

DESCRIPTION OF THE LARVA OF *Amblyomma longirostre* (KOCH, 1844) (ACARI: IXODIDAE) BY LIGHT AND SCANNING ELECTRON MICROSCOPY*

DARCI M. BARROS-BATTESTI¹; MÁRCIA ARZUA²; VANESSA M.M. REBELLO¹;
FÁBIO DA S. BARBIERI³; KÁTIA M. FAMADAS³

ABSTRACT:- BARROS-BATTESTI, D.M.; ARZUA, M.; REBELLO, V.M.M.; BARBIERI, F. DA S.; FAMADAS, K.M. **Description of the larva of *Amblyomma longirostre* (Koch, 1844) (Acari: Ixodidae) by light and scanning electron microscopy.** [Descrição da larva de *Amblyomma longirostre* (Koch, 1844) (Acari: Ixodidae) por microscopia ótica e eletrônica de varredura.] *Revista Brasileira de Parasitologia Veterinária*, v. 14, n. 2, p. 51-57, 2005. Instituto Butantan, Laboratório de Parasitologia, Av. Vital Brasil 1500, São Paulo, SP 05503-900, Brazil. E-mail: dbatatesti@butantan.gov.br

The description of the larva of *Amblyomma longirostre* (Koch, 1844) is based on optical and scanning electron microscopy. Larvae (F1) were obtained under laboratory conditions from an engorged *A. longirostre* female, which had been collected on a *Coendu prehensilis* (Linnaeus) (Rodentia: Erethizontidae) from the Jaraguá Mountain, São Paulo municipality, State of São Paulo, Brazil. Several characters are presented including the chaetotaxy of idiosoma, palps and Haller's organ, campaniform sensillum on festoons and measurements. In addition, the relationship of *A. longirostre* larva to other Neotropical *Amblyomma* spp. larvae is discussed.

KEY WORDS: *Amblyomma longirostre*, larva, morphology, *Coendu prehensilis*, Rodentia.

RESUMO

A larva de *Amblyomma longirostre* (Koch, 1844) foi descrita com base em microscopia óptica e eletrônica de varredura. As larvas (F1) foram obtidas sob condições de laboratório a partir de uma fêmea engorgitada de *A. longirostre* coletada em *Coendu prehensilis* (Linnaeus) (Rodentia: Erethizontidae), no Pico do Jaraguá no município de São Paulo, Estado de São Paulo, Brasil. Caracteres como quetotaxia do idiosoma, palpos e órgão de Haller, assim como sensillum campaniforme presente nos festões e medidas são apresentadas. A larva de *A. longirostre* é comparada com outras larvas de *Amblyomma* spp. da região neotropical e suas relações são discutidas.

PALAVRAS-CHAVE: *Amblyomma longirostre*, larva, morfologia, *Coendu prehensilis*, Rodentia.

INTRODUCTION

There are a few more than 100 *Amblyomma* species worldwide of which 57 are found in the Neotropical region and 45 species are restricted to this region (GUGLIELMONE et al., 2003). While morphological descriptions are available for most of African and Australian *Amblyomma* immature ticks, these are at best fragmentary for immature Neotropical species. Of the 53 species from the New World, morphological descriptions are either available for adult specimens, but very incomplete for immature stages (CASICAS et al., 1998). The specific determination of the larval stage of ixodid ticks has been a troublesome problem for systematists. It is because few species reared from identified females are known (KEIRANS, 1992). According to Cliford and Anastos (1960), the deficiencies in our knowledge of larval tick systematics could be attributed to some factors: few consistent characters for inclusion in keys; inadequate descriptions and drawings; relative few species reared in the laboratory from identified females; and the virtual impossibility of associating larvae collected in the

*This work was supported in part by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Biota project (No. 99/05446-8) to DMBB.

¹Instituto Butantan, Laboratório de Parasitologia, Av. Vital Brasil 1500, São Paulo, SP 05503-900, Brasil. E-mail: dbatatesti@butantan.gov.br

²Museu de História Natural Capão da Imbuia, Curitiba, PR.

³Universidade Federal Rural do Rio de Janeiro, 23890-000, Seropédica, RJ.

field with the corresponding adults. Very little information exists in the South America literature regarding to the immature stages of species of *Amblyomma* genus found in this region. Nevertheless, descriptions were made for larvae and nymphs of *A. parvum* Aragão, 1908, *A. pseudoparvum* Guglielmone, Mangold and Keirans, 1990 (GUGLIELMONE et al., 1990), *A. tigrinum* Koch, 1844, *A. neumannii* Ribaga, 1902, *A. testudinis* (Conil, 1877) (ESTRADA-PEÑA et al., 1993) and *A. triste* Koch, 1844 (ESTRADA-PEÑA et al., 2002); and for larvae of following species: *A. nodosum* Neumann, 1899 (AMORIM; SERRA-FREIRE, 1994a), *A. dissimile* Koch, 1844 (AMORIM; SERRA-FREIRE, 1994b), *A. rotundatum* Koch, 1844 (AMORIM; SERRA-FREIRE, 1995), *A. varium* Koch, 1844 (AMORIM; SERRA-FREIRE, 1996), *A. cajennense* (Fabricius, 1787) (FAMADAS et al., 1997), *A. geayi* Neumann, 1899 and *A. auricularium* (Conil, 1878) (AMORIM; SERRA-FREIRE, 1999a), *A. dubitatum* Neumann, 1899 (= *A. cooperi* Nuttall and Warburton, 1908) (AMORIM; SERRA-FREIRE, 1999b), and *A. aureolatum* (Pallas, 1772) (ARZUA, 2002). Unfortunately, most of these descriptions were only based by only light microscope, but many structures would be better observed under scanning electron microscopy. Regarding to the nymph of *Amblyomma longirostre* (Koch, 1844), it was described by Cooley and Kohls (1944) as *Amblyomma avecolens* Cooley and Kohls, 1944. These authors illustrated the ventral surface of idiosoma, including spiracular plate and legs I and IV. Keirans and Durden (1998) illustrated the ventral view of the gnathosoma and the scutum. Therefore, we studied the morphology of the larva of

