Dantas-Torres, Venzal and Labruna, 2012; *O. chironectes* Jones and Clifford, 1972; *O. clarki* Jones and Clifford, 1972; *O. coriaceus* Koch, 1844**; *O. cyclurae* De La Cruz, 1984; *O. darwini* Kohls, Clifford and Hoogstraal, 1969; *O. denmarki* Kohls, Sonenshine and Clifford, 1965; *O. dusbabeki*

Cerný, 1967; O. dyeri Cooley and Kohls, 1940; O. echimys Kohls, Clifford and Jones, 1969; O. elongatus Kohls, Sonenshine and Clifford, 1965; O. epitesicus Kohls, Clifford and Jones, 1969; O. fonsecai (Labruna and Venzal, 2009); O. furcosus Neumann, 1908; O. galapagensis Kohls, Clifford and Hoogstraal, 1969; O. guaporensis Nava, Venzal and Labruna, 2013; O. hasei (Schulze, 1935); *O. jul* Schulze, 1940; *O. kelleyi* Cooley and Kohls, 1941; O. kohlsi Guglielmone and Keirans, 2002; O. knoxjonesi Jones and Clifford, 1972; O. marinkellei Kohls, Clifford and Jones, 1969; O. marmosae Jones and Clifford, 1972; O. microlophi Venzal, Nava and González-Acuña, 2013; O. mimon Kohls, Clifford and Jones, 1969; O. mormoops Kohls, Clifford and Jones, 1969; O. natalinus Cerný and Dusbábek, 1967; O. nattereri Warburton, 1927; O. nicollei Mooser, 1932; O. peropteryx Kohls, Clifford and Jones, 1969; O. peruvianus Kohls, Clifford and Jones, 1969; O. puertoricensis Fox, 1947; O. quilinensis Venzal, Nava and Mangold, 2012; O. rioplatensis Venzal, Estrada-Pena and Mangold, 2008; **O. rondoniensis** (Labruna, Terassini, Camargo, Brandão, Ribeiro and Estrada-Peña, 2008); O. rossi Kohls, Sonenshine and Clifford, 1965; O. rostratus Aragão, 1911; O. rudis Karsch, 1880; O. setosus Kohls, Clifford and Jones, 1969; O. spheniscus



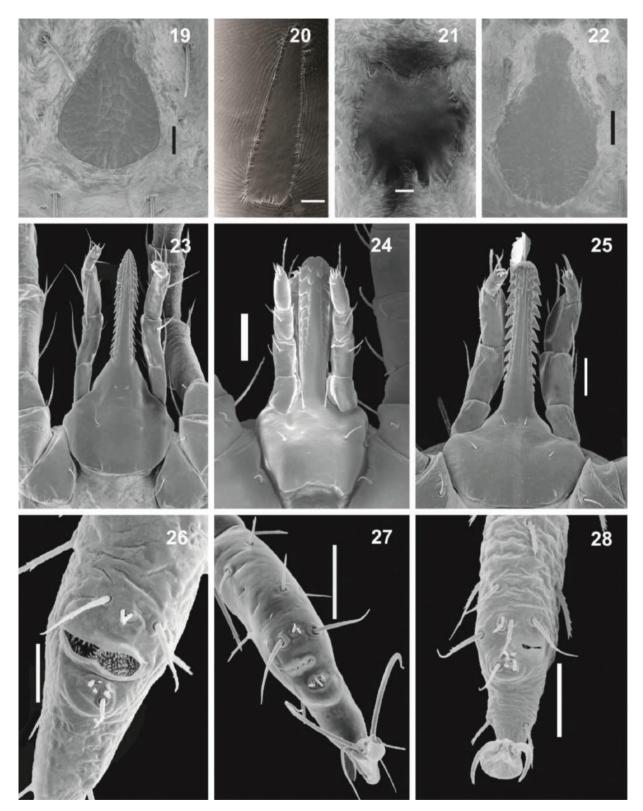
Figures 15-18. Nymph of *Argas.* 15. Idiosoma dorsal view, showing suture distinguishing dorsal surface from ventral surface (arrow). 15a. Discs of the tegument in detail. 16. Idiosoma ventral view. 17. Gnathosoma ventral view. 18. Tarsus I, showing Haller's organ with capsule perforated (arrow). *Scale bars*: 15-16, 300 μm; 15a, 40 μm; 17-18, 60 μm.

