



Systematics, Morphology and Biogeography

Anopheles (Nyssorhynchus) striatus, a new species of the Strodei Subgroup (Diptera, Culicidae)



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ABSTRACT

A new species of the genus *Anopheles* Meigen (Diptera: Culicidae), *Anopheles (Nyssorhynchus) striatus* n. sp., preliminary designated as *Anopheles* CP Form, from Brazil, is here validated and described using morphological characteristics of the egg, fourth-instar larva, pupa, female and male genitalia. The species is morphologically more similar to species of the Strodei Subgroup of *Anopheles (Nyssorhynchus)* than to any other species of the subgenus *Nyssorhynchus* Blanchard. However, adult female that can be misidentified with *Anopheles (Nyssorhynchus) galvaoi* Causey, Deane & Deane if the identification is mainly based on the ratio of dark and white scales of the hindtarsomere 2. In addition, the characterization of the new species includes aspects of its bionomics, and geographical distribution. The new species is known from Espírito Santo, Minas Gerais and Paraná states, in Brazil. Diagnostic characters for the identification of the species are provided.

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Introduction

The genus *Anopheles* Meigen includes 472 formally recognized species, which are classified in eight subgenera, *Anopheles* Meigen, *Baimaia* Harbach, Rattanarithikul & Harrison, *Cellia* Theobald, *Christya* Theobald, *Kerteszia* Theobald, *Lophopodomyia* Antunes, *Nyssorhynchus* Blanchard and *Stethomyia* Theobald (Harbach, 2015). Forty-one species/species complexes of the genus are the dominant vectors of six protozoan species of the genus *Plasmodium* Marchiafava & Celli that cause malaria in humans (Sinka et al., 2012). Malaria in humans is mainly caused by *Plasmodium falciparum* Welch and *Plasmodium vivax* Grassi & Feletti (Oliveira-Ferreira et al., 2010; White et al., 2014), which are transmitted by mosquitoes of the subgenera *Nyssorhynchus* and *Kerteszia* in Brazil (Oliveira-Ferreira et al., 2010; Rezende et al., 2009; Marrelli et al., 2007). Furthermore, the subgenus *Nyssorhynchus* contains the most important vectors in Central and South America (Laporta et al., 2015; Sinka et al., 2012; Bourke et al., 2010). Morphological identification of several vector species of *Nyssorhynchus* is problematic if based only on females because most characteristics employed in identification keys cannot accurately separate them (Rosa-Freitas et al., 1998). Consequently, it can be problematic to incriminate a species as a potential vector involved in the dynamics of malaria

transmission if it belongs to a cryptic species complex (Bourke et al., 2013).

Root (1926) described *Anopheles (Nyssorhynchus) strodei* based on morphological characters of the female, male, fourth-instar larva, and pupa. The type locality is the district of Água Limpa in the municipality of Juiz de Fora, Minas Gerais, Brazil (Root, 1926). Later, *Anopheles (Nyssorhynchus)* variety *ramosi* Unti was described based on differences observed in fourth-instar larvae collected in Lorena municipality, São Paulo, Brazil (Unti, 1940). The following year, Unti (1941) recognized three new varieties of *An. strodei*, i.e. *An. strodei* variety *albertoi*, *An. strodei* variety *arthuri* and *An. strodei* variety *artigasi*, based on morphological characteristics of the eggs. These three nominal forms have the same type locality, i.e. Rio Paraíba, São Paulo, Brazil. *Anopheles strodei* variety *lloydi* was described by Unti (1941) based on morphological characteristics of eggs from Panama illustrated by Rozeboom (1938). Faran (1980) synonymized the four varieties described by Unti (1941), and also *An. strodei* variety *ramosi* described by Unti (1940), with *An. strodei*. Recently, Sallum et al. (2010) removed *An. strodei* variety *albertoi* and *An. strodei* variety *arthuri* from synonymy and recognized them as valid species. In addition, the presence of a new species, preliminary designated as *Anopheles* CP Form, was hypothesized. Recognition of the two originally described varieties and the *Anopheles* CP Form as independent species was confirmed by the results of phylogenetic analyses using DNA sequences of a fragment of the cytochrome c subunit I (*COI*) mitochondrial gene and of the single copy nuclear *white* gene, and morphological

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characters of the male genitalia and the eggs. Subsequently, Bourke et al. (2013) evaluated a multi-loci system for identifying potential malaria vector species of the *An. strodei* subgroup (viz. Strodei Subgroup). The results confirmed that *An. strodei* and *An. albertoi* are valid species, which *An. arthuri* represents a complex composed of four undescribed species preliminary named *An. arthuri* species A–D, and that *Anopheles* CP Form belongs to the Strodei Subgroup.

Considering that *An. strodei* as defined by Faran (1980) represents a species complex, the participation of any species of this subgroup in the dynamics of malaria remains unknown. However, two facts seem to suggest that *An. arthuri* species C can be a vector. First, *An. strodei* was found naturally infected with *P. vivax* in Rondônia State, Brazil (Oliveira-Ferreira et al., 1990). Second, according to Bourke et al. (2013) specimens identified as *An. strodei*, which were captured in Rondônia, belong to the phylogenetic lineage named *An. arthuri* species C.

The present study focuses on the description of a new species of the Strodei Subgroup of *Anopheles* (*Nyssorhynchus*), previously preliminarily recognized as *Anopheles* CP Form. The purpose of this paper is to describe and formally name *An. (Nys.) striatus* n. sp. based on morphological characteristics of the egg, fourth-instar larva, pupa, female, male and male genitalia. In addition, the characterization of the new species includes aspects of its bionomics and geographical distribution.

Material and methods

Anopheles striatus n. sp. was captured in areas inland and costal Atlantic Forest, including in restinga vegetation and Tabuleiro forest, and in transitional areas of the Atlantic Forest and Cerrado, in Brazil: Reserva Natural da Vale do Rio Doce (19° 04' 58" S, 39° 53' 03" W, Datum SAD69), Linhares, Espírito Santo state, Brazil; in three localities in Minas Gerais state, Brazil, as follows: Fazenda Brejão (20° 01' 31.0" S, 49° 04' 35.4" W, Datum SAD69), Frutal; EMBRAPA (21° 38' 08.95" S, 43° 19' 09.36" W, Datum SAD69), Coronel Pacheco; Taquaral (20° 46' 06" S, 44° 52' 42" W, Datum SAD69), Oliveira; Refúgio Biológico Bela Vista (25° 27' 16.5" S, 54° 32' 47.5" W, Datum SAD69), Foz do Iguaçu, Paraná state, Brazil.

Eggs were collected from five females. Blood-fed females were kept in the laboratory for 48 h and one wing was removed to induce oviposition. Eggs were fixed in Bouin's solution 36 h after oviposition and prepared for scanning electron microscopy (SEM) following the protocol described by Sallum et al. (2010). External structure of the eggs was examined in a scanning electron microscope (JEOL 6460LV, Japan) in the Laboratório de Filmes Finos, Departamento de Física Experimental, Instituto de Física, Universidade de São Paulo, Brazil. Morphological characters of the adult female, adult male, fourth instar larva, pupa, and male genitalia were examined. Abbreviations used to denote 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. Specimens used in this study are deposited in the Coleção Entomológica de Referência (FSP-USP), Faculdade de Saúde Pública, Universidade de São Paulo.