A. longirostre and we are presenting its description by means of optical and scanning electron microscopy.

MATERIAL AND METHODS

Larvae of *A. longirostre* were reared from a female collected on a *Coendou prehensilis* (L.) (Rodentia: Erethizontidae) from the Jaraguá Mountain ($23^{\circ}40' S$, $45^{\circ}44' W$), São Paulo County. The female was maintained under $27^{\circ}C$ and high humidity (almost 100%), scotophase. The preoviposition, oviposition, and incubation periods were 10, 30, and 30 days, respectively. The eclosion period (10 days) resulted in 180 (35%) emerged larvae. A sample of 40 individuals was kept without food for 15 days, so that the consolidation of the exoskeleton took place. Subsequently they were placed in water at $70 \pm 10^{\circ}C$ and preserved in 70% ethanol. From these, 20 specimens were prepared for optical microscopy according to Famadas et al. (1996) method, and 20 other specimens were processed for scanning electron microscopy according to the method devised by Keirans et al. (1976). The engorged female was identified according to the keys proposed by Aragão and Fonseca (1961), Jones et al. (1972), and Guimarães et al. (2001). Larval chaetotaxic terminology is that of Clifford and Anastos (1960), Hess and Vlimant (1983) and Woolley (1988). All measurements are given in millimeters. The average is followed by standard deviation, and the interval represents a sample of 20 specimens measured with a Zeiss MC 80 DX optical microscope. The remaining 140 larval specimens as well as the female were

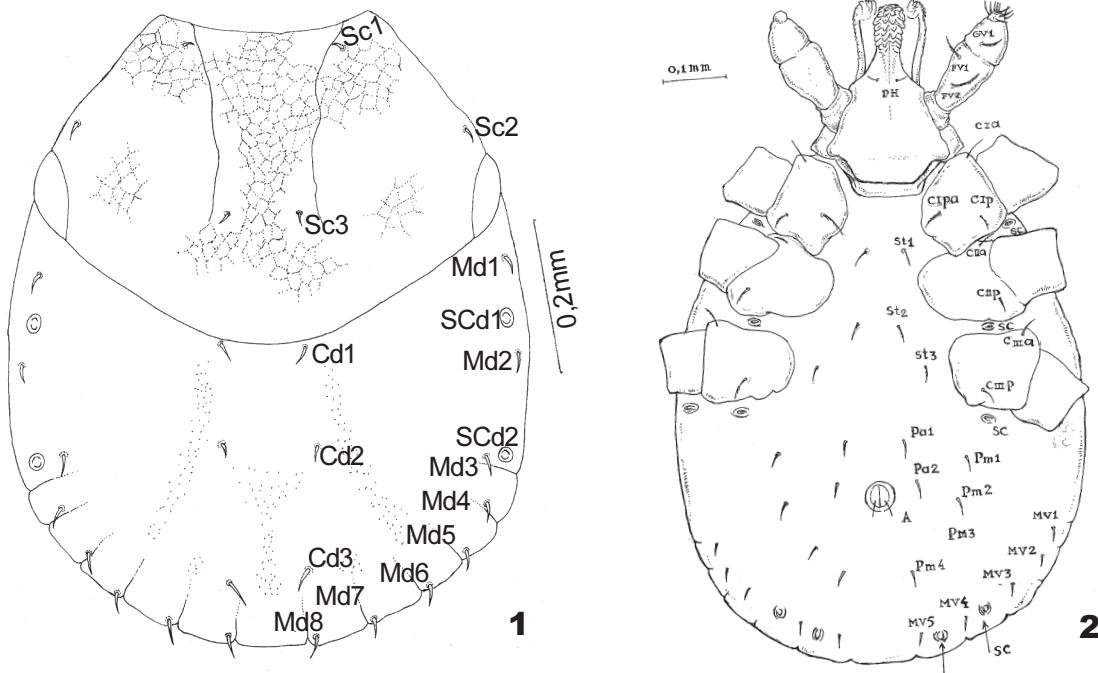


Figure 1-2. *Amblyomma longirostre*, larva, idiosoma (IBSP 7758): 1. dorsal view. Abbreviations: Setae: Sc, scutal; Cd, central dorsal; Md, marginal dorsal; SCd, dorsal campaniform sensillum. 2. ventral view; St, sternal; Pa preanal; Pm, premarginal; Mv, marginal ventral; Scf, sensillum campaniform of festoon.

deposited in the Acari Collection of the Instituto Butantan (IBSP 7480).

