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# *Anopheles goeldii* Rozeboom & Gabaldón (Diptera, Culicidae): a species of the Nuneztovari Complex of Anopheles Meigen

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ABSTRACT

*Anopheles* (*Nyssorhynchus*) *goeldii* Rozeboom & Gabaldón, 1941, a species of the Nuneztovari Complex, was described based on morphological characteristics of the male, female, larva, pupa, and eggs. The type locality is Boa Vista (= Fordlândia), a district in the vicinity of Rio Tapajós, in the municipality of Aveiro, in the state of Pará, Brazil. *Anopheles goeldii* is redescribed based on morphological traits of the fourth instar larva, pupa, egg, and male and female. DNA sequences from the cytochrome oxidase subunit I (COI barcode region) of the mitochondrial genome were utilized for species characterization. Specimens of *An. goeldii* from the Pará, Amapá, and Amazonas states were employed to redescribe the species and to compare with morphologically similar taxa.


## Introduction

*Anopheles* (*Nyssorhynchus*) *goeldii* Rozeboom & Gabaldón, 1940 was described based on morphological characteristics of the male, female, larva, pupa, and eggs. The type specimens utilized for species designation and description were captured in a locality in the vicinity of Rio Tapajós designated as Boa Vista district (= Fordlândia). Townsend captured the type specimens from June 1932 to April 1933 (Townsend, 1934). Currently, the district of Boa Vista (= Fordlândia) is situated in the municipality of Aveiro, state of Pará, Brazil. Later, Floch and Abonnenc (1946) synonymized *An. goeldii* with *Anopheles* (*Nyssorhynchus*) *nuneztovari* Gabaldón, 1940 based on specimens captured in French Guiana. Lane (1953) adopted Floch and Abonnenc's (1946) hypothesis and maintained *An. goeldii* in the synonymy of *An. nuneztovari*. In 1980, Faran also accepted the synonymy of the two species, however, he also argued that *An. goeldii* was likely a valid species. Subsequently, Gabaldón (1981) ressurrected *An. goeldii* from the synonymy and listed several morphological characteristics of the fourth instar larva and male genitalia to distinguish both species.

Results of several taxonomic studies carried out for *An. nuneztovari* showed that morphological features, including characteristics of the polytene chromosomes, could differentiate populations from distinct localities. Conn (1990) named two allopatric populations of *An. nuneztovari* as chromosome type A and chromosome type B. The former population was determined based on individuals captured in the Amazon River basin, whereas the latter population was from areas in western Venezuela and east of the Andes. Later, Conn et al. (1993) described a third population that could be differentiated from type A and type B based on the polytene chromosome-banding pattern. This population was designated as chromosome type C and was found in Colombia, western Venezuela and eastern Andes. Subsequently, Fritz et al. (1994) employed DNA sequences using the internal transcribed spacer 2 (ITS2) of the ribosomal DNA of individuals of *An. nuneztovari* representing three populations from Brazil, Bolivia, Venezuela, Colombia, and Suriname. As a result, Fritz et al. demonstrated the presence of two distinct groups, one group encompassing individuals from Suriname and northern Brazil and a second group formed by populations from eastern and central Brazil. Conn et al. (1998) addressed genetic variation among 12 populations of *An. nuneztovari* from Brazil, Bolivia, Colombia, Suriname, and Venezuela employing restriction fragment length polymorphism (RFLP) of the mitochondrial DNA. Consequently, three genetic groups were defined, one from Venezuela and Colombia, and two from the Amazon River basin. More recently, Bergo et al. (2007), based on morphological characteristics of the male genitalia of specimens from the Amapá state, confirmed *An. goeldii* as a valid species. In addition, Calado et al. (2008), employing DNA sequences of the cytochrome oxidase subunit I (COI) mitochondrial gene, the single copy nuclear

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*white* gene and the second internal transcribed spacer (ITS2) of the ribosomal DNA, formally resurrected *An. goeldii* from the synonymy with *An. nuneztovari*. Gabaldón (1981) had proposed that the species was a valid taxon, without formally resurrecting it from the synonymy. In the present study, *An. goeldii* is addressed employing both morphological characters and COI barcode sequences obtained from specimens captured in the Amapá, Amazonas, and Pará states. Specimens from the state of Pará were from a locality situated in the same ecoregion of the type locality of *An. goeldii*.

## Materials and methods

Twenty-five field-collected females were captured resting in corrals and cattle sheds in Urumanduba, in the Santarém municipality (2°28'56.2" S, 54°39'39.3" W), and in São Domingos (2°45'07.2"S, 55°01'04.9"W), in the Belterra municipality, state of Pará, Brazil. Blood-fed females were kept in laboratory for 48 hours. One wing was removed to induce oviposition (Sallum et al., 2010). Thirty-six hours after oviposition, 20 eggs of each female were taken from the water and fixed in alcoholic Bouin's solution. The remaining eggs were kept in separate vials to obtain adults associated with pupa and fourth instar larva. Males and females were identified as near *An. nuneztovari* using the key proposed by Forattini (2002).

Eggs were prepared for *scanning electron microscopy* (SEM) following the protocol described by Almeida et al. (2014). External morphology of the eggs was examined in a scanning electron microscope (JEOL 6460LV, Japan) in the Laboratory of Thin Films, Department of Physics, Institute of Experimental Physics, Universidade de São Paulo, Brazil. Morphological characters of the female, male, fourth instar larva, pupa, and male genitalia were examined. Abbreviations adopted for the life stages are: F, adult female; M, adult male; G, male genitalia; L, larva; P, pupa; Le, larval exuviae; Pe, pupal exuviae; E, eggs. Terminology adopted for morphological descriptions followed Sallum et al. (2005). Voucher specimens are deposited in the Coleção Entomológica de Referência (FSP-USP), Faculdade de Saúde Pública, Universidade de São Paulo.

## Results

*Anopheles (Nyssorhynchus) goeldii* Rozeboom & Gabaldón, *1941*

*Anopheles goeldii* Rozeboom & Gabaldón (1941): 88-100. Description based on six males and nine females. The holotype male and the paratype males and females are deposited in the National Museum of Natural History (NMNH), USA. The type locality is near Rio Tapajós, Boa Vista district (= Fordlândia), situated in the municipality of Aveiro, state of Pará, Brazil.