Hoogstraal, Wassef, Hays and Keirans, 1985; *O. stageri* Cooley and Kohls, 1941; *O. tadaridae* Cerný and Dusbábek, 1967; *O. talaje* (Guérin–Méneville, 1849); *O. tiptoni* Jones and Clifford, 1972; *O. tuttlei* Jones and Clifford, 1972; *O. viguerasi* Cooley and Kohls, 1941; *O. yumatensis* Cooley and Kohls, 1941; and *O. yunkeri* Keirans, Clifford and Hoogstraal, 1984.

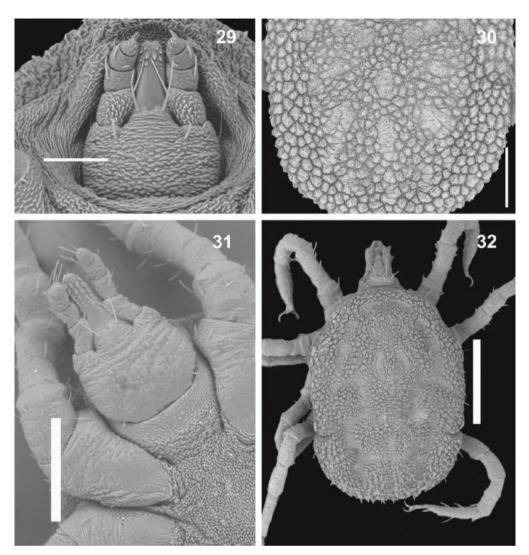
Comments: *The larvae of the *talaje* group species have 17-21 pairs of setae, whereas *O. setosus* larvae have 27-29 pairs of setae (KOHLS et al. 1969). There are around 2-6 nymphal instars usually, but few species present 5-6 instars (KLOMPEN; OLIVER, 1993). *O. peropteryx* has a single nymphal instar (VENZAL et al., 2013b). The species *O. brasiliensis* and *O. rostratus* are included among those with 5-6 nymphal instars. **Larvae of *O. coriaceus* present two pairs of eyes (KLOMPEN; OLIVER, 1993). The diagnoses of larvae and nymphs were based on Cooley and

Kohls (1944); Clifford et al. (1964, 1980); Kohls et al. (1965, 1969); Roberts (1970); De La Cruz (1974); Keirans et al. (1980, 1984); Endris et al. (1989); Venzal et al. (2008, 2012, 2013a, b); Labruna et al. (2008, 2011); Labruna and Venzal (2009); Nava et al. (2010, 2013); Barros-Battesti et al. (2011, 2012); and Dantas-Torres et al. (2012).

Otobius – Larvae (Figures 33-36): integument striated, dorsal surface with 7-10 pairs of setae, dorsal plate large, elongate tapering slightly posteriorly; two pairs of eyes; ventral surface with 5 pairs of setae + 1 pair on valves; pulvilli present on all tarsi, not enlarged, claws present, Haller's organ with capsule aperture large and rounded, with posterior projections; hypostome long without corona, dental formula 2/2. Nymphs (Figures 37-41): camerostome and hood absent; hypostomal dentition 4/4; idiosoma panduriform, integument striated and spinous; spiracular plate



