



## A new species of the Arthuri Complex of the Strodei Subgroup of *Nyssorhynchus* (Diptera: Culicidae)

DENISE CRISTINA SANT'ANA<sup>1,2\*</sup> & MARIA ANICE MUREB SALLUM<sup>1,3</sup>

*Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo SP 01246-904, Brazil.*

<sup>2</sup>✉ [deniseecs@usp.br](mailto:deniseecs@usp.br); <https://orcid.org/0000-0001-6883-6611>

<sup>3</sup>✉ [masallum@usp.br](mailto:masallum@usp.br); <https://orcid.org/0000-0002-7051-2891>

\*Corresponding author.

### Abstract

*Nyssorhynchus* (*Nyssorhynchus*) *rondoniensis*, a new species of the Arthuri Complex of the Strodei Subgroup, is described and validated using morphological characters of the adult male and female, male genitalia, fourth-instar larva and pupa. The new species is recorded in the municipalities of Campo Novo de Rondônia and Monte Negro, Rondônia State, Brazil. Based on DNA sequence data, the new species (as *Ny. arthuri* species C) was found to belong to a separate lineage within the Arthuri Complex. Morphological characteristics of the male genitalia and fourth-instar larva confirmed that the new species shared morphological similarities with other species of the Arthuri Complex, but it can be distinguished by characteristics of the male genitalia, adult female and larva. *Nyssorhynchus rondoniensis* may be involved in malaria transmission because females can be easily misidentified as *Ny. oswaldoi* (Peryassú, 1922) *s.l.* or *Ny. konderi* (Galvão & Damasceno, 1942) *s.l.* Both species were previously hypothesized to be local vectors in Acre and Rondônia States.

**Key words:** Anophelinae, description, malaria, *An. rondoniensis* n. sp., malaria vector

### Introduction

The subfamily Anophelinae is a monophyletic lineage of the Culicidae, however there are some disagreements regarding the internal classification of genera and subgenera. Recently, based on the results of phylogenetic analyses, a new classification of the subfamily was proposed by Foster *et al.* (2017). Accordingly, the monophyletic phylogenetic lineages *Kerteszia* Theobald, 1905, *Lophopodomyia* Antunes, 1937, *Nyssorhynchus* Blanchard, 1902 and *Stethomyia* Theobald, 1902 were elevated to genus level and the genus *Nyssorhynchus* currently includes subgenera *Myzorhynchella* Theobald, 1907 and *Nyssorhynchus*. Although the nomenclatural changes proposed by Foster *et al.* (2017) have been questioned by Harbach (2018a) and Marinotti (2021), and were not adopted by Harbach (2018b) and Wilkerson *et al.* (2021), they were defended by Lamas *et al.* (2021). Herein, we are employing classification of Foster *et al.* (2017) because it is based on the results of robust phylogenetic analyses and is supported by morphological hypothesis (Sallum *et al.* 2000).

The Strodei Subgroup of the genus *Nyssorhynchus* includes *Ny. (Nys.) albertoi* (Unti, 1941), *Ny. (Nys.) arthuri* (Unti, 1941), *Ny. (Nys.) rondoni* (Neiva & Pinto, 1922), *Ny. (Nys.) striatus* (Sant'Ana & Sallum, 2017) and *Ny. (Nys.) strodei s.s.* (Root, 1926). All of these species were previously classified in the subgenus *Nyssorhynchus* of the genus *Anopheles* Meigen, 1818.

*Nyssorhynchus strodei s.s.* was described by Root (1926) based on morphological characters of the adult 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. The morphological description of the egg of *Ny. strodei* was based on specimens obtained from females collected in São Paulo municipality by Galvão & Lane (1936). Subsequently, Unti (1940) described *Ny. strodei* var. *ramosi* based on differences observed in fourth-instar larvae collected in Lorena municipality, São Paulo, Brazil. Later, Unti (1941) described four morphological varieties based on characteristics of eggs from females collected in Rio Paraíba, São Paulo, Brazil — *Ny. strodei* var. *albertoi*, *Ny. strodei* var. *arthuri*, *Ny. strodei*

var. *artigasi* and *Ny. strodei* var. *lloydi* from Panama. In a revision of the Albimanus Section of *Nyssorhynchus*, Faran (1980) synonymized all of Unti's varieties with *Ny. strodei* until additional specimens and more data related to bionomics and genetics were available. Later, *Ny. strodei* var. *albertoi* and *Ny. strodei* var. *arthuri* were removed from synonymy and recognized as valid species, whereas *Ny. strodei* var. *ramosi* and *Ny. strodei* var. *lloydi* remained in synonymy with *Ny. strodei*, and *Ny. artigasi* was transferred to the synonymy of *Ny. arthuri* by Sallum *et al.* (2010). Recently, seven distinct lineages within the Strodei Subgroup, *Ny. albertoi*, *Ny. arthuri* s.s./*Ny. arthuri* A, *Ny. arthuri* B, *Ny. arthuri* C, *Ny. arthuri* D, *Ny. striatus* and *Ny. strodei* were molecularly identified in Brazil (Bourke *et al.* 2013, Greni *et al.* 2018).

The recent findings in the Strodei Subgroup revealed the need for further taxonomic investigations to achieve accurate species identifications, and to establish the public health importance of *Ny. strodei* and other species of the subgroup. Females of *Ny. strodei* were found naturally infected with *Plasmodium vivax* and the species was incriminated as a secondary vector in Ariquemes, Rondônia, Amazon Region (Oliveira-Ferreira *et al.* 1990). According to Bourke *et al.* (2013), it is likely that specimens identified as *Ny. strodei* belong to the phylogenetic lineage of *Ny. rondoniensis* n. sp. (previously informally named *An. arthuri* C).