**Description.** *Female.* Integument dark brown, with contrasting light and dark areas; integument pruinose. **Head:** Integument brown to dark brown. Proboscis with dark, decumbent scales and short setae, length 1.89-2.25 mm (mean = 2.08 ± 0.1) (n = 10); maxillary palpus length 1.84-2.39 mm (mean = 2.0 ± 0.17) (n = 10). Maxillary palpomeres 1 and 2 predominantly with semi-erect dark scales, palpomere 2 with narrow, apical white band, palpomere 3 mostly dark-scaled with white scales at apex, palpomere 4 mostly white-scaled at dorsal and outer surfaces, with dark scales at base, palpomere 5 predominantly white-scaled with dark scales at base. **Thorax:** Anterior promontory with long, white setiform scales, usually not extending far dorsad onto acrosthical area. **Legs:** Fore tarsomeres 2 and 3 with pale scales at apex, tarsomere 2 length 0.10-0.17 mm (mean = 0.14 ± 0.02) (n = 9), tarsomere 3 length 0.12-0.19 mm (mean = 0.16 ± 0.02) (n = 9); tarsomere 4 variable, totally dark or with a small, pale, apical band, tarsomere 5 dark, with a yellowish, apical band. Midtarsomeres 1 and 2 with pale, apical band, segments 3 and 4 usually dark-scaled, occasionally with a few pale scales at apex, segment 5 with pale, apical scales. Hind tarsomere 2 dark-scaled in basal 0.23-0.38 (mean = 0.28 ± 0.04) (n = 10), tarsomere 5 dark-scaled in about basal 0.5, pale-scaled at apex. **Wing:** Length 2.82-3.35 mm (mean = 3.12 ± 0.2) (n = 10), wing spot measurements in Table 1; veins with dark and pale, yellowish spots; vein costa always with basal plus prehumeral pale, prehumeral dark, humeral pale, humeral dark, presector pale, presector dark, distal sector dark, subcostal pale, preapical dark, preapical pale, and apical dark spots; sector pale, sector dark, and accessory sector pale spots present in 60% of specimens examined; ratio of length of humeral pale spot/length of prehumeral dark spot: 0.77-1.16 (mean = 0.96 ± 0.12) (n = 10); ratio of length of subcostal pale spot/ length of distal sector dark spot 0.21-0.41 (mean = 0.33 ± 0.08) (n = 10). **Abdomen:** integument dark brown; terga II-V with pale scales in sub-triangular pattern, pale scales evenly distributed on terga VI-VIII; dark posterolateral scale tufts present on segments II-IV; sternum I with a few, moderately long to long setae.

*Male.* Essentially the same as in female except for secondary sexual characters. Wing generally paler, with reduced scaling, pale spots usually longer than in female. **Head:** proboscis length 2.33-2.70 mm (mean = 2.53 ± 0.13) (n = 10); maxillary palpi length 2.16-2.70 mm (mean = 2.42 ± 0.22) (n = 6), mostly dark-scaled, with pale spots; palpomere 2 with erect, dark scales and a few pale scales; palpomere 3 with erect, dark scales basally, with pale, apical band; palpomere 4 dark-scaled, with pale scales at base and apex. *Male genitalia* (Figs. 1

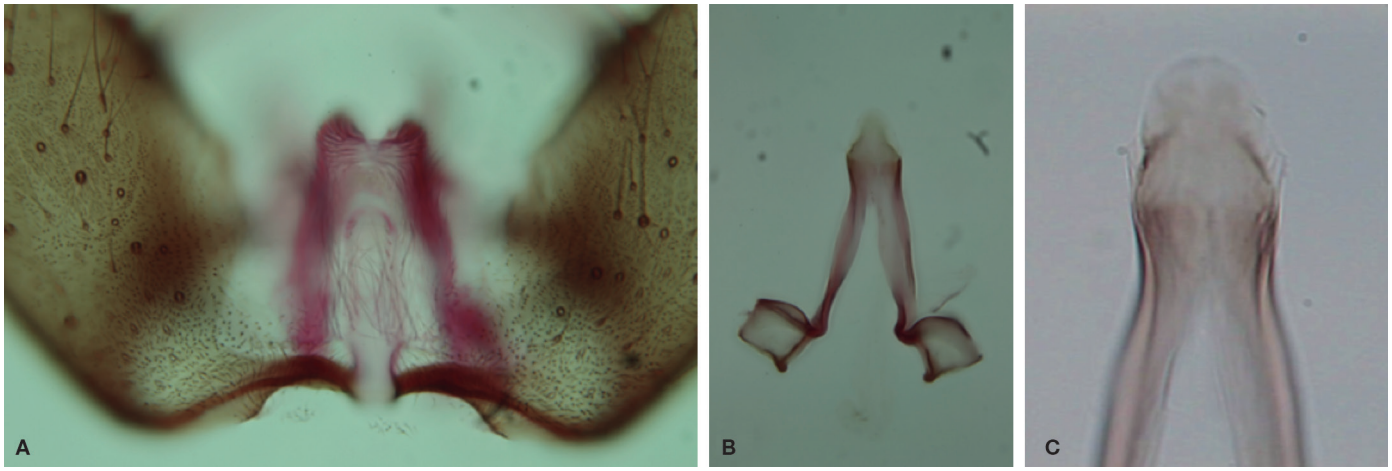


**Figure 1.** Male genitalia of *Anopheles goeldii* obtained from one male collected in the Belterra municipality, in the state of Pará, Brazil. A, ventral claspette. B, dissected aedeagus showing the position of the ventromesal subtriangular projection. C, the aedeagal leaflets.

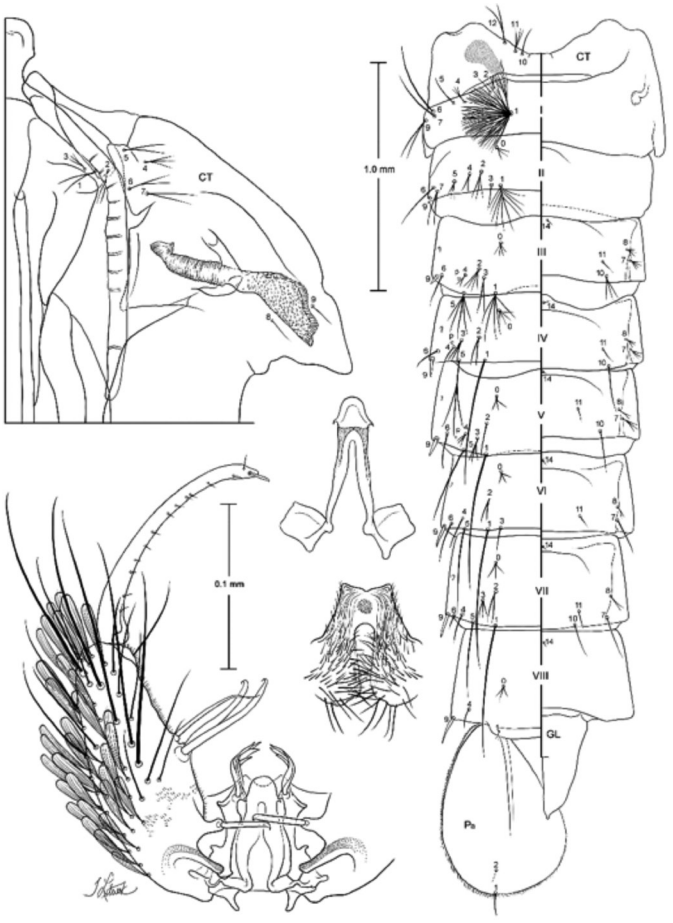


**Figure 2.** Pupa and male genitalia of *Anopheles goeldii*. Pupa, CT: cephalothorax; Pa: paddle; I-IX: abdominal segments. Male genitalia. Scales in mm.