Figures 19-28. Larva of *Ornithodoros.* 19. *O. mimon*, showing dorsal plate with piriform shape. 20. *O. marinkellei*, showing dorsal plate with elongated subrectangular shape. 21. *O. rostratus*, showing dorsal plate with subrectangular shape presenting concavity anteriorly and posteriorly. 22. *O fonsecai*, showing dorsal plate with piriform shape. 23. Capitulum of *O. fonsecai*, showing pointed hypostome and dentition 3/3 near to the apex. 24. Capitulum of *O. rostratus*, showing spatulated hypostome and dentition 2/2. 25. Capitulum of *O. mimon*, showing spatulated hypostome and dentition 4/4 near to the apex. 26. Haller's organ of *O. brasiliensis*, showing capsule aperture transversely slit-like and large. 27. Haller's organ of *O. rostratus*, showing capsule aperture transversely slit-like, occupying part of dorsum. 28. Haller's organ of *O. mimon*, showing small capsule with aperture transversely slit-like, occupying part of dorsum. *Scale bars*: 19, 30 μm; 20, 60 μm; 21, 20 μm; 22, 60 μm; 23-24, 60 μm; 25, 40 μm; 26, 30 μm; 27, 50 μm; 28, 40 μm.



Figures 29-32. Nymph of *Ornithodoros* (first nymphal instar). 29. Gnathosoma of *O. mimon*. 30. Idiosoma dorsal view of *O. mimon*. 31. Gnathosoma of *O. brasiliensis*. 32. Idiosoma dorsal view of *O. brasiliensis*, with dorsoventral grooves present (arrow). *Scale bars*: 29, 100 μm; 30, 250 μm; 31, 250 μm. 32, 500 μm.

cone-shaped; Haller's organ with capsule aperture transversely slit-like, elevated and large, bordered with prolonged pointed projections and with small setae internally.

List of species (N = 1): *O. megnini* (Dugès, 1883). There are two species but only this one occurs in the Neotropical region.

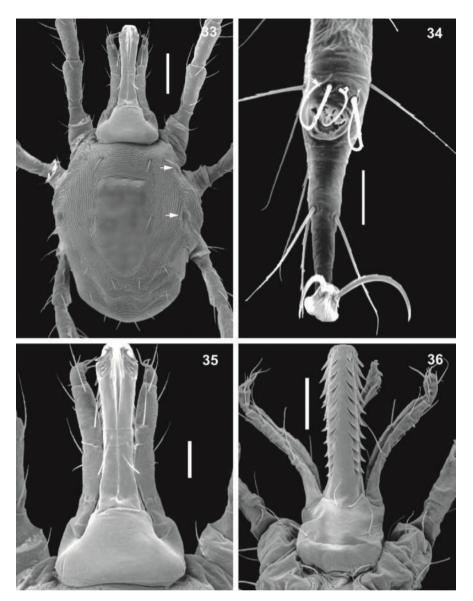
Comments: There have been isolated reports of *O. megnini* in Brazil (FLECHTMANN, 1985; DINIZ et al., 1987); however, there has been no indication that this species is established in Brazil, even though it is established in several neighboring countries (GUGLIELMONE et al., 2003).

Argasids from Brazil

Genus Antricola. This occurs in hot and humid caves inhabited by bats (Chiroptera), from southern United States to northern Mexico (A. coprophilus), throughout Cuba and the Caribbean areas, to South America (Colombia, Venezuela and northern and northeastern Brazil), mainly on the guano. Many species

are known only from the adult stage described in Cuba. Adult ticks have mouthparts incompatible with blood feeding, and there is no evidence of blood feeding in the late nymphal instars (ESTRADA-PEŇA et al., 2008). The larvae, in turn, have a long and terminal hypostome and present well-developed pulvilli that facilitate climbing cave walls. Adults of three species are known in Brazil: *A. inexpectata*, described from a cave at the locality of Brejinho, municipality of Araripe (13° 47' S, 59° 49' W), state of Ceará; and *A. delacruzi* and *A. guglielmonei*, both from a cave in Itabaiana (10° 50' S, 37° 27' W), state of Sergipe (ESTRADA-PEŇA et al., 2004). The latter two species were also collected from a cave in the municipality of Porto Velho (08° 40' S, 63° 51' W), state of Rondônia (LABRUNA et al., 2008).