The accuracy of mosquito species identification is key for both vector incrimination and the design of control interventions in areas where malaria is endemic. For this reason, the objective of the present study was to describe and formally name *Ny. rondoniensis* new species. For species definition, morphological characters of the fourth-instar larva, pupa, adult female, adult male and male genitalia are described and illustrated. In addition, ecological and distribution data are provided for the new species.

## Material and methods

*Nyssorhynchus rondoniensis* n. sp. was collected in the municipality of Campo Novo de Rondônia, Rondônia State, Amazon Region, Brazil. Field collections were carried out in Fazenda Marechal Rondon (10° 38' 15.5" S, 65° 29' 59.4" W, Datum SAD69) and Monte Negro, Rondônia State (10° 16' 07.1" S, 63° 33' 19.4" W, Datum SAD69). Larvae and pupae were collected from ground-pool habitats to obtain adults with associated larval and pupal exuviae. After emergence, adults were maintained for 12 h before being euthanized with ethyl acetate vapor and isolated in separate minute plastic vials with silica gel. Both larval and pupal exuviae were preserved in 80% ethanol prior to slide-mounting. Male genitalia were dissected and mounted on microscope slides in Canada balsam. Larval and pupal setae were examined, measured and branches counted for the description. Morphological characters of the female, male, fourth-instar larva, pupa and male genitalia were examined and are illustrated. Abbreviations used to denote life stages are F, adult female; M, adult male; G, male genitalia; L, larva; P, pupa; Le, larval exuviae; Pe, pupal exuviae. Terminology for the morphological descriptions is that of Harbach & Knight (1980, 1982), except for the wing veins and spots which follow Wilkerson & Peyton (1990).

### *Nyssorhynchus (Nyssorhynchus) rondoniensis* Sant'Ana & Sallum, n. sp.

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*Anopheles arthuri* C of Bourke *et al.* 2013: 1, 3, 6–16 (phylogeny); Foster *et al.* 2017: 5 (phylogeny),

*Nyssorhynchus arthuri* C of Greni *et al.* 2018: 1–4, 6–9 (phylogeny, distribution).

**Female.** Integument light to dark brown, pruinose. *Head:* Vertex posterior to frontal tuft with erect, white spatulate scales and a few long, white setae, remainder of vertex and occiput with dark brown erect forked scales. Proboscis dark-scaled. Maxillary palpomere 1 with erect, dark scales; palpomere 2 with semi-erect, dark scales, pale scales at apex; palpomere 3 with decumbent, mostly dark scales, white scales at apex; palpomere 4 with decumbent, mostly white scales, dark scales at base, sometimes a few dark scales laterally and at apex; palpomere 5 with decumbent, predominantly white scales, dark scales at base. *Thorax:* Integument pruinose with dark areas between dorsocentral area and lateral margin, at posterior edge of scutal fossa and posteriorly on prescutellar area. Anterior promontory with long, white setiform scales, usually not extending far dorsad onto acrostichal area; acrostichal setae strong; dorsocentral setae long; scutellum with long dark setae on posterior margin and white spatulate scales anteriorly. Antepnotum with dark setae. Prepiracular area bare; upper mesokatepisternum without setae and scales, lower

mesokatepisternum with white spatulate scales and brownish setae. *Wing* (Fig. 1a): Veins covered with dark and pale scales. Dorsal spots as follows: Costa with basal pale and prehumeral pale (BP and PHP), prehumeral dark (PHD), humeral pale (HP), humeral dark (HD), presector pale (PSP), presector dark (PSD), sector pale (SP), sector dark (SD), accessory sector pale (ASP), sector dark (SD), subcostal pale (SCP), preapical dark (PD), preapical pale (PP), apical dark (AD) and apical pale (AP) spots; vein  $R_s$  mostly dark-scaled;  $R_{2+3}$ ,  $R_{4+5}$  and M predominantly pale-scaled. *Legs*: Tarsomeres Ta-I<sub>1,3</sub> and Ta-I<sub>5</sub> with white-scaled apices, Ta-I<sub>4</sub> dark-scaled. Tarsomeres Ta-II<sub>1,2</sub> and Ta-II<sub>5</sub> dark-scaled with apical white band, Ta-II<sub>3</sub> and Ta-II<sub>4</sub> dark-scaled. Hindleg (Fig. 1b): Tarsomeres Ta-III<sub>1</sub> predominantly dark-scaled, pale-scaled at apex, Ta-III<sub>2</sub> dark-scaled approximately on proximal 0.23, pale-scaled approximately on distal 0.77, Ta-III<sub>3</sub> and Ta-III<sub>4</sub> entirely white-scaled, Ta-III<sub>5</sub> dark-scaled on approximately proximal 0.5, white-scaled on distal 0.5. *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 on terga II–VII. Sternum I bare; sterna II–VII with a few sparse pale scales; sternum VIII with pale scales and a few dark scales.

