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## New records of *Amblyomma goeldii* (Acari: Ixodidae) and description of the nymphal stage

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### Abstract

Since its original description from the Amazonian region, the tick species *Amblyomma goeldii* Neumann, 1899 has been misidentified with *Amblyomma rotundatum* Koch, 1844 in different countries of the Neotropical region. Because of this, some authors have considered that the only confirmed records of *A. goeldii* were from French Guyana. Herein, we reviewed all specimens of *A. goeldii* that have been deposited at two tick collections in Brazil. In addition, we describe the nymphal stage of *A. goeldii* for the first time. A total of 10 unpublished records of the adult stage of *A. goeldii* are recorded from the Amazonian region of Brazil, confirming the occurrence of *A. goeldii* in this country. Except for one record on the snake *Boa constrictor* Linnaeus, all records of *A. goeldii* reported in the present study were from anteaters (Pilosa: Myrmecophagidae). Our results, in conjunction with previous literature records, indicate that anteaters and large snakes are important hosts for the adult stage of *A. goeldii*. The nymph of *A. goeldii* is morphologically similar to the nymphs of *Amblyomma romitii* Tonelli-Rondelli, 1939, *Amblyomma dissimile* Koch, 1844, and *A. rotundatum*. We present a modification of a previously published taxonomic key of *Amblyomma* nymphs from Brazil, in order to perform taxonomic identification of the nymph of *A. goeldii* based on external morphology. The geographical distribution of *A. goeldii* appears to be restricted to the Amazonian region. There were no previous host records for the immature stages of *A. goeldii*, thus it is expected that the present nymphal description will facilitate further works on the ecology of this poorly studied tick species.

**Key words:** tick, nymph, description, identification key, Brazil

### Introduction

The tick genus *Amblyomma* (Acari: Ixodidae) is currently represented in Brazil by 31 established species (Martins *et al.* 2010; Nava *et al.* 2014; Krawczak *et al.* 2015). The species *Amblyomma goeldii* Neumann, 1899 was described based on 6 males and 1 female collected on an anteater (*Myrmecophaga tetradactyla* Linnaeus)

[=*Tamandua tetradactyla* (Linnaeus)], 4 females collected on a toad (*Bufo agui* Latreille) [= *Rhinella marina* (Linnaeus)] from the state of Pará, eastern Brazilian Amazon, 1 female from British Guyana, and 1 female from the Brazil-French Guiana border (Neumann 1899; Robinson 1926). Since its description, considerable taxonomic problems have occurred with the female stage of *A. goeldii*, which is thought to be in part a synonym of *Amblyomma rotundatum* Koch, 1844 because Neumann (1899) apparently confused the female of *A. rotundatum* (a parasite of toads and reptiles) with *A. goeldii* (Guglielmone *et al.* 2003, 2014). For example, Aragão (1936) recognized that his previous reports of *A. goeldii* females from toads and snakes at different parts of Brazil (Aragão 1911) were in fact *A. rotundatum*. According to Floch & Fauran (1958), the first description of a genuine female of *A. goeldii* was published by Floch & Abonnenc (1941) under the name *Amblyomma ininii* Floch and Abonnenc, 1941, based on specimens collected from an anteater at French Guiana.

While the immature stages of *A. goeldii* remain undescribed (Martins *et al.* 2010, 2013), there has been a number of literature reports of the adult stage parasitizing mammals, mostly anteaters (order Pilosa) or cold-blooded animals (mostly toads and snakes) in Brazil (Neumann 1899; Aragão 1911, 1936; Soares *et al.* 2015), French Guiana (Floch & Fauran 1958), Surinam (Voltzit 2007), Guyana (Neumann 1899, Robinson 1926), Colombia (Robinson 1926), and Jamaica (Thompson 1950). However, because of the taxonomic problems mentioned above, many of these records remain doubtful and require confirmation. In fact, Guglielmone *et al.* (2003) considered that the only confirmed records of *A. goeldii* were those from French Guyana. More recent studies reported reliable records of *A. goeldii* in Surinam (Voltzit 2007) and from two Amazonian States of Brazil, Pará (Soares *et al.* 2015) and Amazonas (Ogrzewalska *et al.* 2010). Herein, we revised all records of *A. goeldii* that have been deposited at two tick collections of Brazil. In addition, we describe the nymphal stage of the *A. goeldii* for the first time and propose the inclusion of an *A. goeldii*-step in the taxonomic key for *Amblyomma* nymphs from Brazil, recently published by Martins *et al.* (2010).

## Material and methods

We performed a morphological examination under a stereoscope microscope of all collections of *A. goeldii* that are currently deposited at the National Tick Collection (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, and at the Acari Collection of the Instituto Butantan (IBSP), São Paulo, Brazil. Taxonomic identifications of the ticks were confirmed with the morphological descriptions or redescrptions of Neumann (1899), Floch & Abonnenc (1941), Voltzit (2007), and the specific identification keys of Aragão & Fonseca (1961), Jones *et al.* (1972), and Onofrio *et al.* (2006).