**Table 1.**
Wing spot measurements (in mm) for male and female of *Anopheles goeldii* collected in Santarém and Belterra, state of Pará, Brazil.

| Wing spot | Range | Mean | SD (±) | n = |
|---|---|---|---|---|
| Female | | | | |
| Basal pale + prehumeral pale | 0.155-0.266 | 0.192 | 0.022 | 10 |
| Prehumeral dark | 0.102-0.225 | 0.159 | 0.034 | 10 |
| Humeral pale | 0.11-0.184 | 0.145 | 0.019 | 10 |
| Humeral dark | 0.094-0.18 | 0.126 | 0.026 | 10 |
| Presector pale | 0.04-0.118 | 0.089 | 0.02 | 10 |
| Presector dark | 0.278-0.397 | 0.343 | 0.035 | 10 |
| Sector pale | 0.081-0.11 | 0.098 | 0.008 | 6 |
| Sector dark | 0.036-0.102 | 0.059 | 0.02 | 6 |
| Accessory sector pale | 0.073-0.135 | 0.101 | 0.018 | 6 |
| Distal sector dark | 0.532-0.688 | 0.603 | 0.042 | 10 |
| Subcostal pale | 0.127-0.27 | 0.2 | 0.042 | 10 |
| Preapical dark | 0.52-0.651 | 0.588 | 0.036 | 10 |
| Preapical pale | 0.155-0.327 | 0.241 | 0.037 | 10 |
| Apical dark | 0.065-0.127 | 0.093 | 0.017 | 10 |
| Male | | | | |
| Basal pale + prehumeral pale | 0.151-0.196 | 0.167 | 0.014 | 10 |
| Prehumeral dark | 0.102-0.225 | 0.162 | 0.031 | 10 |
| Humeral pale | 0.094-0.192 | 0.147 | 0.028 | 10 |
| Humeral dark | 0.065-0.204 | 0.134 | 0.039 | 10 |
| Presector pale | 0.024-0.143 | 0.086 | 0.033 | 10 |
| Presector dark | 0.336-0.442 | 0.396 | 0.027 | 10 |
| Sector pale | 0.028-0.122 | 0.081 | 0.022 | 10 |
| Sector dark | 0.04-0.163 | 0.107 | 0.032 | 10 |
| Accessory sector pale | 0.09-0.282 | 0.158 | 0.047 | 10 |
| Distal sector dark | 0.34-0.594 | 0.508 | 0.07 | 9 |
| Subcostal pale | 0.204-0.311 | 0.249 | 0.028 | 9 |
| Preapical dark | 0.389-0.561 | 0.485 | 0.053 | 9 |
| Preapical pale | 0.188-0.274 | 0.231 | 0.03 | 10 |
| Apical dark | 0.049-0.114 | 0.077 | 0.02 | 10 |

and 2) – Segment VIII: tergum and sternum moderately narrow, with spatulate scales and long setae. Segment IX: sternum moderately long, subtrapezoidal. Dorsal claspette: pedicel long, moderately broad to moderately narrow, base rounded; leaflets broad, length 0.05-0.09 mm (mean = 0.06 ± 0.03) (n = 10); dorsal leaflet with prominent, large basomesal projection. Ventral claspette: moderately short, lateral margins not strongly tapering toward apex; apex broad, width at apex about 0.44-0.53 (mean = 0.49 ± 0.03) (n = 7) length of claspette; basal lobule moderately expanded laterally, rounded distally; spicules along basal margin short, evenly distributed over basal surface; spicules long, more abundant on basomesal margin; ventral and lateral surfaces, including basal lobule, with short spicules, extending to or nearly to apex; apex with abruptly angled, rounded, sclerotized, lateral margins; preapical plate moderately small, circular, weakly sclerotized. Phallosome: Aedeagus length 0.13-0.19 mm (mean = 0.18 ± 0.02) (n = 8); apex moderately rounded, wider than long; subapical leaflets variable, present or absent (when present, small, membranous, weakly sclerotized, non-serrated).

*Pupa* (Fig. 2). Position and development of setae as figured; range and modal number of branches in Table 2. All measurements are from 20 specimens unless otherwise indicated. Integument without distinctive pattern of dark spots, mostly yellowish; sternum II with central pentagonal dark area, with minute spicules anteriorly on ventral surface. **Cephalothorax:** setae 1,2-CT usually double, 2-CT shorter than 1,3-CT, 4-5-CT usually triple, 6,8-CT single or double, 7,9-CT usually double, 10,12-CT single to triple. Trumpet: length 0.35-0.43 mm (mean = 0.38 ± 0.02), width 0.08-0.10 mm (mean = 0.09 ± 0.02) (n = 20); pinna moderately pigmented, light brown, about 2.0-4.0 (mean = 2.98 ± 0.52) (n = 15) length of meatus. **Abdomen:** length 2.26-2.79 mm (mean = 2.54 ± 0.13). Seta 1-I dendritic, number of branches not counted, 5-I single to triple, long, 9-I single, rarely double, long; 1-II, III moderately long, developed; 1-IV-VII single, long, strongly developed, pigmented; 9-II short, unpigmented, 11-II single, present or absent; 5-III long, developed, 9-III short, lightly pigmented; 5-IV frequently triple , 9-IV short, thick, heavily pigmented, 1.72-3.25 (mean = 2.3 ± 0.44) length of seta 9-III; 5-V, VI single to triple, usually single, long, developed, 9-V thick, pigmented, acuminate, lightly curved, 1.44-2.22 (mean = 1.78 ± 0.22) length of seta 9-IV, 9-VI strong, pigmented, acuminate, curved, 0.85-1.40 (mean = 1.13 ± 0.15) length of 9-V; 5-VII frequently single, long, 9-VII thick, pigmented, acuminate, curved, about 1.08-1.68 (mean = 1.30 ± 0.18) length of 9-VI; 9-VIII strong, pigmented, about 0.95-1.39 (mean = 1.16 ± 0.12) length of 9-VII. Paddle: length 0.67-0.78 mm (mean = 0.72 ± 0.03), width 0.49-0.55 mm (mean = 0.52 ± 0.02), obovate, with round apex, weakly emarginated at insertion of 1-Pa, lightly pigmented, buttress slightly darker, midrib faint, outer basolateral serration prominent, filamentous spicules on outer apical margin and most of inner margin prominent, setae 1-Pa strong, dark-pigmented, 2-Pa single or double.