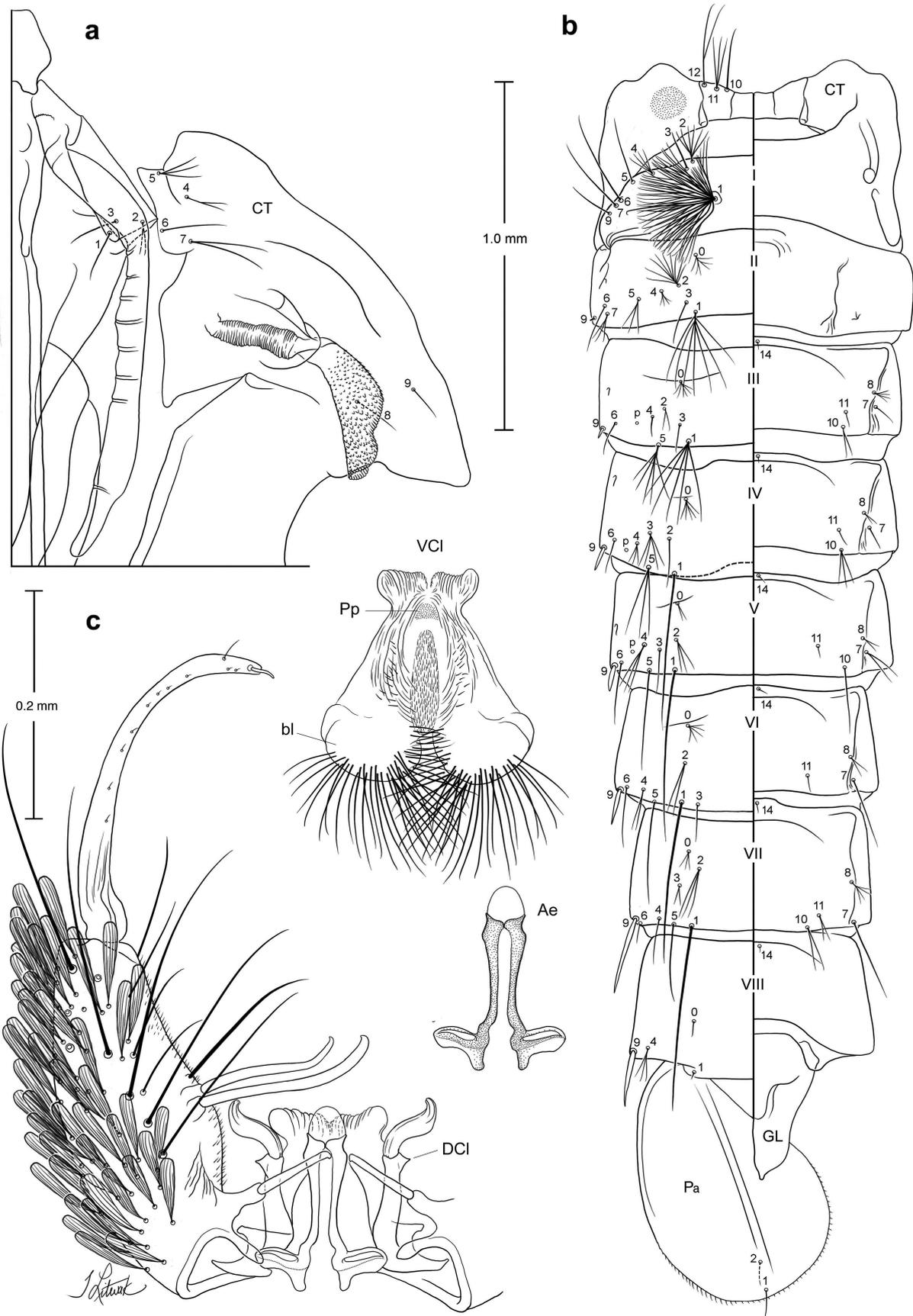


**FIGURE 1.** Wing (a) and hindtarsus (b) of *Nyssorhynchus rondoniensis* n. sp. Costal wing spots: AD, apical dark; AP, apical pale; BP, basal pale; HD, humeral dark; HP, humeral pale; PD, preapical dark; PHD, prehumeral dark; PHP, prehumeral pale; PP, preapical pale; PSD, presector dark; PSP, presector pale; SD, sector dark; SCP, subcostal pale; SP, sector pale; Ta-III<sub>1</sub>–Ta-III<sub>5</sub>, hindtarsomeres 1–5.