DESCRIPTION

Amblyomma longirostre Koch, 1844, LARVA

IDIOSOMA (Figs. 1, 2, 6, 8, 9) *Dorsal surface* (Figs. 1, 8, 6) - length from apices of scapulae to posterior margin of body 0.797 ± 0.054 (0.625-0.773); greatest width 0.689 ± 0.057 (0.625-0.773); outline oval, with 11 festoons. Presence of two pairs of campaniform sensilla, SCd₁ between Md₁ and Md₂ and SCd₂ above the first festoon near to Md₃. *Setae*: 3 central pairs (Cd₁-Cd₃); 8 marginal pairs (Md₁-Md₈) with Md₁ and Md₂ pairs before SCd (Figs. 1,8), and Md₃ pair located in the inner side close to the sensillum (SCd₂), and the remaining (Md₄-Md₈) pairs posterior to sensillum, each one in a different festoon. *Scutum*: outline subtriangular; length 0.374 ± 0.013 (0.355-0.395), breadth 0.553 ± 0.030 (0.510-0.593) up to the eyes'line. Integument with irregular hexagonal ornamentation. Eyes slightly bulging and shallow; cervical grooves extending parallel to the proximities of setae Sc₃, but slightly diverging at the end. *Setae*: 3 scutal pairs (Sc₁-Sc₃).

Ventral surface (Fig. 2, 9) - with 4 pairs of campaniforme sensilla; 1 pair located on the outer margin of the coxa I, 1 pair behind coxa II and 1 pair behind coxa III. In addition there are more 2 pairs of campaniform sensilla on the 4th and 5th festoons (SCf). Central festoon without seta, greatest width 0.101 ± 0.001 (0.100-0.103). *Setae*: 3 sternal pairs (St₁-St₃); 2 preanal pairs (Pa₁, Pa₂); 4 premarginal pairs (Pm₁-Pm₄); 5 margin-

nal ventral pairs (Mv1-Mv5). Anal aperture on central portion of opisthosoma with 1 pair of setae on the valva (A₁).

Gnathosoma (Figs. 3,4,7,10): *Dorsal*: *Basis capituli* (Figs. 3,7) - triangular in outline; length from palpal apices to posterior margin 0.255 ± 0.009 (0.238-0.269), width 0.241 ± 0.014 (0.225-0.265). Posterior margin straight, cornua absent. *Basis capituli* on median line with 1 sensillum pair. Palpal grooves segment well defined. Palpi length from apices of tibiotarsal segment (IV) to posterior margin of trochanter 0.185 ± 0.006 (0.173-0.191); femur (II) 4.0 times longer than trochanter (I); combined length of femur and genu (III) 0.165 ± 0.007 (0.150-0.174). Femur with sensillum near seta Fa₁.

Ventral: *Basis capituli* as illustrated (Figs. 4,10). *Hypostome*- compact, spatulate, length from apices to post hypostomal seta (Ph1) 0.128 ± 0.008 (0.113-0.137), dental formula 2/2, 5 teeth per file, apical corona with 9 denticles. *Palpal setae* - 12 setae on tibiotarsus, 8 terminal (Tt₁-Tt₈), 2 paraxial (Tp₁, Ttp₂) and 2 antiaxial (Tta₁, Tta₂); 6 genual setae, 1 paraxial (Gp₁), 1 antiaxial (Ga₁), 3 dorsal (Gd₁-Gd₃), and 1 ventral (Gv₁); 6 femoral setae, 1 paraxial (Fp₁), 2 antiaxial (Fa₁, Fa₂), 1 dorsal (Fd₁) and 2 ventral (Fv₁, Fv₂); trochanter 0.

LEGS (Figs. 2,9,11,12): Coxa I with 1 triangular sharp-pointed spur; coxa II and III each with 1 short spur (Figs 2,9). *Setae*-3 on coxa I; coxa II e III each with 2 setae. Trochanter lacking spur. *Tarsus I* (Fig. 12) 0.277 ± 0.007 (0.262-0.284) long. *Setae*: *Dorsal*- 2 in dorsal I group (dI₁, dI₂), 7 dorsal II (dII₁-dII₇) (Fig.11), 2 dorsal III (dIII₁, dIII₂), 2 dorsal IV (dIV₁, dIV₂), 0 dorsal V and 2 dorsal VI (dVI₁, dVI₂); *Ventral*- 2 ventral I (vI₁, vI₂), II (vII₁, vII₂), III (vIII₁, vIII₂); *Lateral anterior*- 1 in I group