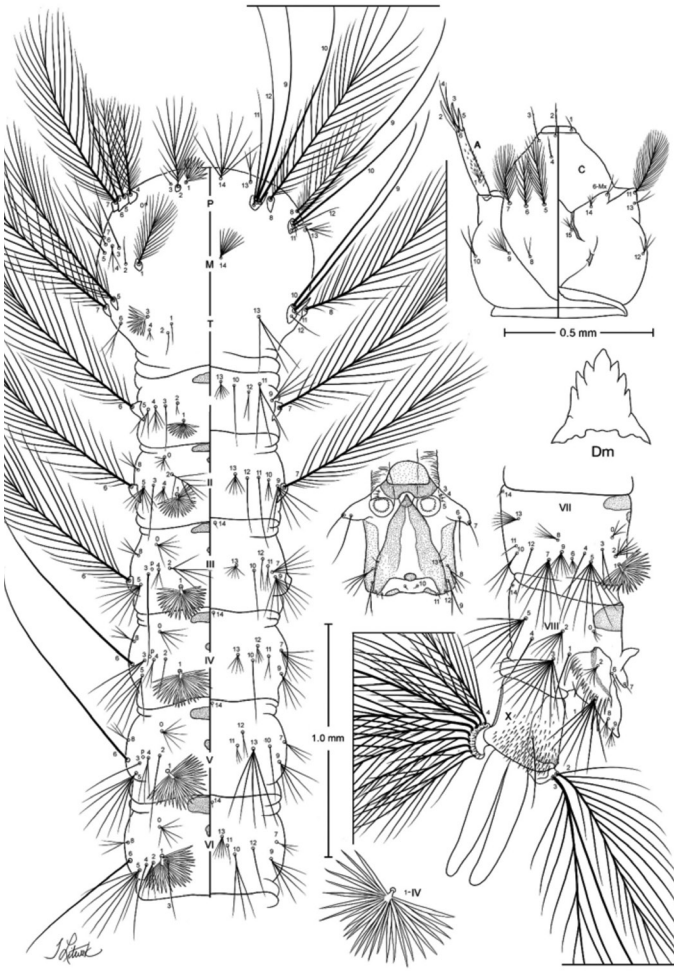


**Figure 3.** Fourth-instar larva of *Anopheles goeldii*. A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; T: metathorax; Vm: ventromentum; I-VIII: abdominal segments; X: anal lobe. Scales in mm.

*Fourth instar larva* (Fig. 3). Position and development of setae as figured; range and modal number of branches in Table 3. All measurements from 20 specimens, unless otherwise indicated. **Head:** integument light brown to yellowish, with dark spots, not forming distinct pattern; length 0.42-0.54 mm (mean = 0.49 ± 0.04) (n = 11), width 0.54-0.58mm (mean = 0.56 ± 0.01) (n = 11). Setae 2,3-C single, barbed, 2-C length 0.14-0.18mm (mean = 0.14 ± 0.05) (n = 10), 3-C length 0.11-0.14 mm (mean = 0.13 ± 0.01) (n = 11); 0.04-0.06 mm (mean = 0.04 ± 0.01) (n = 10) distance between bases of 2-C; 0.06-0.08 mm (mean = 0.06 ± 0.02) (n = 10) distance between bases of 2-C and 3-C; 4-C frequently double, forked; 5-C long, plumose; 9-C with 4-8 branches. Collar dark brown, heavily pigmented. **Antenna:** length 0.19-0.29 mm (mean = 0.25 ± 0.03) (n = 11), width 0.03-0.04 mm (mean = 0.04 ± 0) (n = 11), slightly pigmented; 1-A small, inserted 0.05-0.07 mm (mean=0.06 ± 0.01) (n = 11) distant from base. **Thorax:** setae 1-2-P normally on separate tubercles, 1-P palmate, lanceolate, moderately narrow, pigmented leaflets; 3-T palmate, moderately narrow, semitransparent leaflets. **Abdomen:** setae 0-II-VII moderately long; 1-I-VII palmate, 1-I moderately narrow, semitransparent leaflets, 5-I moderately short, usually triple, 13-I short; 1-II, VII with leaflets slightly smaller than those from setae 1-III-VI; 2-IV, V usually single; 13-V larger than 13-IV. Pecten plate with 4-6 long spines alternating with 10-14 short spines, long spines length 0.09-0.1 mm (mean = 0.1 ± 0) (n = 11), short spines length 0.04-0.05 mm (mean = 0.04 ± 0) (n = 11); setae 1-X long, single, inserted at ventral border of saddle.

*Eggs* (Fig. 4). Length 361-400 µm (mean = 389 ± 10.37); width 104-110 µm (mean = 107 ± 1.7), ratio of length/width 3.37-3.74 (mean = 3.63 ± 0.1) (n = 13).

*Overall appearance*. Black in color, boat-shaped in dorsal and lateral views (Figs. 4A and 4C), in lateral view the contour is slightly concave dorsally and curved ventrally (Fig. 4C). Floats lateral in position, long, well developed, not reaching the end of the egg (Figs. 4D and 4E), dorsal frill in the anterior and posterior poles, frill of the anterior pole is more developed than the posterior pole, connected to the float (Fig. 4A-B, 5C). *Dorsal surface*. Deck with anterior end wider than the posterior end (Fig. 4B). Deck with tubercles irregularly shaped with tiny tubercles intermixed with larger ones (Figs 4F

**Table 2.**
Number and range (mode) of setal branches of the pupa of *Anopheles goeldii* collected in Santarém and Belterra, state of Pará, Brazil (n = 40).

