

## Original article

Morphological description of the nymphal stage of *Amblyomma geayi* and new nymphal records of *Amblyomma parkeri*Thiago F. Martins<sup>a</sup>, Alessandra Scofield<sup>b</sup>, Wanessa B.L. Oliveira<sup>b</sup>, Pablo H. Nunes<sup>c</sup>, Diego G. Ramirez<sup>a</sup>, Darci M. Barros-Battesti<sup>d</sup>, Lilian R.M. Sá<sup>a</sup>, Fernanda Ampuero<sup>a</sup>, Julio C. Souza Jr.<sup>e</sup>, Marcelo B. Labruna<sup>a,\*</sup><sup>a</sup> Faculty of Veterinary Medicine, University of São Paulo, São Paulo, SP, Brazil<sup>b</sup> Faculty of Veterinary Medicine, Federal University of Pará, Castanhal, PA, Brazil<sup>c</sup> Institute of Biosciences, São Paulo State University, Rio Claro, SP, Brazil<sup>d</sup> Laboratory of Parasitology, Butantan Institute, São Paulo, SP, Brazil<sup>e</sup> Regional University of Blumenau, FURB, Blumenau, SC, Brazil

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## ABSTRACT

The external morphology of the nymph of *Amblyomma geayi* Neumann is described by optical and scanning electron microscopy. Unfed nymphs were obtained from an engorged *A. geayi* female, which had been collected on a sloth (*Bradypus variegatus*) from Belém municipality, State of Pará, northern Brazil, and was kept under laboratory conditions. With the present description, we propose a modification of a taxonomic key published in 2010 for the *Amblyomma* nymphs that occur in Brazil, through the inclusion of *A. geayi*. The nymph of *A. geayi* is morphologically very similar to the nymph of *Amblyomma parkeri* Fonseca and Aragão, with only slight morphological differences related to scutal surface and punctuations (more shagreened and less punctuated in *A. geayi*). These 2 nymphs differ from all other known *Amblyomma* nymphs from Brazil by the combination of auriculae present as small posterolateral rounded projections, eyes located at the level of the scutal midlength, and a rounded hypostome. These nymphal similarities as well the morphology of the adult stage corroborate previous studies that showed that *A. geayi* and *A. parkeri* are genetically closely related. Unpublished host records of the nymphs of both *A. geayi* and *A. parkeri* are provided. Established populations of *A. geayi* and *A. parkeri* seem to be geographically separated, since all confirmed records of *A. geayi* are from the northern half of South America (mainly the Amazonian region) and Central America, whereas all known records of *A. parkeri* are from the Atlantic rainforest biome in northeastern, southeastern, and southern Brazil.

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## Introduction

The genus *Amblyomma* is currently represented by 29 established species in Brazil (Dantas-Torres et al., 2009), from which the nymphal stage of only 2 species, namely *Amblyomma geayi* Neumann, 1989, and *Amblyomma goeldii* Neumann, 1899, remains undescribed (Martins et al., 2010). The current geographical distribution of *A. geayi* is restricted to Central and South America, with records for Brazil, Colombia, Costa Rica, French Guiana, Guyana, Panama, Peru, Surinam, and Venezuela (Alvarez et al., 2005; Guglielmone et al., 2003; Clavijo et al., 2009). In Brazil, it has been reported from the northern states, within the Amazon basin (States of Tocantins, Amazonas, and Pará), where the tick adult