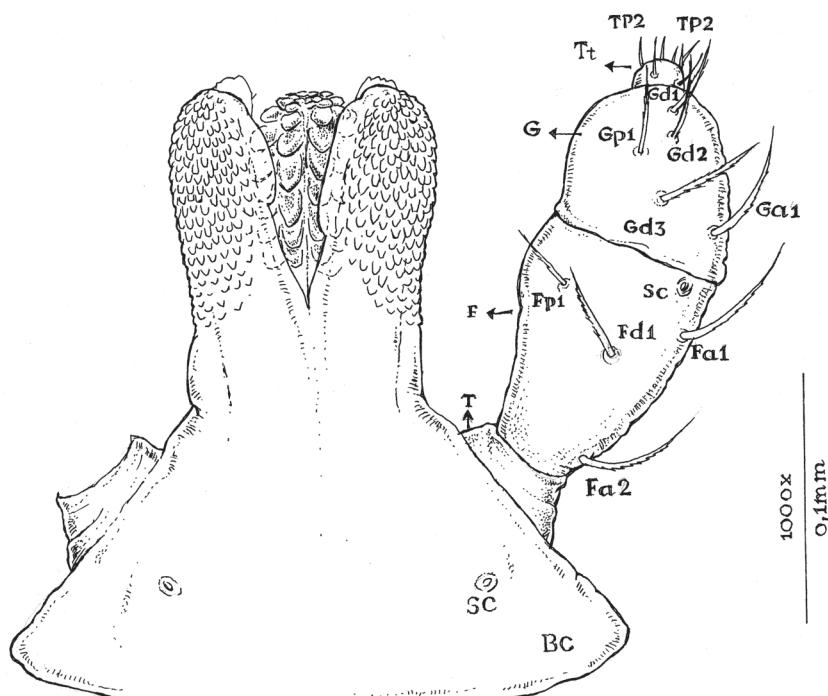


Figura 3. *Amblyomma longirostre*, larva (IBSP 7758d), Gnathosoma dorsal. Abbreviations: a, antiaxial; p, paraxial; T, trochanter; F, fêmur; G, genu; Tt, tibiotarsus; PH, post hypostomal setae; SC, campaniform sensillum.

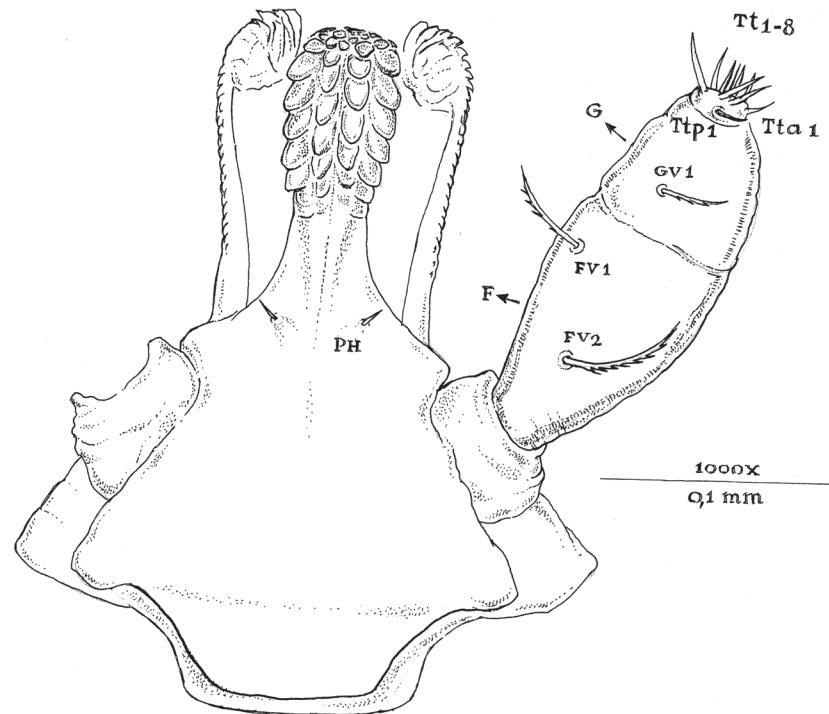


Figura 4. *Amblyomma longirostre*, larva (IBSP 7758d), Ginathosoma ventral. Abbreviations: a, antiaxial; p, paraxial; T, trochanter; F, fêmur; G, genu; Tt, tibiotarsus; PH, post hypostomal setae; SC, campaniform sensillum.