| Seta | Cephalothorax | Abdominal segments | | | | | | | | | Paddle |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | CT | I | II | III | IV | V | VI | VII | VIII | IX | P |
| 0 | – | – | 2-6 (4) | 4-8 (5) | 4-7 (5) | 3-6 (4) | 2-5 (4) | 3-7 (4) | 1, 2 (1) | – | – |
| 1 | 2, 3 (2) | n.c.[a] | 7-12 (9) | 4-8 (5) | 1 | 1 | 1, 2 (1) | 1 | – | n.c. | 1 |
| 2 | 2, 3 (2) | 4-7 (5) | 4-8 (6) | 2-6 (4) | 1-3 (2) | 1-3 (2) | 1-3 (2) | 1-4 (2) | – | – | 1, 2 (2) |
| 3 | 2-4 (3) | 1, 2 (1) | 1, 2 (1) | 1-3 (1) | 3-6 (5) | 1-4 (3) | 1-3 (2) | 2-5 (4) | – | – | – |
| 4 | 2-5 (3) | 2-5 (4) | 2-5 (4) | 2-7 (3) | 2-5 (4) | 1-5 (3) | 1-4 (2) | 1-3 (1) | 2, 3 (3) | – | – |
| 5 | 2-5 (3) | 1-3 (1) | 2-6 (4) | 4-9 (6) | 1-6 (3) | 1-3 (1) | 1-3 (1) | 1, 2 (1) | – | – | – |
| 6 | 1, 2 (2) | 1, 2 (1) | 1-3 (1) | 1-3 (1) | 1, 2 (1) | 1, 2 (1) | 1, 2 (1) | 1, 2 (1) | – | – | – |
| 7 | 1-3 (2) | 1-4 (3) | 1-6 (3) | 2-6 (4) | 2-5 (4) | 1-4 (3) | 1 | 1, 2 (1) | – | – | – |
| 8 | 1, 2 (1) | – | – | 2-5 (3) | 1-4 (3) | 1-4 (2) | 1-4 (3) | 3-6 (3) | – | – | – |
| 9 | 1-3 (2) | 1, 2 (1) | 1, 2 (1) | 1 | 1 | 1 | 1 | 1 | 1 | – | – |
| 10 | 1-3 (1) | – | – | 1-4 (3) | 1 | 1 | – | 1, 2 (1) | – | – | – |
| 11 | 4-8 (5) | – | 1 | 1, 2 (1) | 1, 2 (1) | 1, 2 (1) | 1, 2 (1) | 1-3 (2) | – | – | – |
| 12 | 1-3 (2) | – | – | – | – | – | – | – | – | – | – |
| 13 | – | – | – | – | – | – | – | – | – | – | – |
| 14 | – | – | – | – | 1 | 1 | 1 | 1 | 1 | – | – |

[a] n.c. = not counted.

**Table 3.**
Number and range (mode) of setal branches of the fourth instar larva of *Anopheles goeldii* collected in Santarém and Belterra, state of Pará, Brazil (n = 40).

| | Head | | Thorax | | Abdominal segments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | C | P | M | T | I | II | III | IV | V | VI | VII | VIII | X |
| 0 | 1 | 1 | – | – | – | 3-8 (6) | 3-7 (5) | 4-8 (5) | 5-8 (6) | 4-8 (5) | 3-8 (5) | 2-5 (3) | – |
| 1 | 1 | 11-16 (13) | 23-34 (29) | 1, 2 (1) | 8-17 (14) | 19-27 (23) | 21-32 (26) | 21-29 (26) | 20-32 (26) | 19-28 (25) | 18-29 (25) | 1 | 1, 2 (1) |
| 2 | 1 | 12-23 (17) | 1-3 (1) | 1 | 2-4 (3) | 4-8 (6) | 3-6 (4) | 1 | 1-3 (1) | 3-9 (5) | 5-9 (6) | 5-10 (8) | 15-22 (19) |
| 3 | 1 | 1 | 1 | 10-20 (14) | 1, 2 (1) | 1-3 (1) | 1 | 2-4 (3) | 1 | 1 | 2-4 (3) | 7-16 (12) | 6-12 (8) |
| 4 | 1-3 (2) | 14-21 (17) | 1-4 (3) | 2-5 (3) | 3-6 (4) | 4-7 (5) | 2-4 (3) | 2-4 (3) | 2-5 (3) | 1 | 1 | 1 | 8 |
| 5 | 14-23 (16) | 26-41 (32) | 1 | 29-42 (37) | 2-4 (3) | 5-10 (8) | 5-12 (7) | 3-5 (4) | 4-7 (6) | 5-9 (7) | 5-10 (8) | 5-10 (6) | – |
| 6 | 11-22 (15) | 1 | 2-6 (3) | 2, 3 (2) | 30-40 (32) | 31-43 (35) | 21-36 (26) | 1 | 1 | 1, 2 (1) | 3-6 (5) | 1-S | 4-8 (6) |
| 7 | 15-24 (18) | 27-43 (31) | 3-5 (3) | 28-40 (36) | 25-38 (28) | 26-40 (30) | 3-5 (4) | 3-5 (4) | 2-4 (3) | 1-3 (3) | 4-7 (5) | 2-S | 3-7 (5) |
| 8 | 1-4 (3) | 28-40 (35) | 17-29 (22) | 29-43 (35) | – | 2, 3 (3) | 2-4 (3) | 2, 3 (3) | 2-4 (3) | 2-4 (3) | 4-8 (5) | 6-S | 1, 2 (1) |
| 9 | 4-8 (6) | 1 | 1 | 1 | 3-7 (5) | 6-11 (7) | 6-12 (10) | 5-11 (8) | 6-12 (9) | 5-13 (9) | 6-12 (8) | 7-S | 1-3 (2) |
| 10 | 1-4 (3) | 1 | 1 | 1 | 1 | 2-4 (3) | 1 | 1 | 1 | 2-4 (3) | 3-6 (5) | 8-S | 2-5 (4) |
| 11 | n.c.[a] | 1-3 (2) | – | – | 2-5 (3) | 1 | 2, 3 (2) | 2, 3 (2) | 1-3 (2) | 1-3 (2) | 1-3 (2) | 9-S | 2-5 (4) |
| 12 | 2-4 (3) | 1 | 1, 2 (1) | 1-3 (2) | 1-3 (2) | 1 | 2-4 (3) | 2-5 (3) | 1, 2 (2) | 1 | 1, 2 (1) | – | – |
| 13 | 2-5 (4) | 3-4 (3) | 4-9 (6) | 2-4 (2) | 4-8 (6) | 6-11 (7) | 6-10 (7) | 4-8 (5) | 4-7 (5) | 6-14 (8) | 4-6 (5) | – | – |
| 14 | n.c. | 5-10 (7) | 6-13 (10) | – | – | 1 | 1 | 1 | 1 | 1 | 1 | 1 | – |
| 15 | 3 | – | – | – | – | – | – | – | – | – | – | – | – |

[a] n.c. = not counted.