Genus *Argas*. Among the 12 species in the Neotropical region, the genus *Argas* is represented in Brazil only by *A. miniatus*, for which all stages have been described. The first record of *A. miniatus* in Brazil was in the state of Rio de Janeiro (MARCHOUX; SALIMBENI, 1903). Rohr (1909) referred to *A. miniatus* as *A. persicus*, with



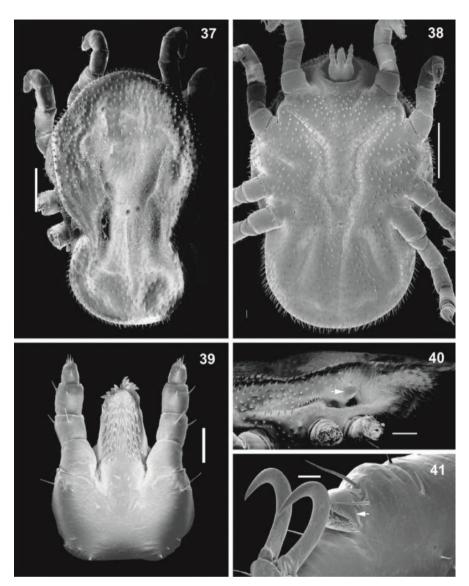
Figures 33-36. Larva of *Otobius*. 33. Idiosoma dorsal view, showing two pairs of eyes (arrow). 34. Haller's organ, showing branch-like posterior projections and very long posthalleral setae (arrow). 35. Gnathosoma dorsal view. 36. Gnathosoma ventral view. *Scale bars*: 33, 90 μ m; 34, 30 μ m; 35, 40 μ m; 36, 60 μ m.

occurrence in the municipality of Campinas (state of São Paulo) and in Rio de Janeiro (referred to as the "Federal District"). Aragão (1936) considered this tick species to be *A. persicus* var. *dissimile* and mentioned its distribution in the states of Paraná, Santa Catarina, São Paulo, Rio de Janeiro, Minas Gerais, Espírito Santo, Mato Grosso, Pernambuco, Paraíba, Maranhão, Ceará, Pará and Bahia. Cançado et al. (2008) included the Pantanal region of Mato Grosso do Sul in its distribution area. Besides Brazil, *A. miniatus* is distributed in Colombia, Guyana, Panama, Trinidad & Tobago, Cuba, Jamaica, Puerto Rico, Venezuela and the Nearctic region (GUGLIELMONE et al., 2003). This species occurs mainly on chickens but may be found on other birds. It is a vector of *Borrelia anserine*, the agent of fowl spirochetosis. Although *A. persicus* has been recorded in many countries of South America, this species is originally from the Palearctic region. However, the Neotropical

species *A. persicus* is probably a sibling species closely related to a true Palearctic species (GUGLIELMONE et al., 2003).

Genus Nothoaspis. Until recently, this genus was represented by a single species, *N. redelli*, in Mexico. In Brazil, a second species, *N. amazoniensis*, was recently found in caves in Rondônia (NAVA et al., 2010). The descriptions include larvae, nymphal instars and adults.

Genus *Ornithodoros*. Most species are known only from the larval stage, and therefore, the keys for specific diagnosis, although older, refer to this stage (KOHLS et al., 1965; 1969). Currently, the genus comprises around 118 known species around the world (VIAL; CAMICAS, 2009; NAVA et al., 2009; GUGLIELMONE et al., 2010; DANTAS-TORRES et al., 2012; HEATH, 2012; VENZAL et al., 2013a, b); 55 species occur in the Neotropical region, and 16 in Brazil. The first species