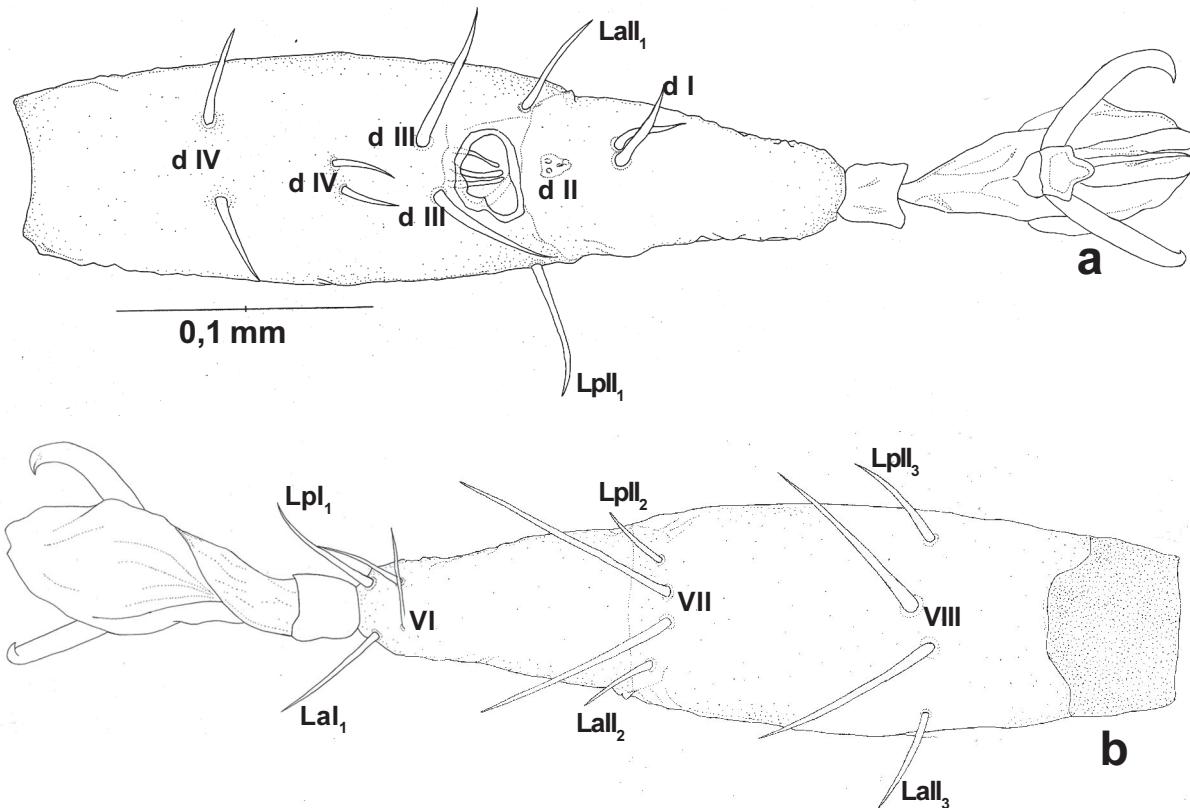


Figura 5. *Amblyomma longirostre*, larva (IBSP 7758d), Tarso I, 5a dorsal; 5b ventral. Abbreviations: d, dorsal; v, ventral; la, lateral anterior; lp, lateral posterior.

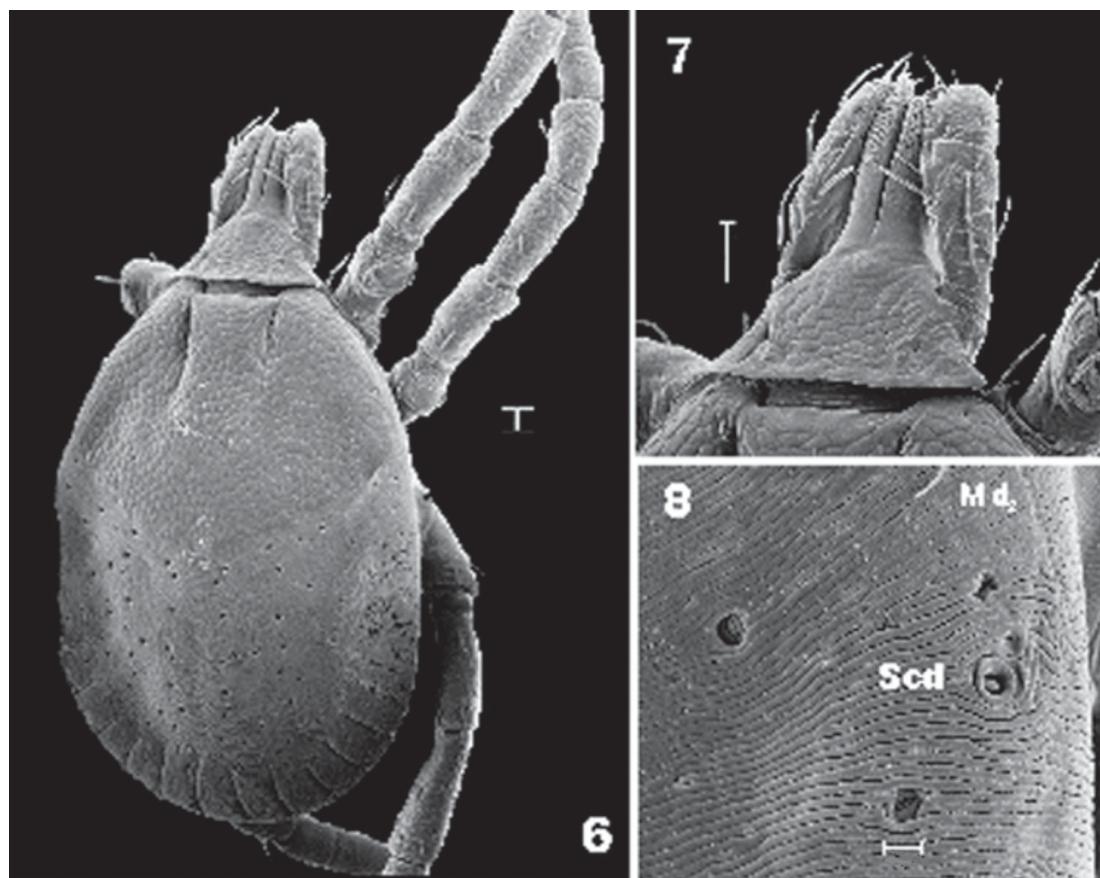


Figura 6-8. *Amblyomma longirostre*, larva, dorsal view (IBSP 7758d) 6. Idiosoma; 7. Gnathosoma; 8. Detail of campaniform sensillum on lateral margin of alloscutum. Abbreviations: Md₂, marginal dorsal seta; Scd, campaniform dorsal sensillum. Scale bars: 6 = 30 μm; 7 = 10 μm; 8 = 9 μm.