For morphological description of the nymphal stage, unfed nymphs were obtained in the laboratory. For this purpose, six engorged females were collected from a collared anteater (*T. tetradactyla*) at Manaus municipality (03°06'S, 60°01'W), State of Amazonas, Brazil, on 17/XII/2013. In the laboratory, these engorged females were held in an incubator (27°C and 95%RH) in order to lay eggs, which were pooled. A sample of ≈500 hatched larvae was allowed to feed inside a cotton sleeve glued to the shaved back of a domestic rabbit *Oryctolagus cuniculus* (Linnaeus); another sample of ≈500 larvae was allowed to feed freely on the body of a snake *Boa constrictor* Linnaeus. After natural detachment, engorged larvae were held in the same incubator mentioned above, where they molted to nymphs. Unfed nymphs, 15–30 days old, were killed in hot water (70–80°C) and immediately preserved in 70% alcohol until further processing for description. Ten nymphs were measured with the use of the Image-Pro Plus 5.1 program for analysis of images and morphometry, fitted to an Olympus SZX stereoscope microscope (Olympus Corporation, Tokyo, Japan). Four nymphs were prepared for scanning electron microscopy (SEM) following techniques described by Corwin *et al.* (1979). Micrographs were taken with a Hitachi TM3000 scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan). In the description that follows, all measurements are given in millimeters; first the mean ± standard deviation, followed by the range in parentheses. These nymphs have been deposited at the CNC tick collection under the accession number (CNC-2783). Finally, we propose a modification of the taxonomic key reported by Martins *et al.* (2010) for the *Amblyomma* nymphs that occur in Brazil. In this case, only the step 16 from that taxonomic key was modified in order to include *A. goeldii*.

## Results

The CNC tick collection contains the following unpublished records of *A. goeldii* adults, all collected in Brazil: Amazonas State - 3 males, anteat, Manaus, IV/1997 (CNC-157); 2 females, *T. tetradactyla*, Manaus, 31/VIII/2007 (CNC-1394); 43 males and 8 females, *T. tetradactyla*, Manaus, 22/XI/2005 (CNC-1395); 12 males and 15 females, *T. tetradactyla*, Beruri (4°22'S, 60°57'W), I/2010 (CNC-1640); 5 males and 12 females, *T. tetradactyla*, Manaus, XII/2013 (CNC-2589).

The IBSP tick collection contains the following unpublished lots of *A. goeldii* adults, all collected in Brazil: Amazonas State - 8 males and 3 females, *T. tetradactyla*, Codajás (3°50'S, 62°3'W), 17/X/1938 (IBSP-1529); Pará State - 3 males and 1 female, *T. tetradactyla*, unknown municipality, 14/IX/1936 (IBSP-882); 6 males and 1 female, *T. tetradactyla*, unknown municipality, II/1935 (IBSP-1091); 27 males and 9 females, *T. tetradactyla*, Manaus, 21/X/1957 (IBSP-5728); 2 males, *B. constrictor*, Serra dos Carajás, 15/XII/1970 (IBSP-6855).

No engorged larvae of *A. goeldii* were recovered from the domestic rabbit; all unfed larvae refused to attach to this host. In contrast, ≈100 engorged larvae were recovered from the *B. constrictor*; these larvae fed on the snake under a controlled environmental temperature of 27°C. Most of these larvae successfully molted to nymphs, from which a sample was used for the following morphological description.

## Description of the nymph of *Amblyomma goeldii* Neumann, 1899

(Fig. 1)

*Idiosoma*. Length from apices of scapula to posterior body margin 1.501±0.063 (1.432–1.614), maximum breadth 1.146±0.059 (1.055–1.253), outline oval, with 11 festoons without tubercles. *Scutum*. Length 0.768±0.031 (0.724–0.812), breadth 0.933±0.045 (0.869–0.998), breadth/length ratio 1.215±0.032 (1.175–1.262), inornate, numerous deep punctations evenly distributed. Eyes not orbited at lateral scutal angles at level of scutal midlength. Cervical grooves ending at level of scutal midlength, deeper at anterior half. Spiracular plate triangular with rounded angles, with discrete dorsal prolongation; length 0.255±0.015 (0.232–0.290), breadth 0.136±0.008 (0.121–0.149). *Gnathosoma (capitulum)*. Length from palpal apices to posterior margin 0.350±0.018 (0.329–0.390), breadth 0.312±0.012 (0.297–0.335). Basis capituli sub-triangular, posterior margin slightly convex, without cornua; posterior margin straight ventrally, without auriculae. Palpi length 0.290±0.010 (0.273–0.310), article I with small ventral prolongation; article II 0.170±0.010 (0.146–0.192) long, article III 0.089±0.012 (0.064–0.107) long. Hypostome rounded apically; length 0.231±0.024 (0.206–0.296); length of toothed portion 0.127±0.012 (0.108–0.153); dentition 2.5/2.5 (3/3 apically, 2/2 at the base) with 6–7 teeth in external and median rows, 2–3 teeth in internal row. *Legs*. Coxa I with 2 slender, separated, diverging spurs of similar length; coxae II–IV with a small triangular spur. Trochanters without spur; tarsus I 0.441±0.024 (0.402–0.485) long, 0.106±0.005 (0.095–0.118) broad; tarsus IV 0.338±0.012 (0.318–0.351) long, 0.074±0.004 (0.069–0.080) broad.