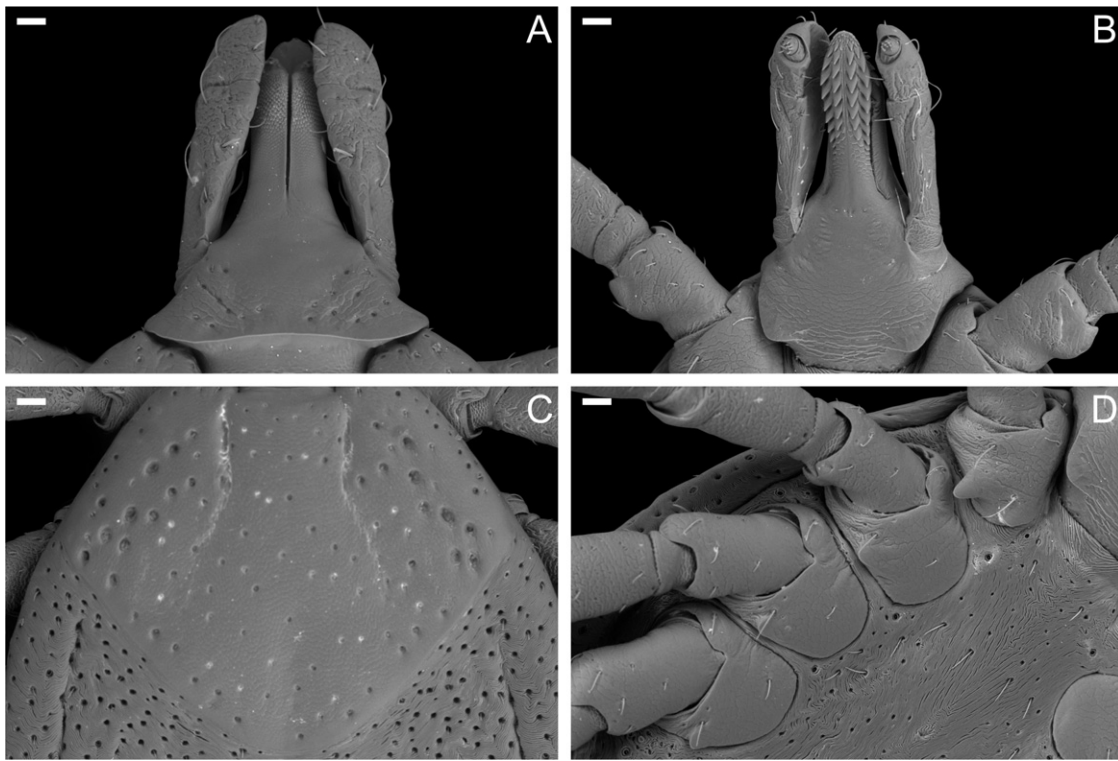
stage is found parasitizing mammals of the order Xenarthra, chiefly sloths (Serra-Freire et al., 1993; Labruna et al., 2009). Herein, we describe the nymphal stage of *A. geayi* for the first time. In addition to providing scanning electron micrographs of the nymphs of both *A. geayi* and a close-related species, *Amblyomma parkeri* Fonseca and Aragão, 1952, we also propose the inclusion of an *A. geayi*-step in the taxonomic key for *Amblyomma* nymphs from Brazil, recently published by Martins et al. (2010).

## Materials and methods

On 16th July 2010, an engorged female of *A. geayi* was collected from a sloth, *Bradypus variegatus*, in Belém municipality (01°27'S, 48°30'W), state of Pará, northern Brazil. In the laboratory, the engorged female oviposited under controlled conditions (25 °C, 95% RH), the hatched larvae were allowed to feed on a tick-naïve rabbit, and flat nymphs molted from engorged larvae under controlled conditions. These unfed nymphs, when 15–30 days old, were killed in hot water (70–80 °C) and immediately preserved in

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**Fig. 1.** *Amblyomma geayi* nymph. (A) Dorsal gnathosoma (capitulum) (bar: 50  $\mu$ m). (B) Ventral gnathosoma (bar: 50  $\mu$ m). (C) Scutum (bar: 50  $\mu$ m). (D) Coxae I–IV (bar: 50  $\mu$ m).

70% alcohol until further processing for description. After oviposition, the engorged female was deposited in the tick collection “Coleção Nacional de Carrapatos” (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo under the accession number CNC-1652. Taxonomic identification of this female was confirmed according to Aragão and Fonseca (1961) and Labruna et al. (2009).