(laI₁) and 3 in II group (laII₁-laII₃); Lateral posterior- 1 in I group (lpI₁) and 3 in II group (lpII₁-lpII₃). Ambulacrum as illustrated (Fig.5).

DISCUSSION

The measurements of the idiosoma, gnathosoma, and tarsus I of larva of *A. longirostre* were compared to other previously described species such as *A. parvum*, *A. pseudoparvum* (GUGLIELMONE et al., 1990), *A. tigrinum*, *A. testudines*, *A. neumannni* (ESTRADA-PEÑA et al., 1993), *A. triste* (ESTRADA-PEÑA et al., 2002), *A. varium* (AMORIM; SERRA-FREIRE 1996), *A. cajennense* (FAMADAS et al., 1997), *A. dubitatum* (AMORIM; SERRA-FREIRE 1999a), *A. dissimile* (AMORIM; SERRA-FREIRE 1999b), and *A. aureolatum* (ARZUA, 2002). It was observed that the larva of *A. longirostre* is larger than the other species of *Amblyomma*, but its dimensions are very close to those of *A. varium*, *A. testudinis*, and *A. neumannni*. The chaetotaxy of larvae of *A. longirostre* and *A. cajennense* palpi is similar, but both species differ from those larvae of *A. aureolatum*, *A. varium*, *A. dubitatum*, *A. parvum* and *A. pseudoparvum* by having two additional setae on the tibiotarsus segment (article IV). They also differ from *A. parvum* and *A. pseudoparvum* that present 5 setae and *A. neumannni* with 3 setae on the genu segment (article III) rather than 6.

For the first time, a third pair of setae (Cd₃) was observed on the central dorsal region of an *Amblyomma* tick. The percentage of occurrence of Cd₃ pair was 1.5% (3/20).

Clifford and Anastos (1960) described the presence of four pairs of sagittiform sensilla (= campaniform; DASGUPTA; RAY, 1949) on the idiosoma. One pair located dorsally on the posterior lateral margin of the body, whereas, the others are located behind each coxae. Posteriorly, Famadas et al. (1997) recorded, for the first time, a pair of this campaniform sensillum on the 5th festoon of *A. cajennense* and Arzua (2002) has observed these sensilla on the 5th and also on the 4th festoons of *A. aureolatum*. For the first time is observed a second pair of campaniform sensillum on dorsal surface of idiosoma in larva of *Amblyomma* tick.

Of all the species studied until now, only *A. aureolatum* and *A. longirostre* present the campaniform sensilla on the 5th and 4th festoons. Both species can be separated by the presence of only one pair of campaniform sensillum on dorsal surface of idiosoma *A. aureolatum* whereas in *A. longirostre* two pairs are present.

All other sensilla (on palpi, on dorsal basis of capituli, and located on the outer margin of the coxa I, and behind coxa II and III) are present in *A. longirostre*. But the presence of two pairs of post hypostomal setae on larvae