Taxonomy

Anopheles (*Nyssorhynchus*) *striatus* n. sp.

Anopheles strodei of Bourke et al. (2010).

Anopheles CP Form of Sallum et al. (2010); Bourke et al. (2013); Foster et al. (2013).

Entomology – The name *striatus* is derived from Latin to denote the wrinkled surface of the ventral claspette of the male genitalia. *Striatus* is the perfect passive participle of *strio*, meaning furrowed, grooved, fluted, or striated.

Diagnosis – A combination of morphological character states allows the identification of *An. striatus* as follows. Female: terga II–VII with dark caudolateral tufts of semi-erect scales; hindtarsomere 2 dark-scaled on basal 0.6–0.7, white-scaled on apical 0.3–0.4; pre-humeral dark spot of the wing smaller than the basal pale and humeral pale spots. Male: Genitalia with apex of ventral claspette wide, rounded, deeply rugose and striated, expanded laterally into conspicuous rounded lobes, striae parallel and perpendicular to longitudinal axis of genitalia; apex strongly expanded laterally into large, rounded lobes; apicolateral lobes with convex basal, lateral, and apical margins, with sparse spicules laterally. Basal portion with minute, sparse spicules on ventral surface, extending from base to level of apicolateral angle, spicules on lateral surface extending toward apex, reaching apicolateral angle. Preapical plate moderately developed and sclerotized, circular, well defined. Basal lobule small, short, with sparse spicules, similar in size and development (Sallum et al., 2010). Fourth-instar larva: seta 2-C inserted close to longitudinal median line, distance between seta 2-C and seta 3-C wide, ratio of distance between 2-C and 3-C greater than 3 times distance between 2-C; seta 1-P palmate; seta 1-I–VII palmate, leaflets with smooth lateral margins; median plate of spiracular lobe with short, pointed anterior lateral arms, posterolateral arms moderately pigmented, poorly pigmented at median longitudinal area. Egg: Frill completely enclosing exposed tubercles at both anterior and posterior ends, forming 2 crowns, one at each end of dorsal surface; deck area narrow, extending along approximately 0.5 length of egg, floats moderately broad, fused dorsally at anterior and posterior areas of deck surface.

Female – Integument light to dark brown with grayish pollinose. **Head.** Vertex posterior to frontal tuft with erect, white spatulate scales and a few long, pale yellow setae, remainder of vertex and occiput with erect dark brown to black spatulate scales; postgena with tuft of black spatulate scales and a few semi-erect, white spatulate scales at junction of eyes; clypeus dark brown, bare. Proboscis dark-scaled; length 1.89–2.19 mm (mean = 1.98 mm ± 0.13) ($n = 5$), length of maxillary palpus 1.73–1.93 mm (mean = 1.84 ± 0.08) ($n = 5$). Maxillary palpomere 1 dark-scaled; palpomere 2 mostly dark-scaled with a few white scales at apex of dorsal surface; palpomere 3 mostly dark-scaled with white scales at apex of dorsal surface, and a few white scales at mid-length of dorsal surface; palpomere 4 mostly white-scaled with dark scales at base, sometimes with a few dark scales at lateral surface and apex; palpomere 5 predominantly white-scaled with dark scales at base; scales erect on palpomeres 1 and 2, semi-erect and decumbent on dorsal surface, and erect on ventral surface of palpomere 3. **Thorax.** Integument pruinose with darker area between dorsocentral area and lateral margin, at posterior edge of scutal fossa, and posteriorly on prescutellar area, extending posteriorly to median scutellar lobe. Anterior promontory with long setiform, white scales, usually not extending dorsad onto acrostichal area; acrostichal setae strong; scutal area with white scales; scutellum with long dark setae along posterior margin, with white spatulate scales anteriorly to setae; integument of median scutellar lobe brown. Anteprepronotum with long dark setae; prespiracular area without setae and scales; prealar area with setae intermixed with white spatulate scales; upper mesokatepisternum without setae and scales, lower mesokatepisternum with white spatulate scales and brownish setae. **Wing.** Length 3.26–3.46 mm (mean = 3.36 ± 0.09; $n = 5$). Veins covered with dark and pale scales. Costa with the following spots: basal and prehumeral pale, prehumeral dark, humeral pale, humeral dark, presector pale, presector dark, accessory sector pale, distal sector dark, subcostal pale, preapical dark, preapical pale and apical dark; sector pale and sector dark spots present in 35% of specimens examined ($n = 10$); vein R_s mostly dark-scaled; R_{2+3} predominantly pale-scaled; R_{4+5} predominantly pale-scaled with dark scales at

base; M mostly pale-scaled. *Halter*. Scabellum and pedicel with pale brown integument; capitellum dark-scaled with patch of pale scales at base. *Legs*. Foretarsomeres 1–3 with white scales at apex, foretarsomeres 4 and 5 dark-scaled. Midtarsomeres 1 and 2 dark-scaled with apical white band, midtarsomeres 3–5 dark-scaled. Hindtarsomere 1 predominantly dark-scaled, pale-scaled at apex,

hindtarsomere 2 dark-scaled on approximately basal 0.6, white-scaled on approximately apical 0.4, hindtarsomeres 3 and 4 entirely white-scaled, hindtarsomere 5 dark-scaled on approximately basal 0.5, white-scaled at apex. *Abdomen*. Integument light to dark-brown; terga II–VIII covered with narrow pale scales, mostly scales disposed in a subtriangular pattern on terga II–V, terga VI–VII more