The nymphal stage of *A. geayi* was described based on optical microscopy, following Martins et al. (2010). For this purpose, 10 unengorged specimens of *A. geayi* were measured using the Image-Pro Plus 5.1 program for analysis of images and morphometry, fitted to an Olympus SZX stereoscope microscope. In the description that follows, all measurements are given in millimeters; first the mean  $\pm$  standard deviation, followed by the range in parentheses. These 10 nymphs have been deposited in the CNC (CNC-2183). Additional nymphs of *A. geayi* were prepared for scanning electron microscopy (SEM) following techniques described by Corwin et al. (1979). Micrographs were taken using a HITACHI TM3000 Scanning Electron Microscope.

Martins et al. (2010) redescribed the nymph of *A. parkeri* based on a single engorged specimen with broken hypostome, based solely on optical microscopy. Because the nymph of *A. geayi* was shown to be very similar to *A. parkeri* (see below), herein we provide for comparison purposes SEM photographs of an unengorged specimen of *A. parkeri* recently collected on a human (*Homo sapiens*) in Cotia municipality (23°36'S, 46°55'W), state of São Paulo, southeastern Brazil, in February 2012. This nymphal specimen has been deposited in the tick collection “Coleção Acarológica do Instituto Butantan” under the accession number IBSP-10895.

Finally, we propose a modification of the taxonomic key presented by Martins et al. (2010) for the *Amblyomma* nymphs that occur in Brazil. In this case, only the step 14 from that taxonomic key was modified in order to include *A. geayi*.

## Results

### Description of *Amblyomma geayi* Neumann, 1899 (Fig. 1)

**Idiosoma.** Length from apices of scapula to posterior body margin  $1.152 \pm 0.039$  (1.089–1.206), maximum breadth  $0.914 \pm 0.032$  (0.860–0.956); outline oval, with 11 festoons without tubercles ().

**Scutum.** Length  $0.587 \pm 0.021$  (0.558–0.614), breadth  $0.656 \pm 0.023$  (0.624–0.692), breadth/length ratio  $1.118 \pm 0.038$  (1.062–1.171), inornate, surface extensively shagreened (rugose); moderate punctations, larger and deeper laterally. Eyes not orbited, at lateral scutal angles at the level of scutal midlength. Cervical grooves reaching the scutal midlength, deep anteriorly. Spiracular plate triangular with rounded angles, with a discrete dorsal prolongation; length  $0.090 \pm 0.010$  (0.080–0.110), breadth  $0.045 \pm 0.005$  (0.035–0.055).

**Gnathosoma (capitulum).** Length from palpal apices to posterior margin  $0.289 \pm 0.021$  (0.256–0.320), breadth  $0.248 \pm 0.010$  (0.234–0.261). Basis capituli sub-triangular, posterior margin straight, without cornua; posterior margin convex ventrally, auriculae as small posterolateral rounded projections, lateral margin projected laterally. Palpi length  $0.257 \pm 0.012$  (0.232–0.274), article I without ventral prolongation; article II  $0.170 \pm 0.008$  (0.161–0.188) long, article III  $0.061 \pm 0.008$  (0.052–0.084) long. Hypostome rounded apically; length  $0.243 \pm 0.009$  (0.226–0.255); length of toothed portion  $0.140 \pm 0.006$  (0.128–0.147); dentition 2/2 with 7–8 teeth per row.

**Legs.** Coxa I with 2 short spurs, the external one broader and longer, the internal one obsolete; coxae II–IV with a small triangular spur. Trochanters without spur; tarsus I  $0.312 \pm 0.015$  (0.285–0.331) long,  $0.071 \pm 0.004$  (0.065–0.080) broad; tarsus IV  $0.272 \pm 0.022$  (0.237–0.304) long,  $0.066 \pm 0.005$  (0.061–0.080) broad.

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