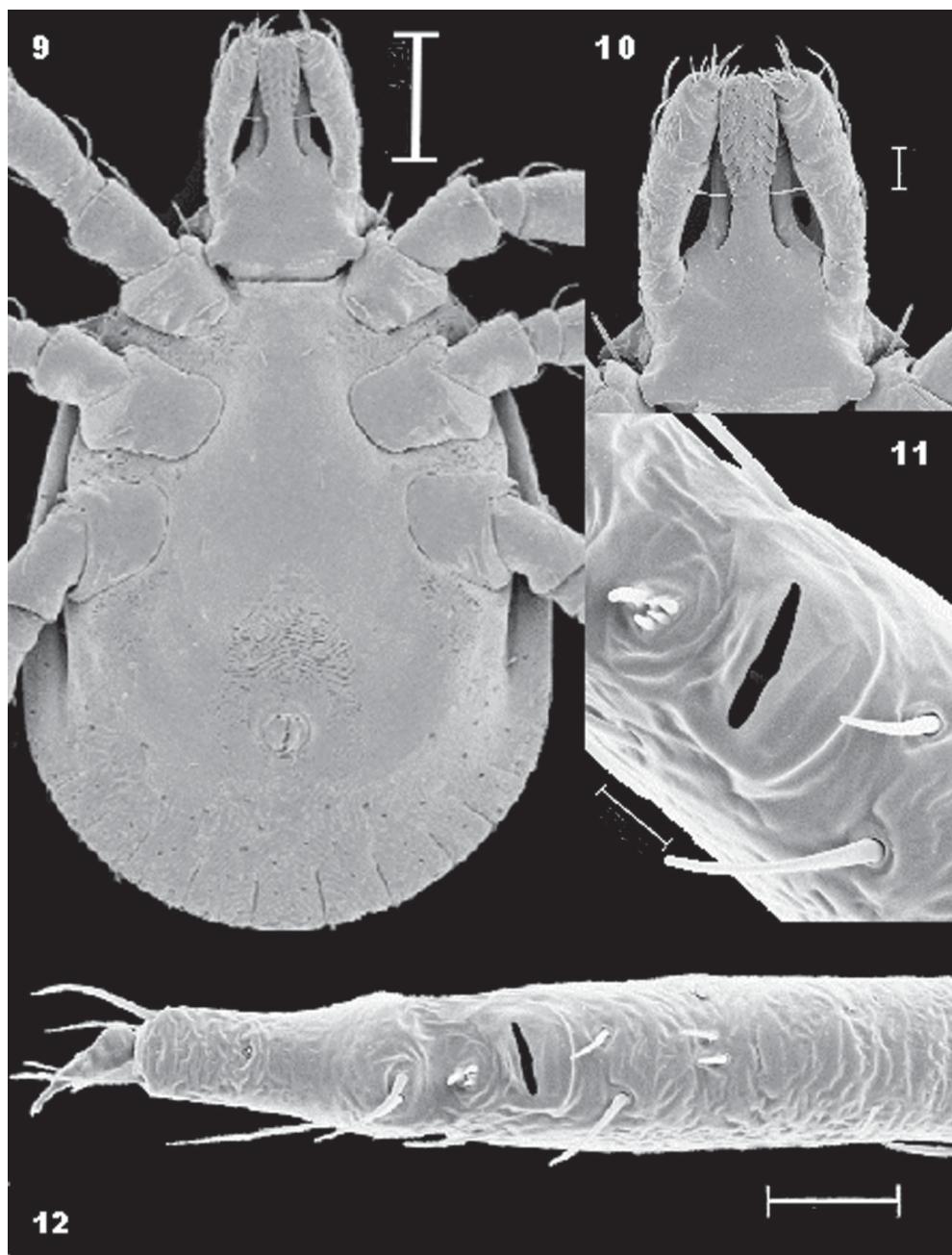


Figura 9-12. *Amblyomma longirostre*, larva (IBSP 7758d) 9. Idiosoma ventral; 10. Gnathosoma ventral; 11. Detail of Haller's organ; 12. Tarsus I, dorsal view. Scale bars: 9 = 120 µm; 10 = 30 µm; 11 = 120 µm; 12 = 40 µm.

of *A. dubitatum* (described as *A. cooperi*), *A. geayi*, and *A. auricularium* (described as *A. auriculare*) (AMORIM; SERRA-FREIRE, 1996), and the three pairs of central dorsal setae on *A. longirostre* as well as the differences present on palpi of some species, show that the chaetotaxy can not be ruled out as taxonomic character until more data are collected.

On the other hand, the larger dimensions of *A. longirostre* larva (only similar to *A. varium*, *A. testudinis*, and *A. neumannii* larvae) as well as the presence of a supplementary pair of sensilla on the idiosoma in addition to the morphology could be enough to separate it from the other species. However it

would be also necessary to use these characters with caution because most larvae of the *Amblyomma* genus from Neotropical region are still unknown.

Acknowledgements:- To Adriana Joppert da Silva, Departamento de Parques e Áreas Verdes da Prefeitura Municipal de São Paulo (DEPAVE), for donation of the *Amblyomma longirostre* engorged female.