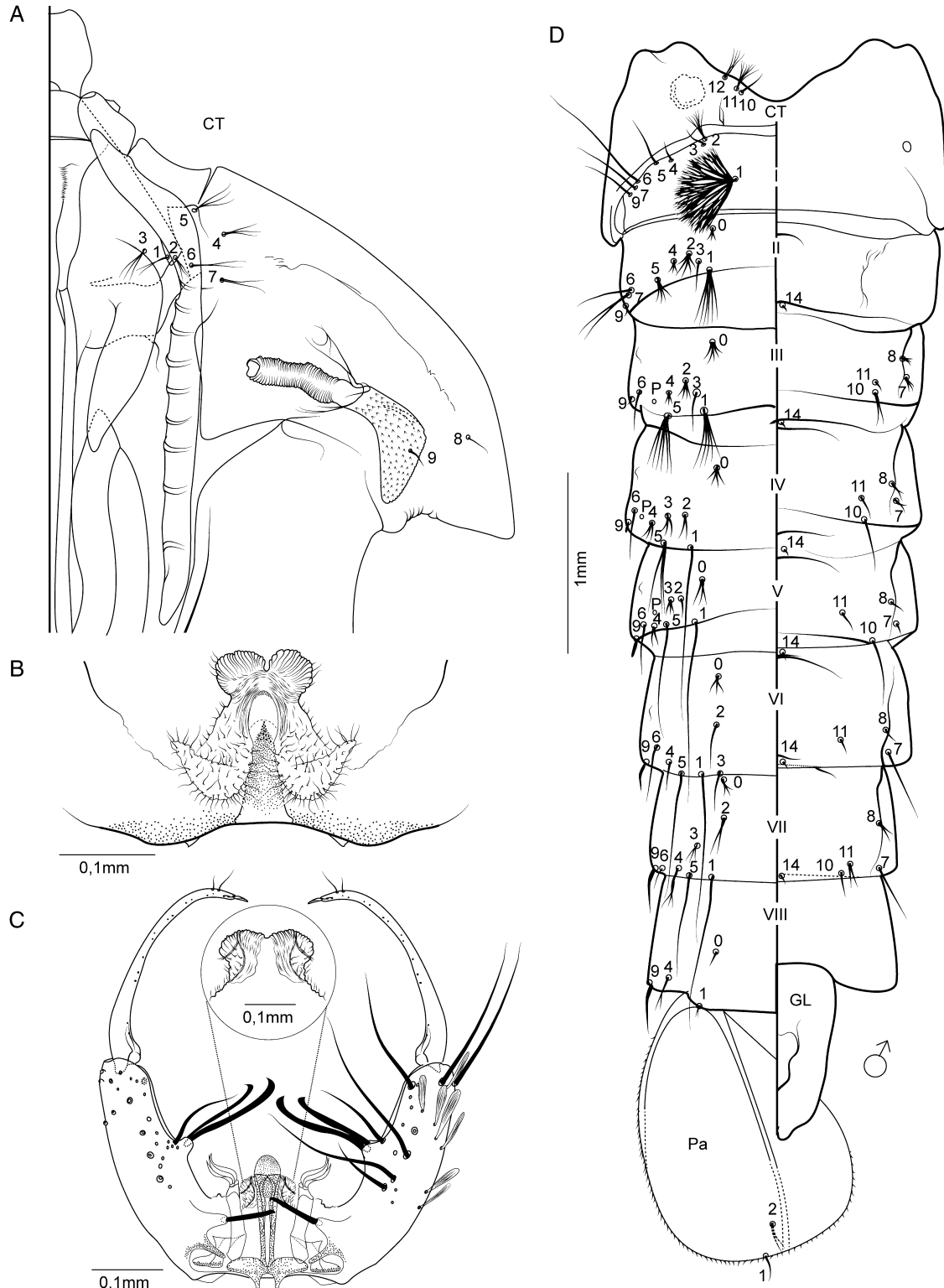


Fig. 1. Pupa and male genitalia of *Anopheles (Nyssorhynchus) striatus* n. sp. Pupa. (A) CT: cephalothorax; (B) Detail of ventral claspette, in ventral view; (C) male genitalia in dorsal view, with a detail of ventral claspette in dorsal view, enclosed by circle. (D), Pa: paddle; I–IX: abdominal segments. Male genitalia. Scales in mm.

evenly covered with pale scales; dark posterolateral scale-tufts on terga II–VII. Sternum I bare; sterna II–VII with a few pale scales; sternum VIII totally covered with pale scales and a few dark scales.

Male (Fig. 1) – Similar to female except for sexual differences. Proboscis length 2.28–2.41 mm (mean = 2.36 ± 0.08) ($n = 5$). Maxillary palpus pale and dark-scaled, scales semi-erect on basal 0.3 of palpomere 2, decumbent on remainder of palpomere 2 and palpomeres 3–5; palpomere 2 dark-scaled with white scale band at junction of palpomere 3; palpomere 3 white-scaled at base and apex; palpomere 4 mostly white-scaled with sub-basal and subapical bands of dark scales extending from dorsal to ventral surface, ventral surface dark-scaled; palpomere 5 mostly white-scaled on dorsal surface with basal band of dark scales, lateral and ventral surfaces mostly dark-scaled. **Genitalia (Fig. 1)**. Tergum and sternum of abdominal segment VIII with spatulate scales and long setae; tergum IX membranous, produced laterally, sternum well developed, sub-rectangular. Dorsal claspette with long pedicel, moderately broad, base rounded, leaflets broad and curved. Apex of ventral claspette wide, rounded, deeply rugose and striated, expanded laterally into conspicuous rounded lobes, striae parallel, perpendicular to longitudinal axis of genitalia; apex strongly expanded laterally into large, rounded lobes; apicolateral lobes with convex basolateral and apical margins. Apex with sparse spicules on lateral surface. Basal portion with minute, sparse spicules on ventral surface, extending from base to level of apicolateral angle, spicules on lateral surface extending toward apex, reaching apicolateral angle. Preapical plate moderately developed and sclerotized, circular, well defined. Basal lobule small, short, with sparse spicules, spicules similar in size and development.

Pupa (Fig. 1) – Position and development of setae as figured. All measurements were based on 5 specimens; seta branches counting were based on a single specimen. **Cephalothorax:** Integument weakly pigmented; legs darker; trumpet angusticorn with meatal cleft; pinna moderately pigmented. **Abdomen:** Length 2.17–2.44 mm (mean = $2.27 \text{ mm} \pm 0.10$); seta 2-I with 5 branches; 3-I single as long as 2-I; 4-I with 4 branches; 5,6-I single, long; 7-I with 2 branches, shorter than 6-I; 9-I single, as long as 7-I; 0-II-VII moderately developed, 0-II-IV with 4 branches, 0-V-VII 3-branched; 1-II, III well developed, with 7 and 5 branches, respectively, 1-IV-VII always single, strong, long, extending beyond following segment; 3-IV with 3 branches, never reaching caudal margin of segment; 3-V double or triple, never reaching caudal margin of segment; 5-III with 5 branches, shorter than 0.5 length of following segment, 5-IV with 2 branches, 5-V-VII normally single, long; 6-II most often single, 6-III single, 6-IV-VII single; 7-II with 2 branches, 7-III,V normally double, 7-IV often double, 7-VI,VII single; 8-III with 2 branches, 8-IV-VI normally double, 8-VII normally triple; 9-II minute, lightly pigmented, 9-III short, stout, 9-IV tick, dark, 9-V strong, 9-VI strong, weakly curved, 9-VII strong, weakly curved, sharply pointed, 9-VIII straight; 10-III normally triple, 10-IV,V single, long; 4-VIII with 2 branches. **Genital lobe:** Thick at base, with sides sloping toward apex, apex with mammiliform protuberance. **Paddle:** Obovate, index 1.38–1.40 (mean = 1.37 ± 0.03) longer than wide, length 0.68–0.72 mm (mean = 0.70 ± 0.02), width 0.48–0.53 mm (mean = 0.51 ± 0.02); outer margin distal of buttress with very fine, minute spicules, extending around apex and becoming sparse along inner margin; seta 1-Pa stronger than 2-Pa, 2-Pa single or double.