Figures 37-41. Nymph of *Otobius*. 37. Idiosoma dorsal view, showing integument with spines. 38. Idiosoma ventral view, showing spines absent in the area surrounding the capitulum. 39. Capitulum, showing hypostomal dentition 4/4. 40. Idiosoma lateral view, showing spiracular plate conical (arrow). 41. Tarsus I, showing Haller's organ with capsule aperture transversely slit-like, elevated and large, bordered superiorly with prolonged pointed projections (arrow). *Scale bars*: 37, 600 μm; 38, 800 μm; 39, 90 μm; 40, 400 μm; 41, 30 μm.

recorded in Brazil was *O. rostratus* (ARAGÃO, 1911) and the second was *O. brasiliensis* (ARAGÃO, 1923), followed by *O. nattereri* (WARBURTON, 1927), *O. jul* (SCHULZE, 1940), *O. hasei* cited as *O. dunni* Cooley and Kohls (1944), *O. rudis*, *O. capensis*, *O. stageri* (from specimens deposited in the USNTC) (JONES et al., 1972), *O. setosus* (KOHLS et al., 1969), *O. talaje* (OBA; BAGGIO, 1977), *O. rondoniensis* (LABRUNA et al., 2008), *O. fonsecai* (LABRUNA; VENZAL, 2009), *O. mimon* (BARROS-BATTESTI et al., 2011), *O. marinkellei* (LABRUNA et al., 2011), *O. cavernicolous* (DANTAS-TORRES et al., 2012) and *O. kohlsi* (from larvae deposited in the IBSP collection), Martins et al. (2013).

The species *O. rostratus* described in Brazil also occurs in Argentina, Paraguay and Bolivia (ARAGÃO, 1936; NAVA et al., 2007). Adults and larvae were described (GUGLIELMONE et al.,

2003). It bites humans and several mammal species (ALMEIDA et al., 2012). The larvae feed for few hours. In Brazil specimens of *O. rostratus* have been recorded in the states of São Paulo, Mato Grosso do Sul, Mato Grosso, Goiás and Minas Gerais, generally in association with domestic animals (ARAGÃO, 1936; PARDI; ROCHA, 1954; CANÇADO et al., 2008). The species *O. brasiliensis* is known only from the state of Rio Grande do Sul, where it has been found parasitizing many animals, including humans (MARTINS et al., 2011). Adults and larvae (ARAGÃO, 1923; BARROS-BATTESTI et al., 2012) and all the nymphal instars (LANDULFO et al., 2013) have been described. Some species such as *O. jul* and *O. nattereri* have not been reported since their description, and only the adult stage is known (GUGLIELMONE et al., 2003). *O. jul* was found in a wasp nest used by bats in the municipality of Nova Teutônia, state

of Santa Catarina. The type of *O. jul* was reported to be deposited at the Berlin museum (SCHULZE, 1940); however, it has not been found in this collection. On the other hand, Warburton (1927) mentioned that the type of *O. nattereri* was deposited at the Vienna Museum (label 86) and that the 12 specimens were from Brazil, but the host is unknown. This species resembles *O. rostratus*.

Although *O. capensis* had been considered to be among the Brazilian species of *Ornithodoros* (DANTAS-TORRES et al., 2012), it may be confused with *O. denmarki*, *O. amblus* and *O. talaje*, among others, which form the "capensis" group. It has wide among marine birds in Neotropical coastal areas and islands, and also in the Ethiopian, Nearctic, Oriental and Palearctic regions (KOHLS et al., 1965; GUGLIELMONE et al., 2003). All stages of *O. capensis* have been described (GUGLIELMONE et al., 2003).

The species A. hasei (cited as O. dunni) was originally described in Panama (MATHESON, 1935), and Cooley and Kohls (1944) cited a female of this species from Marajó Island, state of Pará, Brazil, collected in 1941, which was found "living in a tree hole with bats". All stages of O. hasei have been described (GUGLIELMONE et al., 2003). Cooley and Kohls (1941) described O. stageri from adult and immature specimens collected from bats in California, Arizona, Oklahoma and Texas. This species was found in Mexico (KOHLS et al., 1965), and Jones et al. (1972) enlarged the distribution of O. stageri to include Venezuela, Nicaragua and Brazil. Specimens of A. hasei and O. stageri collected in Brazil are deposited at USNTC (JONES et al., 1972).