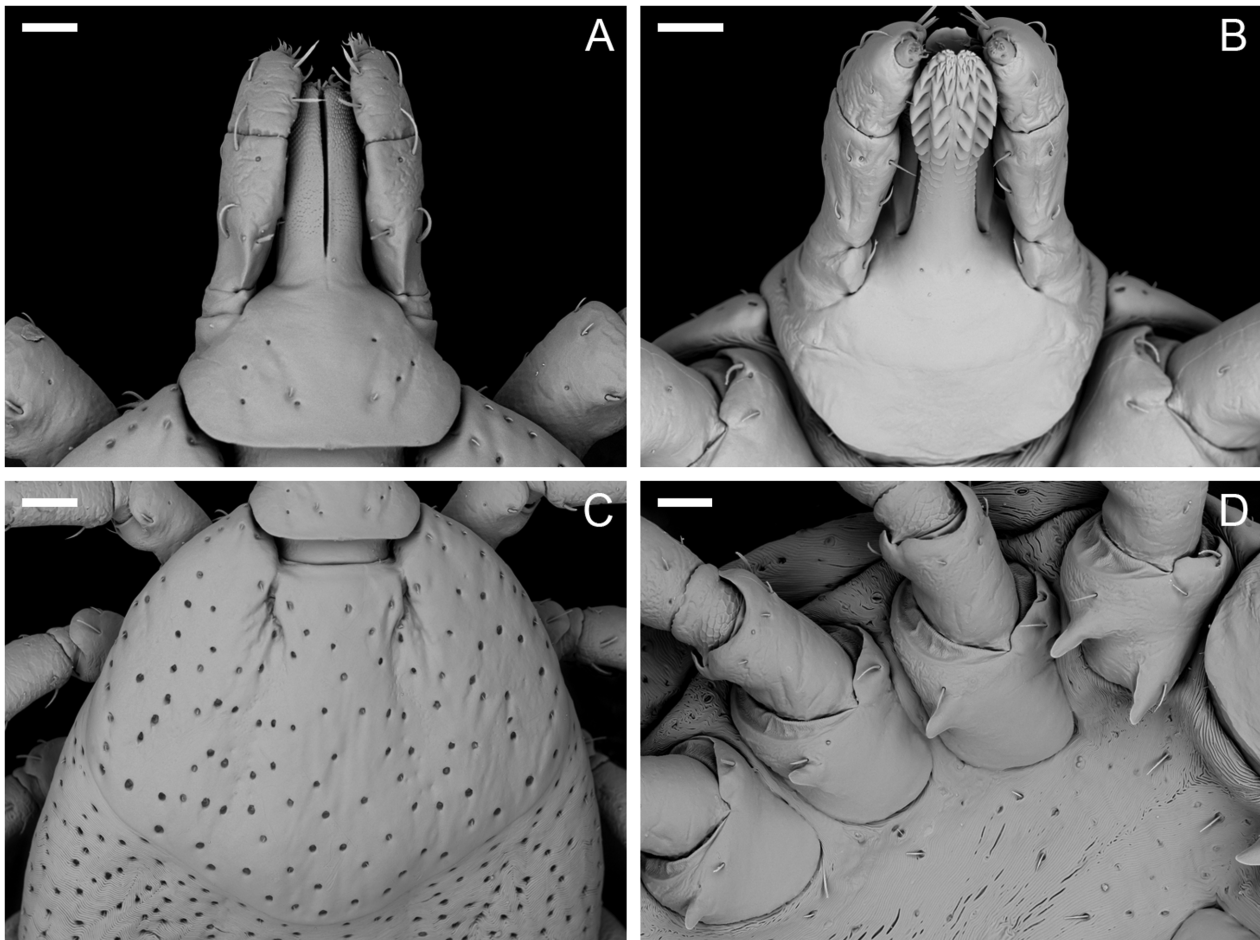
Modification of step 16 of the key to nymphs of the genus *Amblyomma* of Brazil previously reported by Martins *et al.* (2010)