REFERENCES

AMORIM, M.; SERRA-FREIRE, N.M. *Amblyomma nodosum*

- Neumann, 1899 descrição morfológica do estádio de larva. *Revista Brasileira de Parasitologia Veterinária*, v. 3, n. 2, p. 131-142, 1994a.
- AMORIM, M.; SERRA-FREIRE, N.M. Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae). 4. *Amblyomma dissimile* Koch, 1844. *Boletim do Museu Paraense Emílio Goeldi*, v. 10, n. 2, p. 273-288, 1994b.
- AMORIM, M.; SERRA-FREIRE, N.M. Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae). 4. *Amblyomma rotundatum* Koch, 1844. *Parasitología Al Día*, v. 19, n. 1-2, p. 9-19, 1995.
- AMORIM, M.; SERRA-FREIRE, N.M. Morphological description of tick larval stage (Acari: Ixodidae). 3. *Amblyomma varius* Koch, 1844. *Entomología y Vectores*, v. 3, n. 3, p. 67-81, 1996.
- AMORIM, M.; SERRA-FREIRE, N.M. Chave dicotômica para identificação de larvas de algumas espécies do gênero *Amblyomma* Koch, 1844 (Acari: Ixodidae). *Entomología y Vectores*, v. 6, n. 1, p. 75-0, 1999a.
- AMORIM, M.; SERRA-FREIRE, N.M. Descrição morfológica do estádio de larva de carrapato (Acari: Ixodidae). 6. *Amblyomma cooperi* Nuttall & Warburton, 1907. *Entomología y Vectores*, v. 6, n. 2, p. 126-155, 1999b.
- ARAGÃO, H.P.; FONSECA, F. Notas de Ixodologia. VIII. Lista e chave dos representantes da fauna ixodológica brasileira. *Memórias do Instituto Oswaldo Cruz*, v. 59, n. 2, p. 115-129, 1961.
- ARZUA, M. Bioecologia do parasitismo de carrapatos (Acari: Ixodidae) em aves silvestres do Bosque Reinhard Maack, Curitiba, Paraná. Descrição, diagnóstico morfológico e molecular da larva de *Amblyomma aureolatum* (Pallas, 1772). 2002. 88p. Dissertação (Mestrado), Universidade Federal do Paraná, Curitiba, 2002.
- CAMICAS, J.L.; HERVY, J.P.; ADAM, F.; MOREL, P.C. *Légitimes du monde*. Paris: Éditions de L'Orstom, Institut Français de Recherche Scientifique pour le Développement en Coopération, 1998. 233 p.
- COOLEY, R.A.; KOHLS, G.M. The genus *Amblyomma* (Ixodidae) in the United States. *The Journal of Parasitology*, v. 30, n. 2, p. 77-111, 1944.
- CLIFFORD, C.M.; ANASTOS, G. The use of chaetotaxy in the identification of larval ticks (Acarina: Ixodidae). *The Journal of Parasitology*, v. 46, p. 567-578, 1960.
- DINNIK, J.; ZUMPT, F. The integumentary sense organs of the larvae of Rhipicephalinae (Acarina). *Psyche*, v. 56, n. 1, p. 1-17, 1949.
- ESTRADA-PEÑA, A.; GUGLIELMONE, A.A.; MANGOLD, A.J.; CASTELLÁ, J. A description of *Amblyomma tigrinum* Koch, *A. neumanni* Ribaga, and *A. testudinis* (Conil) immatures (Acarina: Ixodidae). *Folia Parasitologica*, v. 40, n. 2, p. 147-153, 1993.
- ESTRADA-PEÑA, A.; Venzal, A.J.; GUGLIELMONE, A.A. *Amblyomma dubitatum* Neumann: description of nymph and redescription of adults, together with the description of immature stages of *A. triste* Koch. *Acarologia*, v. 43, n. 4, p. 323-333, 2002.
- FAMADAS, K.M.; FREIRE, N.M.S.; FACCINI, J.L.H. A note on slide-mounting technique of unfed immature stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). *Memórias do Instituto Oswaldo Cruz*, v. 91, n. 1, p. 139-140, 1996.
- FAMADAS, K.M.; SERRA-FREIRE, N.M.; LANFREDI, R.M. Redescription of the larva of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) using optical and scanning electron microscopy. *Acarologia*, v. 38, n. 2, p. 101-109, 1997.
- GUGLIELMONE, A.A.; MANGOLD, A.J.; KEIRANS, J.E. Redescription of the male and female of *Amblyomma parvum* Aragão, 1908 and description of nymph and larva, and description of all stages of *Amblyomma pseudoparvum* n. sp. (Acari: Ixodida: Ixodidae). *Acarologia*, v. 32, n. 2, p. 143-159, 1990.
- GUGLIELMONE, A.A.; ESTRADA-PEÑA, A.; KEIRANS, J.E.; ROBBINS, R.G. *Ticks (Acari: Ixodida) of the Neotropical Zoogeographic Region*. Atlanta: International Consortium on Ticks and Tick-borne Diseases, 2003. 173 p.
- GUIMARÃES, J.H.; TUCCI, E.C.; BARROS-BATTESTI, D.M. *Ectoparasitos de importância veterinária*. São Paulo: Plêiade/FAPESP, 2001. 213p.
- HESS, E.; VLIMANT, M. The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae: Metastiata). III. Mapping of sensory hairs and evolution of relative importance of sensory modalities during post-embryonic development. *Revue Suisse Zoologie*, v. 90, n. 4, p. 887-897, 1983.
- JONES, E.K.; CLIFFORD, C.M.; KEIRANS, J.E.; KOHLS, G.M. The ticks of Venezuela (Acari: Ixodoidea) with a key to the species of *Amblyomma* in the western hemisphere. *Bingham Young University Science Bulletin Biology Series*, v. 17, n. 4, p. 1-41, 1972.
- KEIRANS, J.E. Systematics of the Ixodida (Argasidae, Ixodidae, Nuttalliellidae): In Fivaz, B.; Petney, T.; Horak, I. *Tick vector biology medical and veterinary aspects* an overview and some problems. Berlin: Springer, 1992. p. 1-21.
- KEIRANS, J.E.; DURDEN, L.A. Illustrated key to nymphs of the tick genus *Amblyomma* (Acari: Ixodidae) found in the United States. *Journal of Medical Entomology*, v. 35, n. 4, p. 489-495, 1998.
- KEIRANS, J.E.; CLIFFORD, C.M. & CORWIN, D. *Ixodes sigelus* n. sp. (Acari: Ixodidae) a parasite of rodents Chile, with a method for preparing of ticks for examination by scanning electron microscopy. *Acarologia*, v. 18, n. 2, p. 217-225, 1976.
- WOOLLEY, T.A. *Acarology. Mites and human welfare*. New York: A Wiley-Interscience Publications, 1988. 484 p.

Received on November 10, 2004.

Accepted for publication on June 20, 2005.