Fourth-instar larva (Fig. 2) – Position and development of setae as figured; all measurements were based on 5 specimens; seta branches counting were based on a single specimen. **Head:** Length 0.63–0.69 mm (mean = $0.66 \text{ mm} \pm 0.02$); width = 0.62–0.67 mm (mean = 0.64 ± 0.03). Integument weakly pigmented, yellowish to light brown, with dark spots, not forming distinct pattern; dorsosentum strongly sclerotized, blackish; median tooth moderately broad, about twice as wide as adjacent

tooth, tapered to point, blunt at apex. Seta 2-C single, with sparse, minute aciculae on distal 0.4, longer than 3-C, seta 2-C inserted close to mate of opposite side; 3-C single, weakly aciculate on distal 0.6; seta 4-C with 5 branches, short, extending approximately halfway to anterior margin of head; 5-C with 15 branches extending well beyond base of 2-C; 6,7-C with 17 branches, 6-C extending beyond base of 3-C, 7-C extending to base of 3-C; 8-C with 3 branches; 9-C with 4 branches; 10-C with 3 branches; 12-C with 4 branches; 13-C with 5 branches. Collar strongly pigmented, dark brown. **Antenna:** Length 0.28–0.33 mm (mean = $0.31 \text{ mm} \pm 0.02$), enlarged toward base, longer than wide; with long and thin spicules on mesal margin, spicules shorter and fewer on dorsal and ventral surfaces, seta 1-A as long as width of antenna at point of insertion, arising at approximately basal 0.3 of flagellum. **Thorax:** Setae 1,2-P arising separate, 1-P palmate, with 11 moderately narrow, lanceolate leaflets, leaflets pointed at apex, 2-P with 16 branches; 3-P single; 14-P with 10 branches, branches arising distinct distance from base, extending beyond anterior margin of thorax; 1-M strongly plumose, usually with 23 branches; 2,3,5-M single, simple; 4-M with 3 branches; 6-M usually with 2 branches; 7-M with 2 or 3 branches; 8-M plumose; 14-M usually with 8 branches; 1,2-T single, simple; 3-T palmate, with narrow, semi-transparent leaflets, usually with 11 leaflets; 4-T small usually with 3 branches; 13-T always double. **Abdomen:** Integument with minute spicules on ventral surface of segments II–VIII; seta 0-IVII moderately large; 1-I-VII palmate, 1-I with 13 leaflets, 1-II-VII with moderately narrow, truncate leaflets; 2-II with 4 branches, strongly developed, large, 2-III frequently triple, stronger than 2-II, 2-IV,V simple, single, 2-VI triple, 2-VII usually with 5 branches; 5-I triple, 5-II usually with 7 branches, 5-III,IV with 5 branches, 5-V,VI with 6 or 7 branches, 5-VII with 7 branches; 6-I-III plumose, inserted on tubercle, 6-IV-VI simple, single, large, 6-VII with 4 branches; 7-I,II plumose, inserted on tubercle, 7-III triple, 7-IV, V with 4 branches, 7-VII double; 8-II-V usually triple, 8-VI double, 8-VII with 5 branches; 9-I with 5 branches, 9-II with 6 branches, 9-III with 9 branches, 9-IV,V with 7 branches; 10-I,III-V simple, single, 10-II double or triple, 10-VI double, 10-VII with 4 branches; 11-I with 3 branches, large; 13-I,II,III with 5, 9 and 8 branches, respectively, 13-IV with 6 branches, shorter than branches of 2-V, 13-VI small, with 8 branches. Pecten with 3 long and 11 short spines; lateral arm of median plate of spiracular apparatus short, directed dorsolaterally, posterolateral arms of median plate; 1-VIII single, 2-VIII with 10 branches, 3-VIII with 8 branches, 4-VIII single, 5-VIII with 5 branches. **Segment X:** Most of segment covered with minute spicules, spicules stronger on posterior margin; seta 1-X as long as saddle, inserted on margin of saddle; anal papillae narrow, longer than saddle. **Spiracular lobe:** Pecten plate usually with 3 long and 11 short spines; median plate predominantly moderately pigmented, darkly pigmented at anterior end with short, pointed lateral arms; seta 1-S strongly developed, with 5 branches, 6-S double, 7-S single, 8-S triple, 9-S with 6 branches, 10-13-S single, minute.

Egg (Figs. 3–5) – **Size and overall appearance:** Length $0.47 \text{ mm} \pm 0.008 \text{ mm}$; width $0.15 \text{ mm} \pm 0.01 \text{ mm}$. Black in color; narrowly boat-shaped in dorsal (upper) view (Fig. 3A), broadly boat-shaped in lateral view (Fig. 3B), tapering toward both anterior and posterior ends (Fig. 3A). **Anterior end (Fig. 3C):** Somewhat broader than posterior end (Fig. 3D). **Float:** Narrow, located laterally, extending anteriorly and posteriorly, never reaching ends of egg, slightly broader than width of egg at midline, width decreasing toward anterior and posterior ends (Fig. 3E). **Frill:** Completely enclosing exposed tubercles at both anterior and posterior ends, forming 2 crowns, one at each end of dorsal surface. Dorsal surface slightly concave, ventral surface strongly concave in lateral view (Fig. 3B). **Dorsal surface:** Deck narrow, extending along approximately 0.5 length of egg (Fig. 3A). Floats fused anteriorly and posteriorly, completely encircling deck. Chorionic cell