The larval morphology of *O. rudis* resembles *O. rostratus* and *O. brasiliensis*, mainly because of the dorsal plate, but it lacks spurs in the dorsal region of palpus I. On the other hand, adults of *O. rudis* may be confounded with other species of the *Alectorobius* group (BARROS-BATTESTI et al., 2012). All stages have been described (GUGLIELMONE et al., 2003).

The species *O. setosus* was described from larvae collected from bats in Piedras Negras, state of Rondônia; the holotype and paratypes were deposited under the number RML 49559, according to Kohls et al. (1969). Larvae have also been collected from bats in Mexico and Venezuela. Only the larval stage is known (GUGLIELMONE et al., 2003).

The species O. talaje forms a species group with wide distribution from the southern United States to Argentina (HOOGSTRAAL, 1985). According to this author, most records before 1950 are questionable because they were based primarily on adult morphology. Venzal et al. (2008) commented that this species may be restricted to Central America, and that the records from South America are probably O. rioplatensis known from Uruguay, or O. puertoricensis, or a yet undescribed closely related species. These authors also commented that the material from Guatemala that they examined had been reared from adults collected close to the type locality of O. talaje. These seem to be the "true" O. talaje larvae, given that the original description of the species by Guérin-Méneville was made from adult specimens collected from a nearby locality. All stages have been described. Two new species were recently described and included in this group: O. guaporensis (larvae and adults, collected from a rocky fissure in the Amazon forest, in Bolivia)

and *O. microlophi* in Chile (larvae collected from lizards of the genus *Microlophus*) (NAVA et al., 2013; VENZAL et al., 2013a).

The species O. rondoniensis is known from its adult stage collected from a cave in the municipality of Porto Velho, state of Rondônia (LABRUNA et al., 2008) and from caves in the state of Pará (HENRIQUE-SIMÓES et al., 2012). The species O. fonsecai and O. cavernicolous were described from the larvae and adults, and from all stages, respectively (LABRUNA; VENZAL, 2009; DANTAS-TORRES et al., 2012). O. fonsecai is only known from specimens collected from bats on the inner walls of São Miguel cave, located in the rural area of Bonito, state of Mato Grosso do Sul. This species has also been found on bats and on walls of a cave named "Gruta Lagoa Azul", located 80 km from the municipality of Nobres, state of Mato Grosso (BARROS-BATTESTI personal communication). On the other hand, the species O. cavernicolous has wide geographical distribution, with occurrences on bats and in caves in the states of Pará, Ceará, Rio Grande do Norte, Bahia Goiás and Minas Gerais (DANTAS-TORRES et al., 2012).

Larvae of *O. mimon* were originally collected from bats in Bolivia, with records also from Uruguay and Argentina (VENZAL et al., 2004). In Brazil, adults and nymphs of *O. mimon* were first collected from a household in the municipality of Araraquara, state of São Paulo. Larvae were reared from females in a laboratory, and were redescribed along with a description of the adults as well as the biology of this species under laboratory conditions (BARROS-BATTESTI et al., 2011; LANDULFO et al., 2012). Nymphal instars of *O. mimon* were also described (LANDULFO et al., 2013).

The species *O. marinkellei* is known from Brazil, Colombia, Panama and Venezuela (VENZAL et al., 2006; LABRUNA et al., 2011). In Brazil, adults of this species were found in caves in the municipality of Porto Velho, state of Rondônia (LABRUNA et al., 2011), and in the state of Pará (HENRIQUE-SIMÓES et al., 2012). In the same cave in Porto Velho, larvae were collected from bats; few of these larvae molted to nymphs under the conditions of the cave. Adults and the first nymphal instar were described and the larva was redescribed by Labruna et al. (2011). This species is closely related to *O. viguerasi* and *O. moormops*, and all belong to the subgenus "*Subparmatus*".