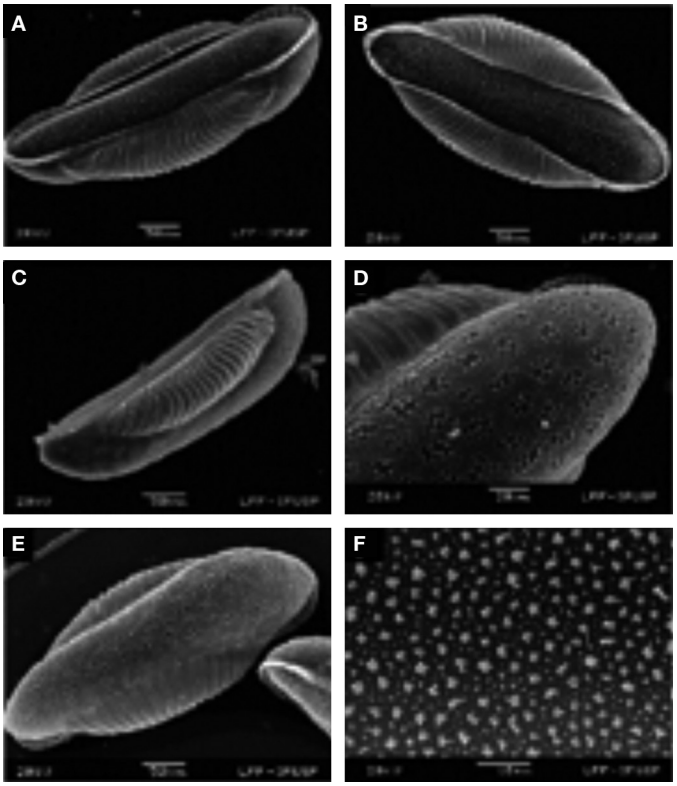


**Figure 4.** *Anopheles goeldii*, scanning electron microscope. A, B, Entire egg, dorsal view. C, Lateral view. D, Ventral view, posterior pole. E, Entire egg, ventral view. F, Central deck, tubercles. Scales in µm.

and 5A); tubercles present on anterior and posterior parts of deck larger and more spacious than those on middle part (Fig. 4B). *Ventral and lateral surfaces*. Composed of chorionic cells (Figs. 4D-E and 5F) with rounded pores of different sizes, central pores larger. Float developed, occupying a lateral position (Figs 4C, 4E, and 5E). *Anterior end*. Rounded (Figs. 4B, 5B, and 5D), frill well developed involving all dorsal anterior end of the egg (Figs. 4A and 4B). Micropylar collar separated from lower frill margin (Figs. 5B and 5D), collar surface smooth, micropylar disk with a continuous ring, with 7, 8 sectors, and limited by short rays (Fig. 5C). *Posterior end*. Narrower than anterior end, with rounded contour, frill developed (Figs. 4A and 4B).

**Molecular characterization.** The inferred phylogeny was presented by Foster et al. (2013) using DNA sequence data from the *white* nuclear gene, the carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) nuclear gene, and the cytochrome oxidase subunit I (COI) mitochondrial gene. The statistical support values for the *An. goeldii* lineage varied depending on the gene and concatenated data sets. Accordingly, the support from the three concatenated genes varied from 0.600 to 0.790 Bayesian posterior probability depending on the data partition, whereas only the COI gene failed to support the monophyly of *An. goeldii* and its closest species, *An. nuneztovari*. The statistical support for the clade composed of *An. nuneztovari* and *An. goeldii* varied depending on the gene and concatenated data sets. The monophyly of *An. nuneztovari* was strongly supported by the *white* gene and the three concatenated genes (see Foster et al., 2013, for further details).

**Distribution.** Considering that *An. goeldii* was resurrected from the synonymy with *An. nuneztovari* by Gabaldón (1981), and the morphological similarity between these two species and *Anopheles* (*Nyssorhynchus*) *dunhami* Causey, 1945, the geographical distribution of *An. goeldii* is incompletely resolved. Calado et al. (2008) proposed that the records from the published literature relative to *An. nuneztovari* cytotype A collected in localities along the Amazon River basin in thenAmazonas, Pará, and Amapá states could also include *An. goeldii*. In addition, because *Anopheles dunhami* can be easily misidentified as either *An. nuneztovari* A or *An. Goeldii*, the distribution of these three species needs to be redefined. Ruiz et al. (2010) recorded the occurrence of *An. dunhami* in Leticia, Colombia, at the Brazilian border, and Foster et al. (2013) confirmed the presence of *An. nuneztovari* A in the Rondônia state. Based on the specimens employed in the present study, *An. goeldii* occurs in localities from the Amazonas, Amapá, and Pará states within the Amazon River basin.

**Medical importance.** It is unknown; however, it is plausible to suppose that this species may be playing an important role, either as

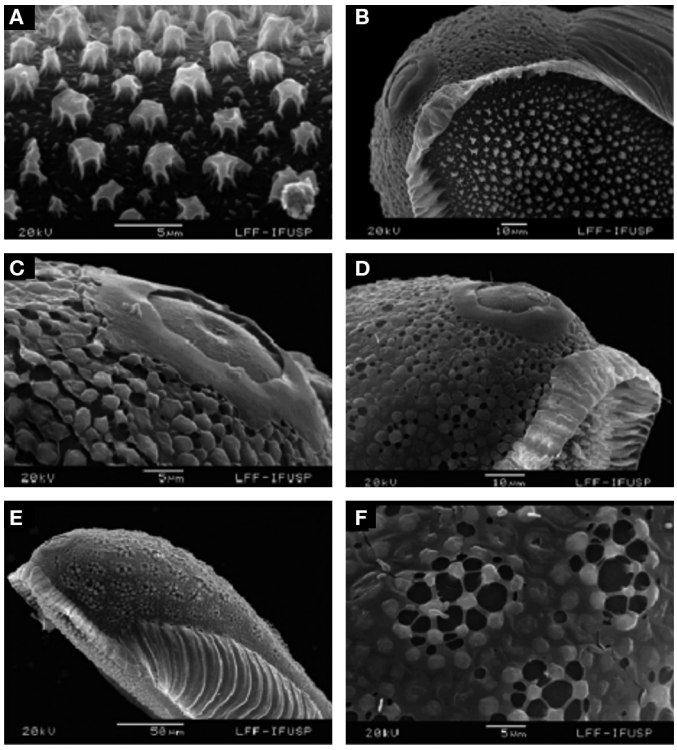


**Figure 5.** *Anopheles goeldii*, scanning electron microscope. A, Detail of the deck tubercles, dorsal view. B, Anterior pole showing the micropyle, dorsoventral view. C, Anterior pole, dorsal view. D, Anterior pole, showing detail of the micropyle, in lateral view. E, Anterior part of the egg, in lateral view. F, chorionic cells. Scales in µm.

a primary or secondary vector, in the dynamics of human malaria in the Amazon River basin. In agreement with this hypothesis, Galardo et al. (2007) demonstrated specimens identified as *An. nuneztovari* from Pará and Amapá as infected with *Plasmodium*. In addition, Tadei and Thatcher (2000) assessed the infectivity of mosquitoes identified as *An. nuneztovari* and found that they were infected with *Plasmodium* (*Plasmodium*) *vivax* Grassi & Feletti, 1890 and *Plasmodium* (*Laverania*) *falciparum* Welch, 1897. Moreover, specimens of *An. nuneztovari* were also found infected with *Plasmodium malariae* (Feletti & Grassi, 1889) along the highway BR-174 (Tadei and Thatcher, 2000). Further studies will be necessary to define the potential association of *An. goeldii* with the dynamics of malaria transmission in the Amazon.