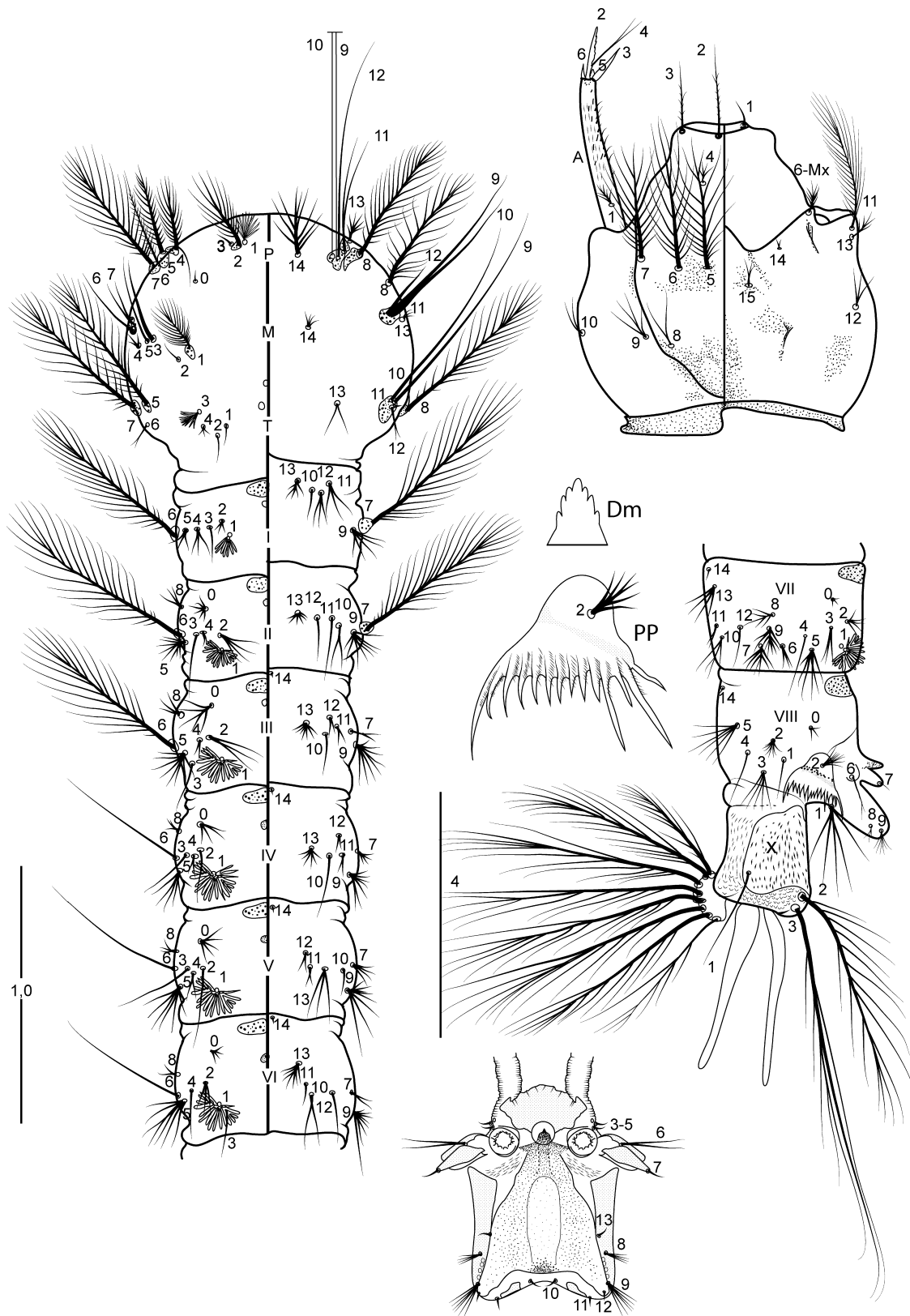


Fig. 2. Fourth-instar larva of *Anopheles (Nyssorhynchus) striatus* n. sp. A: antenna; Dm: dorsomentum; M: mesothorax; P: prothorax; PP: pecten plate; T: metathorax; I–VIII, X: abdominal segments. Scales in mm.

boundaries on deck not visible, deck covered with large tubercles interspersed between smaller tubercles of different size and shape (Figs. 3A and 4A). Large tubercles domed, irregular in shape with buttressed walls (Fig. 4A). Tubercles inside anterior and posterior

crowns (Figs. 3C,D and 4B), larger and more developed than those present on median area of deck (Fig. 4A). *Ventral and lateral surfaces:* Boundaries of cells of outer chorion (plastron) clearly defined on ventral (lower) and lateral surfaces, cell surfaces dominated

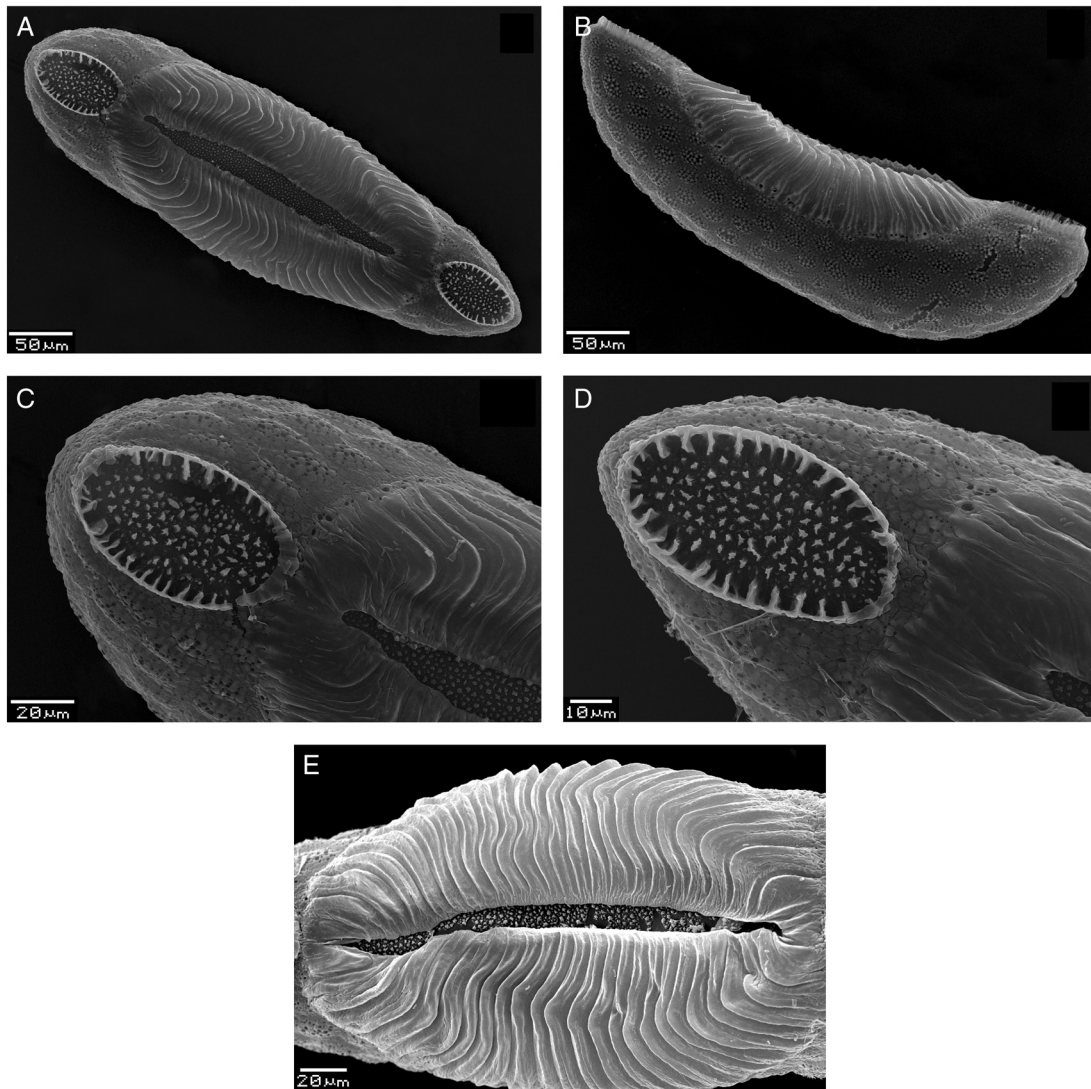


Fig. 3. Scanning electron micrographs of eggs of *Anopheles (Nyssorhynchus) striatus* n. sp. (A) Entire egg, anterior end on the left, upper surface view. (B) Entire egg, anterior end on the right, lateral view. (C) Anterior end, showing the anterior deck, encircled by the fringe, forming a crown on upper surface view. (D) Posterior end, showing the posterior deck, encircled by the frill, forming a crown on upper surface view. (E) Floats enclosing deck, dorsal view. Scales in μm .

by somewhat elliptical, bumpy mounds (Fig. 4C). Pores present in bumpy mounds, with few reduced perforations in areas between mounds (Fig. 4D). Floats moderately narrow (Fig. 3A), fused dorsally at anterior and posterior areas of deck, with approximately 23 ribs, intersection of float borders and ventral plastron clearly defined (Fig. 5A and B). *Anterior and posterior ends:* Anterior and posterior crowns elliptical, tapering toward middle of egg where they are continuous with float margins (Fig. 3A and E). Walls of crowns grooved (Fig. 3C and D). Tubercles inside crowns (Fig. 3C and D) larger than deck tubercles (Fig. 4A). Plastron around micropylar collar and posterior pole of egg with blister-like mounds, less pronounced than those present on ventral plastron (Fig. 5C). Micropylar collar separate from anterior margin of crown; collar surface smooth, inner margin slightly convex, with radial ridges extending from collar into micropylar disk; micropylar disk single layered, supporting a central micropyle within a slightly depressed circular area (Fig. 5D).