Larvae of *O. kohlsi* were collected from bats of the species *Neoplatymops matogrossensis*, which were found in a rock crevice in Monte Negro, state of Rondônia, in 2005. This species was previously described as *O. boliviensis* by Kohls and Clifford (1964), but the name "boliviensis" had been preoccuped. Therefore, Guglielmone and Keirans (2002) proposed the name *O. kohlsi*. This species occurs on bats of the species *Myotis nigricans* and *Molossus* sp. in several localities in Bolivia. According to Kohls et al. (1965), adults and nymphs were found in bat-infested houses, where the tick was found biting humans.

Genus Otobius. This genus is represented by two species in the world, and in the Neotropics only *Otobius megnini* (Dugès, 1883) has been recorded. Although there have been isolated reports from northern and southeastern Brazil (FLECHTMANN, 1985; DINIZ et al., 1987), the species is not established in this country. All stages have been described.

Key to the genera of the larval stage of Argasidae in the	-Integument mammillated or tuberculated, lacking spines $\boldsymbol{3}$
Neotropical region	3. False plate occupying the anterocentral area of dorsum;
1. Eyes present; Haller's organ with capsule aperture large and rounded, with posterior projections like branches, posthalleral setae very long; dorsal idiosoma striated with 7-10 pairs of setae, dorsal plate large, elongate tapering slightly posteriorly; ventral surface with 5 pairs of setae + 1 pair on valvae; hypostome long without corona, dental formula 2/2	ventrally palpi I elongate; large flaps shielding the hypostome, which is pointed; dental formula 4/4 to 5/5 at basis; spiracular plate subcircular and lateral to coxa IV (NAVA et al., 2010)
· ·	4 . Hypostome with a few small denticles, not clearly in a definite
-Eyes absent	row; idiosoma tuberculated; most tubercles on dorsum bearing short setae, some single, others in group; spiracular plate oval; humps on tarsi absent, Haller's organ with capsule small, rounded opening
-Dorsal idiosoma with 13-21 pairs of setae (except for <i>O. setosus</i> with 27-29), hypostome pointed or rounded at apex, claws present or absent on tarsi	merostome present; surface of tarsi mammillated without dorsal humps in bat-associated species, or surface of tarsi flat dorsally, without mammillae and with dorsal humps present Ornithodoros (Figures 29-32)*
3. Pulvilli extended, claws absent (except in <i>A. marginatus</i>); with 14-15 pairs of dorsal setae, dorsal plate large with lateral border parallel, narrowing anteriorly; hypostome pointed at apex, dentition 3/3 extending from posterior third to apex and 2/2 at base, 3 pairs of postcoxal setae	* Nymph of 1st instar; ** nymph of 2nd instar; *** nymphal instar undetermined Key to the larvae of species of Ornithodoros in Brazil* 1.Basis capituli ventral with a pair of cornua-like extensions posteriorly and with a pair of auriculae-like extensions laterally2
4. Dorsal plate with isosceles triangle shape occupying entire length	-Basis capituli ventral without cornua or auriculae-like extensions
of the dorsum (in unfed specimens); dorsal surface with 12-13 pairs of setae; hypostome with apex pointed, dental formula 2/2, corona absent (NAVA et al., 2010)	2.Dorsal plate elongated and triangular in shape, with notched posterior margin; dorsum of body with 27-29 pairs of setae O. setosus (KOHLS et al., 1965)
-Dorsal plate elongated and subrectangular, with anterior extremity narrowed, piriform or triangular; hypostome with apex rounded or pointed, hypostomal dentition 2/2 to 4/4; tarsi surface glabrous or rugous; dorsal surface with 13-21 pairs of setae (except for <i>O. setosus</i> , which has 27-29 pairs)	-Dorsal plate elongated, narrow and triangular in shape, with posterior margin slightly concave, less than one-third as wide as long, dorsum with 13 pairs of setae
Key to the genera of the nymphal stage of Argasidae in the Neotro- pical region	-Dorsum with 16 pairs of setae or more (talaje group)
1.Periphery of the idiosoma flat and structurally different from dorsum, with suture distinguishing dorsal and ventral surfaces	4. Dorsal plate oval, rectangular, elongated or resembling an apple; dorsum with 13-14 pairs of setae (typically 13); ventrally, 8 pairs of setae plus 1 posteromedial seta; presence of short spurs on
-Periphery of the idiosoma not flat, without a suture line separating dorsal and ventral surfaces	dorsal surface of palpal article I
2. Integument with spines; hypostome developed, dentition 4/4; body panduriform; spiracular plate conical <i>Otobius</i>	dorsal surface of palpal article I