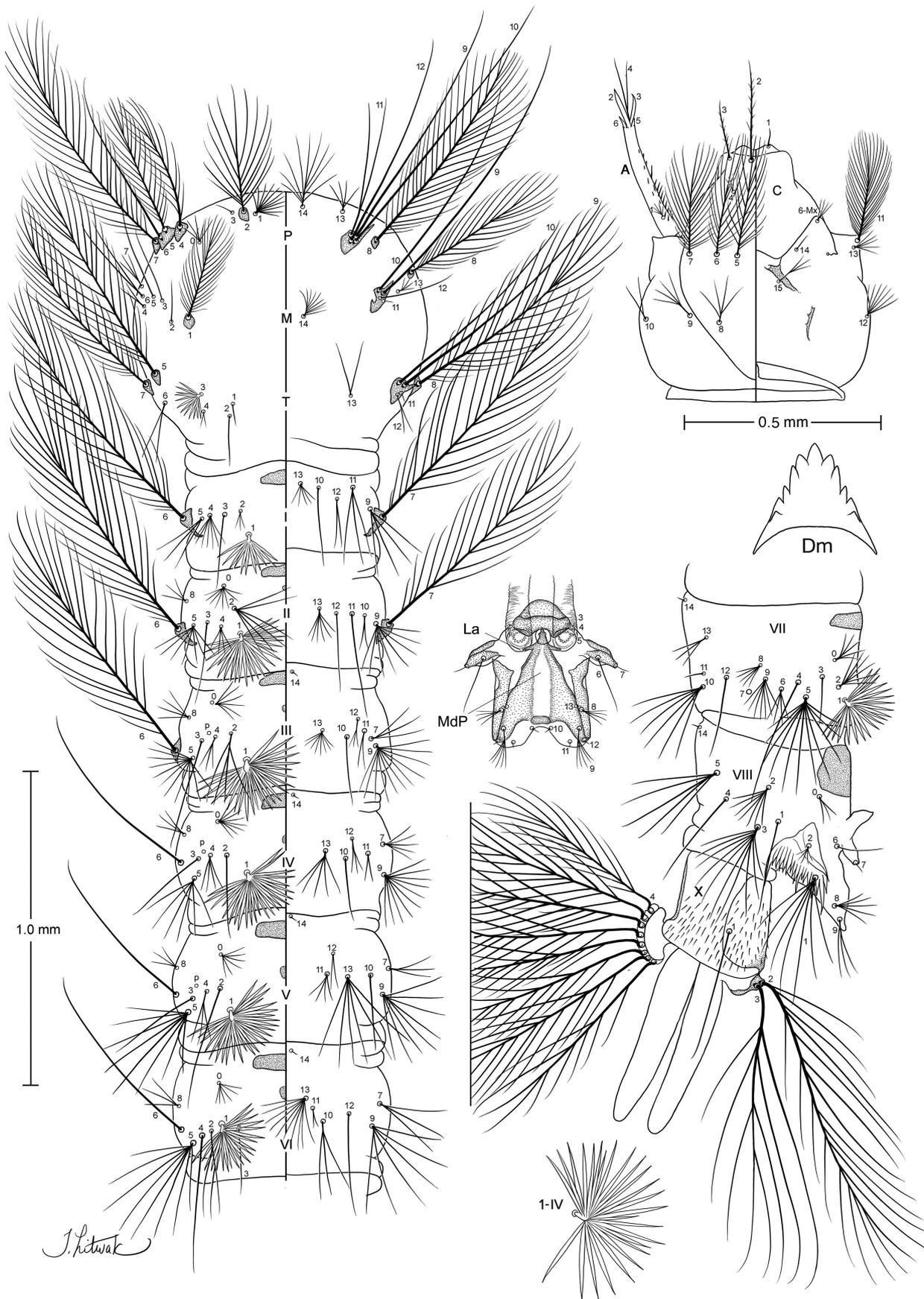
**Male.** Similar to female except for sexual characters. *Head:* Maxillary palpus mostly dark-scaled, with pale spots; palpomere 2 with erect dark scales and a few pale scales; palpomere 3 with erect dark scales basally, with pale apical band; palpomere 4 dark-scaled, with pale scales basally and apically; palpomere 5 mostly white-scaled on dorsal surface, lateral and ventral surfaces mostly dark-scaled. *Genitalia* (Fig. 2c): Segment VIII—Tergum and sternum with spatulate scales and long setae. Segment IX—Sternum well developed, sub-rectangular. Dorsal claspette—Pedicel long, rounded basally, with 3 broad, curved apical leaflets. Ventral claspette—Apex wide, irregular, rugose, striated, striae oriented parallel to longitudinal axis of the genitalia, apex moderately expanded laterally into rounded lobes; apicolateral lobes with convex basal and lateral margins, apical margin weakly concave. Apex without spicules on ventral, lateral and dorsal surfaces. Preapical plate moderately developed, poorly sclerotized, approximately circular, moderately defined. Basal lobule large, slightly expanded laterally at base, spicules on basal margin, long, strong, smaller at internal angle, projecting posteriorly. Phallosome—Aedeagus with broadly rounded apex, leaflets absent.

**Pupa** (Fig. 2a, b). Position and development of setae as figured. All measurements, and number and mode of setal branches are based on 5 specimens. *Cephalothorax* (Fig. 2a): Integument weakly pigmented; trumpet angusticorn with meatal cleft; pinna moderately pigmented. *Abdomen* (Fig. 2b): Length 2.35–2.53 mm (mean 2.42 ± 0.07 mm) seta 1-I dendritic, number of branches not counted; seta 2-I with 2–6 (4) branches; seta 3-I single, as long as 2-I; seta 4-I 3–5 (4) branched; seta 5-I single or double; setae 6,9-I single, 9-I shorter than seta 7-I; seta 7-I usually double, shorter than 6-I; seta 0-II–VII moderately developed, 0-II–IV with 3–6 (5) branches, 0-V, VII with 2–4 (3) branches, 0-VI 2–5 (4) branched; seta 1-II, III well developed, 3–10 (8) and 4–8 (6) branched, respectively, 1-IV–VII always single, strong, long, extending beyond following segment; seta 2-IV, V normally single, with 1–3 branches, 2-VI with 1–3 (3) branches, 2-VII with 1–3 (2) branches; seta 3-IV, V usually triple, 2–4 (3) and 1–3 (3), respectively, 3-VI with 1,2 (2) branches, 3-VII with 1–3 (2) branches; seta 4-IV–VII with 1,2 (2) branches, 4-VIII double, 2,3 (2); seta 5-III, IV with 4–6 (5) and 3–5 (4) branches, respectively, 5-V–VII single, long; seta 6-II–VII single; seta 7-II with 2,3 (3) branches, 7-III with 1,2 (2) branches, 7-IV, V double, 1–3 (2), 7-VI, VII single, 7-VI, VII moderately developed; seta 8-III, IV with 1–3 (2) branches, 8-V, VI with 1,2 (2) branches, 8-VII double, 2,3 (2); seta 9-II minute, lightly pigmented, 9-III short, stout, 9-IV, V stout, dark, 9-VI–VII stout, dark, longer than 9-IV, V; seta 10-III with 1–4 (2) branches, 10-IV, VII with 1,2 (1) branches, 10-V single, 10-VI absent; seta 11-III–VII frequently single. *Genital lobe:* Broad at base, with sides sloping toward apex, apex with mammiliform protuberance. *Paddle:* Length 0.70–0.72 mm (mean 0.71 ± 0.01 mm), width 0.47–0.58 mm (mean 0.51 ± 0.04 mm); obovate, outer margin distad of buttress with very fine, minute spicules, extending around apex and becoming sparse along inner margin; seta 1-Pa single, stronger than seta 2-Pa; seta 2-Pa normally single.