Material examined – Holotype – Deposited in the Coleção Entomológica de Referência, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil, accession number FSP-USP n° E-15583. Pinned adult male with associated Le and Pe and dissected genitalia mounted on microscope slides, specimen field code

MG15(1)-4, bearing the following collection data: Brazil, Minas Gerais State, Coronel Pacheco, Road from Juiz de Fora to Uba municipality, Embrapa, 21° 38' 8.95" S, 43° 19' 9.36" W, coll. 02-Dec-2008, Bergo et al., in Shannon Trap, in a rural area with pasture, mountainous, 900 m altitude. The holotype is derived from a progeny of a female (MG15(1)). *Paratypes* – Minas Gerais State, Coronel Pacheco, Road from Juiz de Fora to Uba, Embrapa, 21° 38' 8.95" S, 43° 19' 9.36" W, coll. 02-Dec-2008, Bergo et al., MG15(1)-1, FSP-USP n° E-15584 [LePeG♂]; MG15(1)-10, FSP-USP n° E-15585 [FLePe]; MG15(2)-3, FSP-USP n° E-15586 [MLePeG]; MG15(3)-2, FSP-USP n° E-15587 [FLePe]; MG15(4)-5, FSP-USP n° E-15588 [MLePeG]; MG15(4)-9, FSP-USP n° E-15589 [FLePe]; MG15(9)-6, FSP-USP n° E-15590 [LePeG♂], which was used for molecular characterization and the COI barcode sequences are deposited in GenBank with accession number KC330243; MG15(13)-1, FSP-USP n° E-15591 [MLePeG]; MG15(24)-3, FSP-USP n° E-15592 [MLePeG]; MG15(24)-14, FSP-USP n° E-15593 [FLePe]; MG15(26)-1, FSP-USP n° E-15594 [MLePeG]. Minas Gerais State, Oliveira, Taquaral, 20° 46' 03" S, 44° 52' 44" W, coll. 11-Apr-2010, Bergo et al., MG38(14)-2, FSP-USP n° E-15595 [MLePeG]; MG38(16)-1, FSP-USP n° E-15596 [FLePe]. Minas Gerais State, Oliveira, Taquaral, 20° 46' 05" S, 44° 52' 40" W, coll. 12-Apr-2010, Bergo et al., MG44(16)-1, FSP-USP n° E-15597

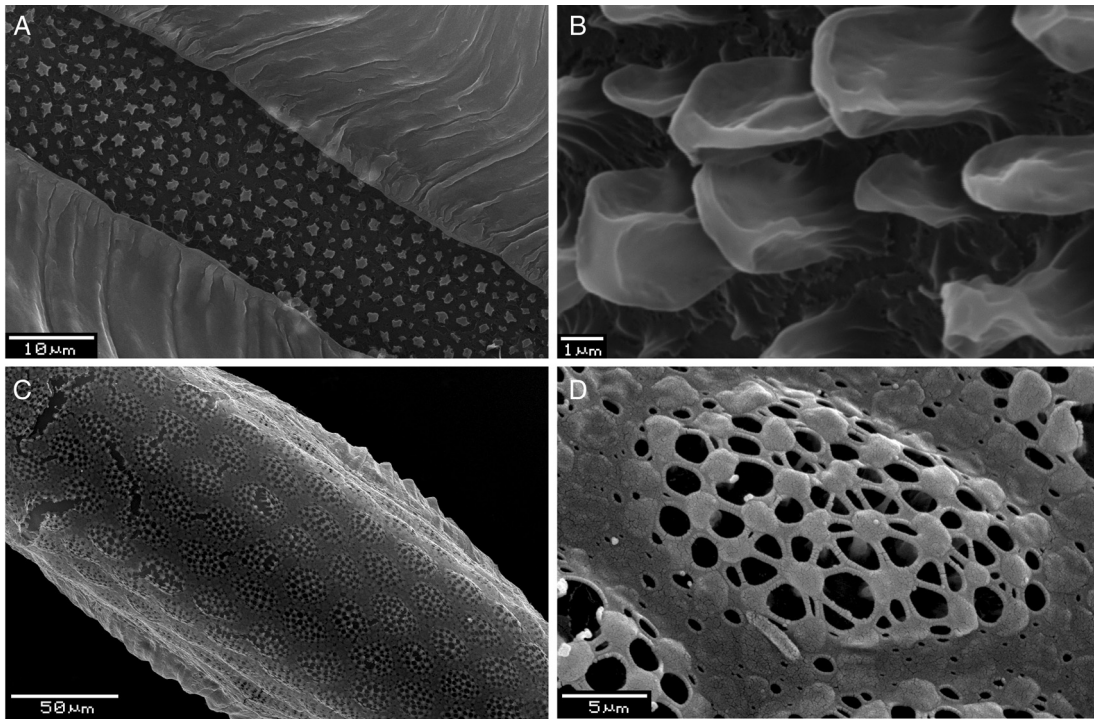


Fig. 4. Scanning electron micrographs of eggs of *Anopheles (Nyssorhynchus) striatus* n. sp. (A) Central deck area, showing the tubercles and margins of the floats, upper surface view. (B) Detail of tubercles of the anterior crown, upper lateral surface view. (C) Plastron, showing the chorionic cells, ventral view. (D) Chorionic cell, showing details of the pores on plastron, lower surface view. Scales in μm .

[FlePe]; MG44(16)- 100, FSP-USP n° E-15598 [MPeG]; MG61(6)-1, FSP-USP n° E-15599 [MLePeG]; MG61(6)- 2, FSP-USP n° E-15600 [FlePe]. Minas Gerais State, Frutal, Road from Frutal to Itapeipe km 14, Fazenda Brejão, Sítio Paraíso, 20° 01' 31.0" S, 49° 04' 35.4" W, coll. 21-Nov2006, Bergo et al., MG07(15)-1, FSP-USP n° E-15601

[FlePe]. Espírito Santo State, Linhares, Reserva Natural do Vale do Rio Doce, Rio Barra Doce, 19° 05' 04.1" S, 39° 53' 03.8" W, coll. 23-Oct-2007, Bergo et al., ES20(4)-3, FSP-USP n° E-15602 [LePe♂]; ES20(4)-4, FSP-USP n° E-15603 [LePe♂]; ES20(4)-7, FSP-USP n° E-15604 [LePe♂]; ES20(4)-8, FSP-USP n° E-15605 [F]. Paraná State,