16. Dorsum of body lacking dense pilosity; spiracular plate not festooned; hypostomal dentition 2.5/2.5 . . . . . 16'  
- Dorsum of body densely pilose; scutum densely punctated; spiracular plate slightly festooned; hypostomal dentition 3/3 . . . . .  
. . . . . *A. romitii*  
16'. Scutum with deep punctations concentrated in the lateral fields; coxa I with external spur clearly longer than internal spur . . .  
. . . . . *A. dissimile*  
- Scutum with deep punctations evenly distributed; coxa I with two spurs of similar length. . . . . *A. goeldii* (Fig. 1)

## Discussion

The present study confirms the occurrence of *A. goeldii* in Brazil, based on several records from the Amazonian region (states of Amazonas and Pará). It is noteworthy to mention that Ogrzewalska *et al.* (2010) reported a mitochondrial 16S rDNA partial sequence (GQ891950) that was generated from an *A. goeldii* male collected from *T. tetradactyla* at Manaus; this male specimen belonged to the CNC-1395 lot, which was examined in the present

study. By BLAST analysis, this *A. goeldii* DNA sequence was at least 10% divergent from all available sequences available in Genbank, including *A. rotundatum* (data not shown).



**FIGURE 1.** *Amblyomma goeldii* nymph. A. Dorsal gnathosoma (capitulum) (bar: 60  $\mu$ m). B. Ventral gnathosoma (bar: 60  $\mu$ m). C. Scutum (bar: 100  $\mu$ m). D. Coxae I–IV (bar: 60  $\mu$ m).

Except for one record on the snake *B. constrictor*, all records of *A. goeldii* reported in the present study were from anteaters. While many of the literature records of *A. goeldii* are doubtful and require confirmation (because of the previous confusion with *A. rotundatum*), many of these records were from anteaters in the Amazonian region of Brazil (Neumann 1899), French Guiana (Floch & Abonnenc 1941; Floch & Fauran 1958), and Surinam (Voltzit 2007). Interestingly, Floch & Abonnenc (1942) and Floch & Fauran (1958) also reported *A. goeldii* adults parasitizing the snakes *Constrictor occidentalis* Serié [= *B. constrictor*] and *Lachesis* spp. in French Guiana. These previous records, in conjunction with the present study, indicate that anteaters and large snakes are important hosts for the adult stage of *A. goeldii*. On the other hand, previous unconfirmed records of *A. goeldii* on toads (Bequaert 1926; Robinson 1926; Floch & Abonnenc 1941) are possibly misidentifications of *A. rotundatum*, since the records of *A. goeldii* on toads from Brazil (Aragão 1911) and Jamaica (Thompson 1950) were later confirmed to be *A. rotundatum* by Aragão (1936) and Kohls (1969), respectively. Additionally, the report of an “*Amblyomma* species, similar to *A. goeldii*” on lizards in southern Brazil (Evans *et al.* 2000) could be misidentification with *Amblyomma fuscum* Neumann, 1907, which is morphologically closely-related to *A. goeldii* (Barros-Battesti *et al.* 2005). Hosts for the immature stages of *A. goeldii* are unknown. In the present study, *A. goeldii* larvae successfully attached and engorged on an unrestrained *B. constrictor*; on the other hand, they refused to attach on a restrained domestic rabbit. While these data represent an isolated observation without replication, our results also suggest that snakes are suitable hosts for *A. goeldii* immature stages under natural conditions.

The nymph of *A. goeldii*, here described for the first time, is morphologically similar to the nymph of *A. romitii*, *A. dissimile*, and *A. rotundatum* (Martins *et al.* 2010). Separation of *A. goeldii* from *A. romitii* and *A.*

*dissimile* can be done following the modification of step 16 of the taxonomic key of Martins *et al.* (2010), as proposed in the present study. The nymph of *A. rotundatum* differs from *A. goeldii* by the presence of a small internal spur on coxa II of the former, which is absent in *A. goeldii*; furthermore, the scutum of *A. rotundatum* consists of punctations concentrated in the lateral fields, in contrast to deep punctations evenly distributed in the scutum of *A. goeldii*.

As reported many decades ago (Aragão 1936), the geographical distribution of *A. goeldii* appears to be restricted to the Amazonian region. The host range of *A. goeldii* adults (Mammalia: Pilosa; and Squamata: Boidae) is rather unusual; however, adults of another Neotropical species, *A. fuscum*, is also known to use either mammals (mostly Cingulata) or Squamata as hosts (Barros-Battesti *et al.* 2005). Similarly, adults of the Afrotropical species *Amblyomma sparsum* Neumann, 1899 is commonly found on reptiles or mammals within its distribution range (Cumming 1998). Because there has been no host record for *A. goeldii* immature stages, it is expected that the present nymphal description will facilitate further works on the ecology of this poorly studied tick species.

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