**Bionomics.** Blood fed females were collected in corrals. Larvae and pupae were found in ground pools in pastures, in flooded areas, artificial ponds, in full sun or partially shaded. The larval habitat consisted of temporary, stagnant, fresh, clear, or turbid water, with abundant floating and emergent vegetation, the water temperature averaging at 28 °C. The mosquito species collected in these habitats with *An. goeldii* were *An.* (*Nyssorhynchus*) *marajoara* (Galvão & Damasceno), 1942; *An.* (*Nyssorhynchus*) *triannulatus* (Neiva & Pinto, 1922); *An.* (*Nyssorhynchus*) *braziliensis*, *An.* (*Nyssorhynchus*) *darlingi* (Root, 1926); *An.* (*Anopheles*) *intermedius* (Peryassu, 1908); *An.* (*Anopheles*) *peryassui* (Dyar & Knab, 1908); *Aedeomyia* (*Aedeomyia*) *squamipennis* (Lynch Arribalzaga, 1878); *Culex* (*Culex*) *mollis* (Dyar & Knab, 1906); *Culex* (*Melanoconion*) *bastagarius* (Dyar & Knab), 1906; *Culex* (*Melanoconion*) *batesi* (Rozeboom & Komp, 1948); *Culex* (*Melanoconion*) *evansae* (Root, 1927); *Culex* (*Melanoconion*) *theobaldi* (Lutz, 1904); *Culex* (*Melanoconion*) *intrincatus* (Brethes, 1916); *Culex* (*Melanoconion) idottus* (Dyar, 1920); *Culex* (*Melanoconion*) *clarki* (Evans, 1924); *Culex* (*Melanoconion*) *eknomios* (Forattini & Sallum, 1992); *Culex* (*Melanoconion*) *vaxus* (Dyar, 1920); *Uranotaenia* (*Uranotaenia*) *pulcherrima* (Lynch Arribalzaga, 1891); and *Uranotaenia* (*Uranotaenia*) *geometrica* (Theobald, 1901).

**Material examined for description.** The specimens of *An. goeldii* used for morphological descriptions were collected in Brazil, state of Pará, Urumanduba municipality, (2°28'56.2"S, 54°39'39.3"W), Bergo et al. coll., 08-Sep-2008, Sallum det., 2008: PA3(6)-2 [MG], PA3(6)-3 [LePe], PA3(6)-5 [F], PA3(6)-9 [F], PA3(6)-12 [LePe], PA3(9)-1 [MG], PA3(9)-4 [FLePe], PA3(9)-6 [LePe], PA3(10)-1 [MG], PA3(10)-7 [LePe], PA3(10)-8 [F], PA3(11)-1 [G], PA3(11)-2 [LePe], PA3(11)-4 [F], PA3(11)-16 [LePe], PA3(11)-20 [LePe], PA3(15) [E], PA3(15)-3 [MG], PA3(15)-11 [LePe], PA3(16)-1 [MG], PA3(16)-16 [LePe], PA3(16)-17 [LePe]. State of Pará, Santarém municipality, Bom Jardim (2°33'7.9"S, 54°35'38.7"W), Bergo et al. coll., 09-Sep-2008, Sallum det., 2008: PA5(4)-1 [MG]. State of Pará, Belterra municipality, São Domingos (2°45'7.2"S, 55°1.0'4.9"W), Bergo et al. coll., 10-Sep-2008, Sallum det., 2008: PA7(2)-1 [MG], PA7(2)-6 [F], PA7(2)-10 [LePe], PA7(2)-17 [F], PA7(2)-19 [LePe], PA7(3)-1 [MG], PA7(3)-8 [G], PA7(4)-2 [MG], PA7(4)-15 [LePe], PA7(6) [E], PA7(6)-1 [G], PA7(7) [L], PA7(7)-4 [LePe], PA7(7)-8 [FLePe], PA7(16)-4 [FLePe], PA7(16)-7 [FLePe], PA7(17)-1 [MG], PA7(17)-12 [LePe]. State of Pará, Belterra municipality, Porto Novo (2°37'58.5"S, 54°58'32.9"W), Bergo et al. coll., 13-Sep-2008, Sallum det., 2008: PA12(1)-3 [LePe].

**Other material examined.** Molecular characterization: The specimens of *An. goeldii* were collected in Brazil, state of Amazonas, municipality of Itacoatiara (3°08'4 8.3"S, 58°23'39.7"W), Hutchings & Sallum coll., 01-June-2005, Sallum det., 2005: BRAM03-1. State of Pará, Prainha municipality, Comunidade Terra Preta (2°4.0'49.3"S, 53°35'27.1"W), Sallum coll., 06-Nov-2005, Sallum det., 2005: BRAM22-101. State of Pará, Belterra municipality, São Domingos (2°45'7.2"S, 55°1.0'4.9"W), Bergo et al., 10-Sep-2008, Sallum det., 2008: PA7(2)-2, PA7(3)-8, PA7(4)-3, PA7(17)-2. Morphology: Specimens were collected in Brazil, state of Amapá, municipality of Macapá, Abacate da Pedreira (0°16'17.5"N, 50°53'53.3"W), Bergo et al. coll., 25-Jul-2006, Sallum det., 2006: AP15. State of Amapá, municipality of Macapá, Abacate da Pedreira (0°17'43.6"N, 50°52'39.8"W), Bergo et al. coll., 25-Jul-2006, Sallum det., 2006: AP16; Abacate da Pedreira (0°16'17.5"N, 50°53'53.3"W), Bergo et al. coll., 27-Jul-2006, Sallum det., 2006: AP20.

## Discussion

Currently, *An. goeldii* is a valid species included in the Nuneztovari Complex of the Albimanus Section with *An. dunhami*, *An. nuneztovari* and *An. nuneztovari* A (Foster et al., 2013). The published literature records relative to these species may include one or even more species under the name *An. nuneztovari* because morphological identification is not an easy task if based on female characteristics only. In addition, *An. goeldii* and *An. dunhami* were both in the synonymy of *An. nuneztovari*. Gabaldón (1981) resurrected *An goeldii*, whereas Peyton (1993) validated *An. dunhami*.