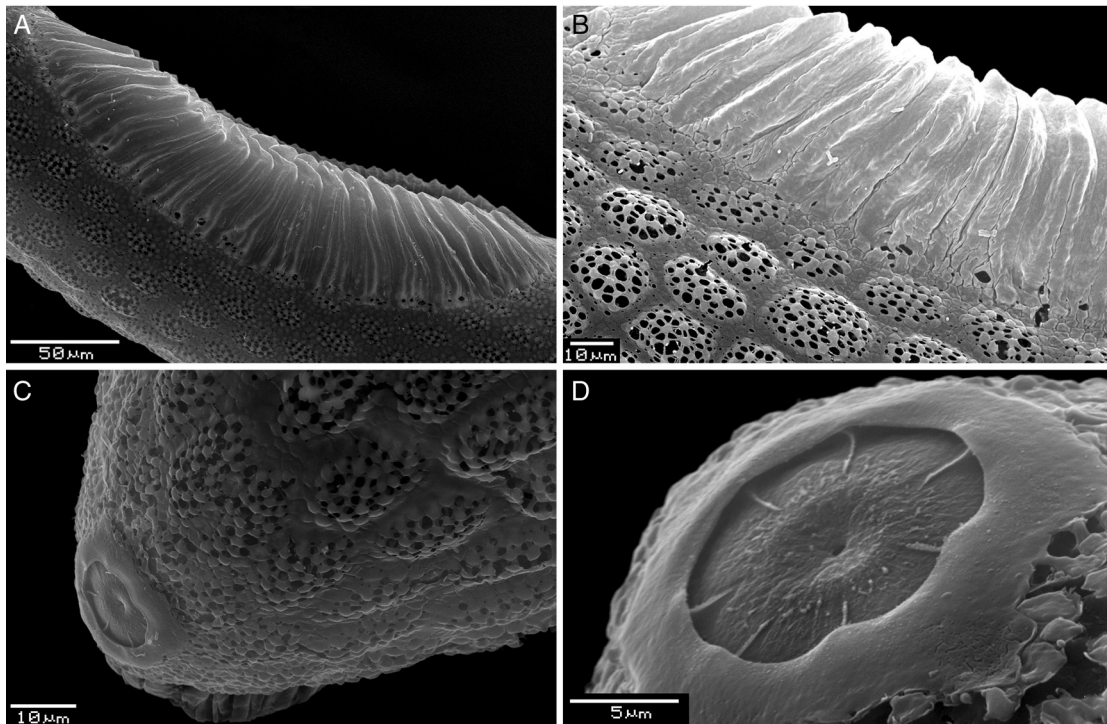


Fig. 5. Scanning electron micrographs of eggs of *Anopheles (Nyssorhynchus) striatus* n. sp. (A) Floats, lateral view. (B) Plastron, showing details of the chorionic cells and junction with the float, upper lateral surface view. (C) Plastron, showing details of the chorionic cells around micropyle, anterior end, lower surface view. (D) Micropyle. Scales in μm .

Foz do Iguaçu, Refúgio Biológico Bela Vista, 25° 27' 16.5" S, 54° 32' 47.5" W, coll. 28-Feb-2007, Bergo et al., PR21-110, FSP-USP n° E-15606 [G♂], which was used for molecular characterization and the *COI* barcode sequences are deposited in Genbank with accession number GU226691. Paratypes are deposited in the Coleção Entomológica de Referência of Faculdade de Saúde Pública, Universidade de São Paulo, Brazil (FSP-USP).

Non-type specimens. Espírito Santo State, Linhares, Reserva Natural do Vale do Rio Doce, Rio Barra Doce, 19° 05' 0.41" S, 39° 53' 03.8" W, coll. 23-Oct-2007, Bergo et al.; Shannon Trap, in Restinga vegetation, on river margin, 3♂, 3♀ (ES20(4)-6, -9, -10, -11, -12, -14) with associated Le and Pe; Minas Gerais State, Coronel Pacheco, Road from Juiz de Fora to Uba, Embrapa, 21° 38' 8.95" S, 43° 19' 9.36" W, coll. 02-Dec-2008, Bergo et al., Shannon Trap, in a rural area with pasture, mountainous, 900 m altitude, 5♂, 4♀ (MG15(1)-4, -8, -9, -11, -12, -13, -14; MG15(1)) 5♂ with associated Le and Pe, 3♀ with associated Le and Pe, 2♀ only; 10♂, 3♀ (MG15(2)-4, -5, -7, -8, -9, -10, -11, -12, -13, -14, -15, -16; MG15(2)) 9♂ with associated Le and Pe, 3♀ with associated Le and Pe, 1♂ adult only; 2♂, 5♀ (MG15(4)-2, -6, -7, -8, 10, 13, 14) all with associated Le and Pe; 14♂, 20♀ (MG15(6)-1, -3, -4, -5, -6, -7, -8, -9, -10, -11, -13, -14, -16, -17, -19, -21, -22, -24, -26, -30, -31), 14♂ with associated Le and Pe, 8♀ with associated Le and Pe, 12♀ adult only; 7♂, 10♀ (MG15(9)-1, -2, -4, -5, -8, -9, -10, -11, -12, -13, -14, -19, -20, -23, MG15(9)) 6♂ with associated Le and Pe, 9♀ with associated Le and Pe, 1♂ adult only, 1♀ adult only; 5♂, 7♀ (MG15(13)-2, -3, -4, -5, -7, -8, -11, -12, -13, -108, MG15(13)) 4♂ with associated Le and Pe, 6♀ with associated Le and Pe, 1♂ adult only, 1♀ adult only; 2♂ (MG15(15)-1, -4)) with associated Le and Pe; 6♀, 1♂ (MG15(24)-2, -4, -5, -6, -7, -8, -9)) with associated Le and Pe; 11♂, 27♀ (MG15(26)-2, -3, -4, -5, -7, -8, -11, -14, -15, -16, -17, -18, -100, -101, MG15(26)) 7♂ with associated Le and Pe, 7♀ with associated Le and Pe, 4♂ adult only, 20♀ adult only.

Molecular characterization. A molecular phylogenetic analysis was conducted by Sallum et al. (2010) using the nuclear single copy *white* gene, and the *white* gene in combination with the mitochondrial cytochrome c oxidase subunit I (*COI*) gene. *Anopheles striatus* n. sp. sequences were clearly separated from those of other individuals (*An. albertoi*, *An. arthuri* and *An. strodei*), however using the *white* gene sequences in combination with those of the *COI* gene, relationships within the ingroup were better resolved than when using sequences of each gene separately. Recently, Bourke et al. (2013) found support for seven clades in the *An. strodei* subgroup (*An. strodei*, *An. albertoi*, *An. arthuri* species A–D and *Anopheles* CP Form) when analyzing sequence data that included a higher number of specimens and several localities not sampled by Sallum et al. (2010). Results from Bayesian phylogenetic analyses that included *COI*, ITS2, and *white* gene sequences corroborated the findings of a new species in the Strodei Complex, and multi-loci *COI*-ITS2 barcode resolved all species in a neighbor joining tree and successfully showed that all species recognized by Bourke et al. (2013) were monophyletic lineages.

Distribution. *Anopheles striatus* n. sp. has been found in Espírito Santo, Minas Gerais and Paraná States, in areas of the interior and coastal Atlantic Forest. The new species occurs in areas of the Restinga vegetation and Tabuleiro forest of the Espírito Santo state, in transitional areas of Atlantic Forest and Cerrado in Minas Gerais state and interior Atlantic Forest in Paraná state.

Medical importance. It is not known whether *An. striatus* n. sp. is of medical importance.

Bionomics. Larvae and pupae were taken from lake margins and ground pools. The water was fresh, clear, permanent, and stagnant; with emergent, floating, sparse vegetation, and green and brown algae; in partial shade. Larval habitats were found in rural areas with pasture; concentration of oxygen dissolved in water was 82.8%, and water temperature at the collection sites was 32.2 °C.

Females were collected in a Shannon trap with humans as collectors, from 18:00 h to 21:00 h. Collections were conducted in secondary environments, i.e. natural ecosystems that were changed by human occupation and activities. The vegetation in areas where the species occurs includes fragments of Restinga vegetation, tropical rain forest, Cerrado vegetation and pasture, in low altitude and mountainous areas, up to 900 m altitude.