**Fourth-instar larva** (Fig. 3). Position and development of setae as figured. All measurements, and number and mode of setal branches are based on 5 specimens. *Head:* Length 0.62–0.64 mm (mean 0.63 ± 0.01 mm), width 0.58–0.63 mm (mean 0.61 ± 0.02 mm). Integument weakly pigmented, yellowish to light brown, with dark spots, not forming distinct pattern. Seta 2-C single, with sparse, minute aciculae distally; seta 3-C shorter than 2-C, single, distance between bases of 2-C 0.010–0.016 mm (mean 0.013 ± 0.005 mm); distance between bases of 2-C and 3-C 0.046–0.060 mm (mean 0.054 ± 0.005 mm), clypeal index (=ratio between distance between bases of 2-C and 3-C on one side and distance between bases of 2-C) 4.15; seta 4-C double, short, usually not reaching base of 2-C; seta 5-C long, plumose, with 15–23 branches extending beyond base of 2-C; seta 6-C with 15–20 branches; seta 7-C with 17–26 branches; seta 8-C with 2–4 (3) branches; seta 9-C 2–4 (4) branched; seta 10-C with 2,3 (2) branches; setae 12,13-C frequently with 4 branches, 3–6 (4) and 4–6 (4), respectively. Collar dark brown, heavily pigmented. *Antenna:* Length 0.27–0.29 mm (mean = 0.28 ± 0.01 mm), enlarged toward base, longer than wide; with long, thin spicules on mesal margin, spicules shorter and fewer on dorsal and ventral surfaces; seta 1-A with 4–7 (6) branches, small, inserted 0.06–0.09 mm (mean = 0.07 ± 0.01 mm) distant from base. *Thorax:* Setae 1,2-P arising separately, 1-P palmate, with 13–16 (13) narrow, lanceolate leaflets, 2-P with 17–22 branches; seta 3-P single; seta 14-P with 8–12 (12) branches, arising distinct distance from base, extending beyond anterior margin of thorax; seta 1-M strongly plumose, with 34–42 branches; seta 2-M normally single, 1,2 (1); setae 3,5-M single; seta 4-M with 2,3 (3) branches; setae 6,7-M with 2,3 (2) and 3,4 (3) branches, respectively; seta 8-M plumose, with 28–36 branches; seta 14-M 8–11 (8) branched; seta 3-T palmate, moderately narrow, with 12–17 (16) semi-transparent leaflets. *Abdomen:* Seta 0-II–VII moderately long; seta 1-I–VII palmate, 1-I with 12–17 (12) narrow, semi-transparent leaflets, 1-II–VII with narrow, pointed leaflets; seta 2-I 3–6 (4) branched, short, 2-II–VII moderately long, 2-II, VII 4–6 (5) branched, 2-III with 3–5 (4) branches, 2-IV, V single; seta 5-I with 3–5 (4) branches, 5-II, V, VI usually with 7 branches,



**FIGURE 2.** Pupa and male genitalia of *Nyssorhynchus rondoniensis* n. sp. a, Cephalothorax; b, abdomen; c, male genitalia. Ae, aedeagus; bl, basal lobule; CT, cephalothorax; DCI, dorsal claspette; GL, genital lobe; Pa, paddle; Pp, preapical plate; VCI, ventral claspette; I–VIII, abdominal segments. Scales in mm.



**FIGURE 3.** Fourth-instar larva of *Nyssorhynchus rondoniensis* n. sp. A, Antenna; C, cranium; Dm, dorsomentum; La, lateral arms; M, mesothorax; MdP, median plate; P, prothorax; T, metathorax; I–VIII, X, abdominal segments. Scales in mm.

7–10 (7), 5–8 (7) and 5–10 (7), respectively, 5-III with 7–10 (8) branches, 5-IV frequently with 6 branches, 5-VII with 7–11 (9) branches; seta 6-I–III plumose, inserted on tubercle, 6-IV–VI simple, single, large, 6-VII with 3,4 (4) branches; seta 13-V larger than 13-I–IV; seta 1-VIII single; seta 2-VIII 6,7 (7) branched, seta 3-VIII with 8–10 (10) branches; seta 4-VIII single; seta 5-VIII 4–6 (5) branched. *Pecten*: With 4 or 5 long and 9–13 short spines; median plate moderately pigmented, darkly pigmented anteriorly, with median, pointed lateral arms; slightly narrowing in portion below the arms. *Segment X*: Seta 1-X inserted on margin of saddle; anal papillae narrow, longer than saddle.