(Figures 37-41)**

-Dorsal plate oval and large without anterior concavity; tarsus I rugous <i>O. brasiliensis</i> (BARROS-BATTESTI et al., 2012) (Figure 26)
6 .Dorsal plate triangular; dorsum of idiosoma with 15 pairs of setae (11 dorsolateral); tarsus I with 24 setae, including 3 basal pairs; presence of short spurs on dorsal surface of palpal article I
-Dorsal plate piriform or rectangular
7.Dorsal plate piriform; dorsum of body with 13-14 pairs of setae (typically 14) and 3 central pairs; venter with 8 pairs of setae plus 1 PMS (posteromedian seta); hypostome long, pointed apically, dental formula 3/3 in the anterior half, 2/2 posteriorly almost to base; dentition: row 1 with 17-18 denticles, row 2 with 15-17, and row 3 with 9-10; occasionally, an accessory row with a single denticle (4/4 at apex)
- Dorsal plate piriform; dorsum with 13 pairs of setae (10 dorso-lateral and 3 central pairs); 6 dorsal pairs anterolaterally, venter 8-9 pairs of setae plus 1 PMS (posteromedian seta); hypostome long, blunt apically; dental formula 4/4 then 3/3 in the anterior third, 2/2 posteriorly almost to base; dentition: row 1 with 17-18 denticles, row 2 with 14-16, row 3 with 4-5 and row 4 with 3
8. Dorsal plate piriform in shape9
-Dorsal plate rectangular, about twice as wide as long, with anterior and posterior slightly concave; dorsum with 16-21 pairs of setae; 12-14 dorsolateral pairs (typically 13) and 4-7 central pairs (typically 5); venter of body with 7 pairs of setae plus a posteromedial seta; hypostome dentition 3/3 anteriorly, 2/2 posteriorly to base
9.Dorsal plate large and piriform, almost pointed anteriorly; dorsum with 22-25 pairs of setae (18-21 dorsolateral and 4 central pairs); hypostome arises from a small subtriangular median extension and tapers to a blunt apex; dentition 5/5 at the apex, then 4/4 near midlength and 2/2 to the basis
-Dorsum with 20 pairs of setae or less
10.Hypostomal dentition 3/311
-Hypostomal dentition 4/4 in the anterior two-thirds, then 3/3 and 2/2 posteriorly to the base; row 1 with 16-18 denticles, row 2 with 15-17, row 3 with 12 or 13 and row 4 with 6-9; hypostome arises from a small, cone-shaped median extension and tapers to a point; dorsal plate moderately large and piriform, widest posteriorly; dorsum with 19 pairs of setae; 15 dorsolateral pairs and 4 central pairs; venter with 8 pairs of setae plus 1 posteromedial seta
11. Hypostome arises from a small subtriangular median exten-

- * For O. jul, O. nattereri and O. rondoniensis, only adults have been described.
- ** modified by Martins et al. (2013).

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