Gabaldón (1981) defended that *An. goeldii* was a valid species because it can be easily differentiated from *An. nuneztovari* by the length of the subapical leaflets of the aedeagus of the male genitalia, in addition to larval characters. In contrast, Faran (1980) considered that the subapical leaflets are either present or absent in *An. nuneztovari*, but when present they are membranous. Savage (1986) examined the type and the paratypes of *An. nuneztovari*, reporting that the leaflets are always present and are well developed. In addition, with the objective of addressing geographical variation of morphological traits of the male genitalia, Hribar (1994) compared the aedeagus of individuals from *An. nuneztovari* cytotype A from Brazil and Suriname, cytotype B from Venezuela, and cytotype C from Venezuela and Colombia. He showed that the subapical leaflets are longer and more sclerotized in *An. nuneztovari* B from Venezuela, whereas they are either poorly developed or absent in *An. nuneztovari* A from the three localities in Brazil. *Anopheles nuneztovari* C from Colombia was considered morphologically more similar to *An. nuneztovari* A than to *An. nuneztovari* B. In contrast, Sierra et al. (2004), using DNA sequence

**Table 4.**
Mean values and ratio of the length of the basal dark area of the hind tarsomere 2 (Ta-III2) and the entire length of the hind tarsomere II; the ratio of the length of the humeral pale spot (HP) and the length of the pre-humeral dark spot (PHD); the ratio of the length of the subcostal pale spot (SCP) and the length of the distal sector dark spot (DSD) in females of *Anopheles goeldii* and *Anopheles nuneztovari.*

| | | | DS-III2 / Ta-III2 | | | | HP /PHD | | | | SCP / DSD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference | Patterns[a] | n | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD | Mean | Min. | Max. | SD |
| Ramos et al. (2008) | I | 267 | 0.32 | 0.21 | 0.42 | 0.05 | 1.28 | 0.38 | 3.25 | 0.42 | 0.47 | 0.21 | 0.70 | 0.08 |
| | II | 37 | 0.30 | 0.24 | 0.35 | 0.03 | 1.34 | 0.75 | 3.00 | 0.50 | 0.47 | 0.29 | 0.57 | 0.07 |
| | III | 62 | 0.31 | 0.23 | 0.39 | 0.04 | 1.51 | 0.60 | 3.00 | 0.56 | 0.51 | 0.25 | 0.71 | 0.08 |
| | IV | 8 | 0.33 | 0.29 | 0.39 | 0.04 | 1.80 | 0.71 | 4.00 | 1.09 | 0.38 | 0.25 | 0.43 | 0.06 |
| | V | 22 | 0.32 | 0.24 | 0.41 | 0.04 | 1.35 | 0.80 | 2.25 | 0.41 | 0.43 | 0.18 | 0.64 | 0.12 |
| Calle et al. (2002) | - | 20 | 0.3 | 0.25 | 0.35 | 0.02 | 1.74 | 1.2 | 2.3 | 0.43 | 0.5 | 0.36 | 0.71 | 0.09 |
| This study | - | 20 | 0.28 | 0.23 | 0.38 | 0.05 | 0.96 | 0.77 | 1.16 | 0.12 | 0.33 | 0.21 | 0.42 | 0.08 |

[a] Numbers I to V represent distinct patterns of wing dark and pale spots.

data from the ribosomal internal transcribed spacer 2 (ITS2), showed that *An. nuneztovari* B and C are conspecific.

Considering the geographical origin of the individuals examined by Hribar (1994), it is plausible to assume that he might have included specimens of *An. goeldii* that were misidentified as *An. nuneztovari* A in the pool of samples from Brazilian localities in the Amapá, Pará, and Amazonas states. Further studies will be necessary to verify this hypothesis. In addition, the discrepancies between Faran's (1980) and Savage's (1986) studies may be likely caused by the fact that within the pool of specimens examined by Faran (1980) there were individuals of *An. nuneztovari*, *An. goeldii* and *An. dunhami* that were misidentified as *An. nuneztovari*, whereas Savage examined and illustrated the holotype and paratypes of *An. nuneztovari*.

Specimens of *An. goeldii* from localities in the Belterra municipality, Pará state, possess aedeagal leaflets variable in development. They can be present or absent; when present they are not easily visible, the leaflets are minute and membranous (Figs. 1 and 2), and can be folded depending on the mounting procedure. Consequently, the presence or absence of aedeagal leaflets do not seem to be a character that can be employed for *An. goeldii* identification. In contrast, the species can be distinguished from *An. nuneztovari* based on the preapical plate of the ventral claspette of the male genitalia, which is moderately small, circular, weakly sclerotized, and by the basal lobule, which possesses long spicules distributed along the basal lobule, but are denser in the inner margin. This finding corroborates the description by Rozeboom and Gabaldón (1941).

Ramos et al. (2008) performed a comparative analysis of eight costal wing spots of females and males of *An. nuneztovari* from three populations from Colombia. They compared the ratio of the length of the humeral pale spot (HP) and the length of the pre-humeral dark spot (PHD), and the ratio of the length of the subcostal pale spot (SCP) and the length of the distal sector dark spot (DSD). Additionally, Ramos et al. also employed a character that is included in identification keys, i.e., the ratio of the length of the basal dark area of the hind tarsomere 2 (Ta-III2) and the entire length of the hind tarsomere II. In the present study, we employed the same set of characters to contrast females of *An. goeldii* with *An. nuneztovari* from Colombia. As a result, the ratio values of wing dark and pale spots (ratio of HP/PHD and SCP/DSD) in *An. goeldii* were lower than those found in *An. nuneztovari* by Ramos et al. (2008). Data obtained herein were also contrasted to those published by Calle et al. (2002), showing that for *An. goeldii* the values of ratio of the wing pale and dark spots were lower than those found for *An. nuneztovari* from Colombia (Table 4). In addition, the ratio of the length of the basal dark scale band in the Ta-$III_2$ and the entire length of the hind tarsomere II did not distinguish *An. goeldii* from *An. nuneztovari* (Table 4). Based on characters of the fourth instar larva, Gabaldón (1981) suggested that *An. goeldii* can be distinguished from *An. nuneztovari* by characteristics of the spiracular lobe. In *An. goeldii*, the median plate of the spiracle possesses small lateral arms that are readily observed, whereas in *An. nuneztovari* these lobes are small (see Sutil Oramas, 1976). Specimens examined for the present study confirm the presence of small lateral arms in *An. goeldii*.

Gabaldón (1981) proposed that specimens of *An. goeldii* from the Brazilian Amazon have been misidentified as *An. nuneztovari*. Several studies carried out to compare populations of *An. nuneztovari* from Venezuela and Colombia with populations from Brazil showed that Venezuela and Colombia populations were similar; however, both were distinct from those populations that occur in the Brazilian Amazon (Conn, 1990; Conn et al., 1993; Conn et al., 1998; Fritz et al., 1994; Kitzmiller et al., 1973; Mirabello and Conn, 2008; Onyabe and Conn, 1999). Results of this study confirmed that *An. goeldii* is a valid species, which can be distinguished from *An. nuneztovari* by characteristics of the fourth instar larva, female, male, and male genitalia. In addition, DNA sequences from the COI barcode gene, when analyzed in combination with DNA sequences from the *white* nuclear gene and CAD nuclear gene, confirm the validation of the species. The COI barcode only does not allow distinction between *An. goeldii* and *An. nuneztovari*.

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## Conflicts of interest

The authors declare no conflicts of interest.

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