**Etymology.** The name *rondoniensis* is derived from the name of Rondônia State, located in the Amazon Region of Brazil, where the species was first recorded.

**Bionomics.** Larvae and pupae of *Ny. rondoniensis* were taken from partially shaded lake margins, ground pools and flooded areas. The water was stagnant or with moderate movement, fresh, clear, with floating, submerged and emergent vegetation. They were also taken in a habitat without vegetation on a rocky outcrop with a waterfall, fully exposed to the sun. It is unknown whether *Ny. rondoniensis* is a local vector of malaria; however, it could be involved since females can be misidentified as *Ny. oswaldoi s.l.* or *Ny. konderi s.l.*, both previously suspected to be local vectors in the States of Acre and Rondônia. Further studies are necessary to verify the potential association of *Ny. rondoniensis* with malaria transmission.

**Distribution.** *Nyssorhynchus rondoniensis* occurs in the municipalities of Campo Novo de Rondônia and Monte Negro, Rondônia State, Brazilian Amazon.

**Material examined.** *Holotype*—Adult male with associated Le and Pe and dissected genitalia mounted on microscope slides, specimen field code RO29-12, FSP-USP n° E-16057, bearing the following collection data: BRAZIL, Rondônia State, municipality of Campo Novo de Rondônia, Fazenda Marechal Rondon, 10° 38' 15.5" S, 65° 29' 59.4" W, coll. 17-Jun-2008, Bergo *et al.*, larvae were collected from ground-pool habitats and kept alive to obtain males associated with larval and pupal exuviae. *Paratypes*—21 specimens with the following information: Rondônia State, municipality of Campo Novo de Rondônia, Fazenda Marechal Rondon, 10° 38' 15.5" S, 65° 29' 59.4" W, coll. 17-Jun-2008, Bergo *et al.*, RO29-1, FSP-USP no. E-16058 [FLPePe]; RO29-5, FSP-USP no. E-16059 [MLPePe]; RO29-9, FSP-USP no. E-16060 [LePeG]; RO29-10, FSP-USP no. E-16061 [LePe]; RO29-11, FSP-USP no. E-16062 [MLPeG]; RO29-13, FSP-USP no. E-16063 [FLPePe]; RO29-17, FSP-USP no. E-16064 [MLPeG]; RO29-101, FSP-USP no. E-16065 [FPe]; RO29-105, FSP-USP no. E-16066 [FPe]; RO29-107, FSP-USP no. E-16067 [MPe]; RO31-2, FSP-USP no. E-16068 [MLPeG]; RO31-4, FSP-USP no. E-16069 [LePeG]; RO31-6, FSP-USP no. E-16070 [LePeG]; RO31-10, FSP-USP no. E-16071 [LePeG]. Rondônia State, municipality of Monte Negro, Gleba Amir Lando, 10° 16' 07.1" S, 63° 33' 19.4" W, coll. 10-Jan-2008, Bergo, RO08-2, FSP-USP no. E-16072 [Le]; RO08-3, FSP-USP no. E-16073 [FLPePe]; RO08-4, FSP-USP no. E-16074 [LePeG]; RO08-105, FSP-USP no. E-16075 [MPe]; RO08-106, FSP-USP no. E-16076 [FPe]; RO08-109, FSP-USP no. E-16077 [MPeG]. Rondônia State, municipality of Monte Negro, Gleba Amir Lando, 10° 17' 27.3" S, 63° 34' 34.9" W, coll. 10-Jan-2008, Bergo, RO07-104, FSP-USP no. E-16078 [PeG]. The holotype and paratypes are deposited in the Coleção Entomológica de Referência (FSP-USP), Faculdade de Saúde Pública, Universidade de São Paulo, Brazil. *Other specimens examined*—Specimens identified as *Ny. strodei* with the following data: San Ramón, Beni Department, Bolivia, coll. 4-Dec-1991, Conn & Fritz [2G].

## Discussion

*Nyssorhynchus rondoniensis* was recognized preliminarily as *Ny. arthuri* C by Bourke *et al.* (2013). Those authors identified seven distinct lineages based on molecular phylogenetic analysis, with four in the Arthuri Complex, *Ny. arthuri s.s.*, *Ny. arthuri* B, *Ny. arthuri* C and *Ny. arthuri* D. Later, Greni *et al.* (2018) confirmed the existence of three putative species under the name *Ny. arthuri s.l.* The four species share morphological similarities in the female, larva, pupa and male genitalia. It is noteworthy considering that characters of the male genitalia are structural and allow a more accurate identification of species (Sallum *et al.* 2020). Thus, further studies should focus on the descriptions and validations of all species included in the Arthuri Complex to verify the morphological characteristics for accurate species identification. Based on available data, these species are likely to exhibit differences in their behavior, ecology and malaria vector status; thus, further investigations will be necessary.