Discussion

Faran (1980) synonymized *An. strodei* variety *albertoi*, *An. strodei* variety *arthuri*, *An. strodei* variety *artigasi*, *An. strodei* variety *ioydi* and *An. strodei* variety *ramosi* with *An. strodei*. Recently, Sallum et al. (2010) resurrected *An. variety albertoi* and *An. variety arthuri* from synonymy and elevated them to species level. In addition, Sallum and colleagues hypothesized the presence of a new species designated *Anopheles* CP Form based on differences observed in the male genitalia of a single individual collected in Foz do Iguaçu in the state of Paraná and sequences of the *COI* mtDNA and the single-copy nuclear *white* gene. Results of a study conducted by Bourke et al. (2013) using three individuals collected in Coronel Pacheco in the state of Minas Gerais confirmed the species status of *Anopheles* CP Form. Consequently, we have herein formally described and name the new species as *An. striatus*, with the aid of additional specimens collected in Frutal and Oliveira municipalities in Minas Gerais state, and Linhares municipality in Espírito Santo state.

Differences in the egg structure of the species comprising the Strodei Subgroup are conspicuous. Galvão and Lane (1936) presented schematic drawings of *An. strodei* eggs from São Paulo state, Brazil. Rozeboom (1938) noted great variation in the structure of the eggs collected from the same larval habitat in Panama. Subsequently, Galvão (1938) found variations in eggs from Brazil, and separated them into two groups by the size of the floats, and Galvão and Barretto (1938) added a third group with eggs without the floats. Unti (1941) described *An. strodei* varieties *arthuri*, *artigasi* and *albertoi* based on characters of eggs from females captured in localities of Vale do Paraiba, São Paulo state. More recently, Sallum et al. (2010) corroborated differences between *An. strodei*, *An. arthuri*, and *An. albertoi* based on comparison of scanning electron micrographs of the eggs with descriptions of eggs available in the published literature. Furthermore, differences observed in the eggs were corroborated by differences found in male genitalia (Table 1), and also the results of phylogenetic analyses of concatenated sequences of the *COI* mitochondrial gene, ITS2 region of rDNA and the nuclear *white* gene.

Anopheles striatus n. sp. is easily identified based on characteristics of the male genitalia (Fig. 1B and C), eggs (Figs. 3–5) and females. The eggs of the new species have the frills completely enclosing the outer chorionic tubercles to form a crown at both the anterior and posterior ends of the dorsal surface (Fig. 3A, C–E), whereas in *An. strodei*, *An. arthuri* and *An. albertoi* the frills are continuous with the floats at both anterior and posterior ends of the egg (Table 1). Based on the male genitalia, *An. striatus* n. sp. is distinguished by the apex of the ventral claspette being expanded into large, rounded, apicolateral lobes, each lobe possessing spicules laterally, and the basal lobule of the ventral claspette slightly expanded laterally with short spicules (Table 1). Furthermore, comparing the aedeagus of *An. strodei*, *An. albertoi* and *An. arthuri* with the aedeagus of the new species, structural differences are observed between them. The height, the width, and the form of the apex of the aedeagi are distinct; likewise the shape of the parameres is distinctive.

Using the morphological keys provided by Forattini (2002), the female of *An. striatus* n. sp. can be misidentified as *An. (Nys.) galvaoui* Causey, Deane & Deane because the basal 0.5 of hindtarsomere 2 is dark-scaled and the prehumeral pale spot (PHP) is usually as large

Table 1
Comparison of the morphological characters of the scanning electron microscope of the eggs and male genitalia of *An. albertoi*, *An. arthuri*, *An. strodei* and *An. striatus* n. sp.

Character/species	<i>An. albertoi</i>	<i>An. arthuri</i>	<i>An. strodei</i>	<i>An. striatus</i> n. sp.
Male genitalia	Apex of ventral claspette moderately expanded laterally into rounded lobes, without spicules on apicolateral lobes. Basal lobule large, expanded laterally, usually bending ventrad at base, spicules over basal margin usually projected posteriorly, long, strong, denser, and more strongly developed on inner angle (Sallum et al., 2010).	Apex of ventral claspette strongly expanded laterally into large, rounded lobes, without spicules on apicolateral lobes. Basal lobule large, expanded laterally, usually bending ventrad at base, spicules over basal margin usually project posteriorly, long, strong, mode dense, and stronger on inner angle (Sallum et al., 2010).	Apex of ventral claspette moderately expanded laterally into somewhat rounded lobes, without spicules on apicolateral lobes. Basal lobule expanded laterally, usually bending ventrad at base, spicules over basal margin similar in size and development, long, moderately strong, usually projecting posteriorly (Sallum et al., 2010).	Apex of ventral claspette strongly expanded laterally into large, rounded lobes, with spicules on apicolateral lobes. Basal lobule slightly expanded laterally, spicules along basal margin short, evenly distributed over basal surface, larger spicules more abundant on basomesal margin and ventral surface (Fig. 1B,C).
Egg	Deck narrow, long, irregular, delimited by a slightly elevated, sinuous, reduced frill; frill not forming a collar; egg without float (Sallum et al., 2010).	Deck large, slightly narrowed at anterior and posterior ends; frill not forming a collar; floats reduced, with 15–18 ribs (Sallum et al., 2010).	Deck large, broad, anterior portion slightly wider and shorter than posterior portion; frill not forming a collar; floats large, broad, with 20–27 ribs (Sallum et al., 2010).	Deck narrow, extending approximately 0.5 length of egg; frill completely enclosing exposed tubercles at both anterior and posterior ends, forming a crown at each end of both anterior and posterior ends, forming a crown at each end of dorsal surface; floats moderately narrow, fused dorsally at anterior and posterior areas of deck, with deck, with approximately 23 ribs (Figs. 3–5).

as the prehumeral dark spot (PHD) on the costa of the wing. In *An. striatus* n. sp., the basal dark scales on hindtarsomere 2 extend beyond the basal 0.5 covering the proximal 0.6 of the tarsomere. However, the prehumeral pale spot is usually smaller than the prehumeral dark spot. It is likely that females of *An. striatus* n. sp. have been largely misidentified as *An. galvaoui* because the basal dark scaling of hindtarsomere 2 is similar in length, and the PHP and PHD spots have been reported to be variable in *An. galvaoui*.

Fourth-instar larvae of *An. striatus* n. sp. can be distinguished from those of *An. strodei* by characteristics of the anterior spiracular lobe, anteromedian process and median plate (Fig. 2). These characteristics were compared with the illustration of *An. strodei* provided by Faran (1980). Assessments of the fourth-instar larva and pupa of *An. arthuri* and *An. albertoi* are required to identify the species with accuracy because these species were incompletely described by Unti (1941), and Sallum et al. (2010) only provided illustrations of the eggs and male genitalia.

Recently, Bourke et al. (2013) recognized four species under the name *An. arthuri*, which were informally designated *An. arthuri* species A–D. However, only two are currently considered to be good species, i.e. *An. arthuri* s.s. and *An. arthuri* species A. It is necessary to conduct comparative morphological studies to elucidate the taxonomic status of the four purported species. Morphological distinctions of the adults, male genitalia, fourth-instar larva and pupa of the *Anopheles* CP Form elucidated in the present study corroborate the molecular studies of Sallum et al. (2010) and Bourke et al. (2013) and confirm the specific status of the form, which is formally described and named herein as *An. striatus* n. sp.

Conflicts of interest

The authors declare no conflicts of interest.

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