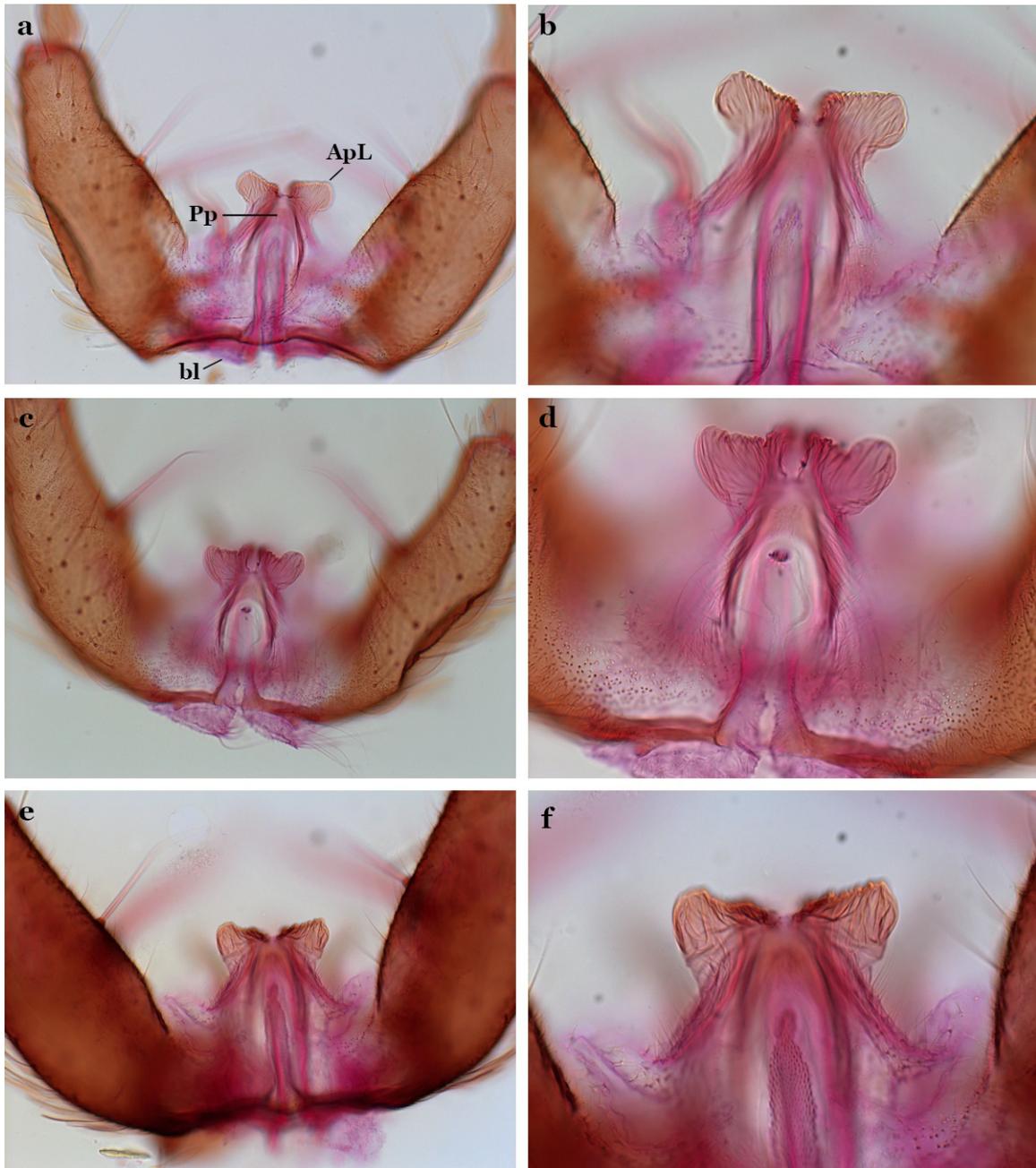
The recognition of *Ny. arthuri* and *Ny. albertoi* as valid species and their resurrection from synonymy with *Ny.*

*strodei* was based on morphological characteristics of eggs and differences in the male genitalia (Sallum *et al.* 2010). Some uncertainty remains relative to species identification and presence of new species, such as the *Anopheles* CP Form that was revealed by molecular analyses along with differences in the ventral claspette of the male genitalia. Recently, Sant'Ana & Sallum (2017) employed specimens collected in Minas Gerais State, Brazil, to validate the CP Form as *Ny. striatus*. The scanning electron microscopy (SEM) of eggs showed that *Ny. striatus* shares similarities with the eggs of *Ny. artigasi* and *Ny. strodei* from Bulo Bulu, Cochabamba Department, Bolivia, described and illustrated by Lounibos *et al.* (1997). The morphological similarities shared among the eggs of *Ny. artigasi*, *Ny. striatus* and *Ny. strodei* from Bulo Bulu indicate that the specimens from Bulo Bulu may belong to a species of the Arthuri Complex. In this study, we examined the genitalia of two males collected in the Beni Department, Bolivia in 1991 that were identified as *Ny. strodei*. It is noteworthy that the ventral claspette of both specimens shares similarities with those of *Ny. rondoniensis*; thus, it is likely that this species may occur in Bolivia, and the specimens from Bulo Bulu, Cochabamba Department, Bolivia belong to *Ny. rondoniensis*. In view of indirect evidence, further investigations are necessary to verify whether specimens from Bolivia belong to *Ny. rondoniensis*.

A combination of morphological characters allows the identification of *Ny. rondoniensis*: Female with dark caudolateral tufts of semi-erect scales on abdominal terga II–VII; foretarsomere 4 entirely dark; hindtarsomere 2 mostly white-scaled, but with dark scales approximately on proximal 0.23, hindtarsomere 3 completely pale; the pale wing scales, at least on anterior veins, white, humeral pale spot longer than twice the length of the pre-humeral dark spot. The fourth-instar larva can be identified by having seta 2-C single, with sparse, minute aciculae distally, seta 3-C shorter than 2-C, single, clypeal index usually 4.15, seta 4-C double, short, usually not reaching base of 2-C, seta 1-P palmate, narrow, with lanceolate leaflets, seta 1-I–VII palmate with smooth leaflets, leaflets of 1-II–VII narrow and pointed apically; spiracular lobe with the lateral arm of the median plate of moderate length, pointed and directed dorsolaterally, slightly narrowing below the arms.

Males of *Ny. rondoniensis* (Figs 4a, b) can be distinguished from those of *Ny. arthuri s.s.* and *Ny. strodei s.s.* by characters of the ventral claspette: Apex with striae oriented parallel to the longitudinal axis of the claspette; spicules absent on ventral, lateral and dorsal surfaces; apex moderately expanded laterally into rounded apicolateral lobes; apicolateral lobes with basal and lateral margins convex, apical margin weakly concave; basal lobule large, slightly expanded laterally at the base with dense spicules covering the basal margin, spicules long, strong, becoming smaller at the inner angle. *Nyssorhynchus arthuri s.s.* (Figs 4c, d) can be distinguished from *Ny. rondoniensis* by the following characters: Apex of the ventral claspette with striae oriented parallel and perpendicular to the longitudinal axis of the claspette; apex strongly expanded laterally into large, rounded lobes; apicolateral lobes with apical margin moderately concave; basal lobule expanded laterally, bending ventrad at base, spicules on the basal margin projected posteriorly, long, strong, denser and stronger on inner angle. *Nyssorhynchus strodei s.s.* (Figs 4e, f) can be distinguished from *Ny. rondoniensis* by the following characters: Apex of ventral claspette with striae oriented parallel and perpendicular to the longitudinal axis of the claspette; with sparse spicules on the ventral surface, spicules on the lateral and dorsal surfaces denser; apex expanded laterally into moderate, somewhat rounded lobes; apicolateral lobes with basal and apical margins weakly convex, lateral margin distinctly convex; basal lobule expanded laterally, bending ventrad at base, spicules on the basal margin similar in size and development, long, moderately strong and sparse, smaller at inner angle (Sallum *et al.* 2010).

Despite the utility of molecular tools to identify species of the Anophelinae, it is necessary to describe all the life stages of a species. An accurate morphological species definition is of utmost importance for those who are focused on ecology, surveillance and the control of species of public health importance. Species of the Strodei Subgroup have been largely misidentified as a single taxon until recently. Detailed comparative morphological studies of all life stages, including the SEM of eggs uncovered morphological features to validate species and to indicate the presence of unknown taxa. Certainly, the use of molecular tools brought evidence to support morphological findings that allowed accurate species delimitation. Further investigations will be necessary to elucidate the taxonomic status of both valid and putative species of the Strodei Subgroup, and the Arthuri Complex as well. The morphological descriptions of the female, male, male genitalia, fourth-instar larva and pupa of *Ny. rondoniensis* add new information that will be useful for morphological identification of the species of the Strodei Subgroup.



**FIGURE 4.** Details of the ventral claspette of the male genitalia. a, b, *Nyssorhynchus rondoniensis* n. sp.; c, d, *Ny. arthuri* s.s.; e, f, *Ny. strodei* s.s.; ApL, apicolateral lobes; bl, basal lobule; Pp, preapical plate.

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### References

- Antunes, P.C.A. (1937) A new *Anopheles* and a new *Goeldia* from Colombia (Dipt. Culic.). *Bulletin of Entomological Research*, 28 (1), 69–73.  
 Blanchard, R. (1902) Nouvelle note sur les moustiques. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société*

*de Biologie*, 54, 793–795.

- Bourke, B.P., Oliveira, T.P., Suesdek, L., Bergo, E.S. & Sallum, M.A.M. (2013) A multi-locus approach to barcoding in the *Anopheles strodei* subgroup (Diptera: Culicidae). *Parasites & Vectors*, 6, 111.  
<https://doi.org/10.1186/1756-3305-6-111>
- Faran, M.E. (1980) Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contributions of the American Entomological Institute*, 15 (7), 1–215.
- Foster, P.G., Oliveira, T.M.P. de, Bergo, E.S., Conn, J.E., Sant'Ana, D.C., Nagaki, S.S., Nihei, S., Lamas, C.E., González, C., Moreira, C.C. & Sallum, M.A.M. (2017) Phylogeny of Anophelinae using mitochondrial protein coding genes. *Royal Society Open Science*, 4 (11), 170758.  
<https://doi.org/10.1098/rsos.170758>
- Galvão, A.A. & Lane, J. (1936) Notas sobre os *Nyssorhynchus* de São Paulo. *Separata da Faculdade de Medicina de São Paulo*, 12, 269–288.
- Greni, S.E., Demari-Silva, B., Oliveira, T.M.P. de, Suesdek, L., Laporta, G.Z. & Sallum, M.A.M. (2018) A multi-gene analysis and potential spatial distribution of species of the Strodei Subgroup of the genus *Nyssorhynchus* (Diptera: Culicidae). *Journal of Medical Entomology*, 55 (6), 1486–1495.  
<https://doi.org/10.1093/jme/tjy137>
- Harbach, R.E. (2018a) An *Anopheles* by any other name ...? *Journal of Medical Entomology*, 55 (5), 1069–1070.  
<https://doi.org/10.1093/jme/tjy108>
- Harbach, R.E. (2018b) *Culicipedia: Species-group, genus-group and family-group names in Culicidae (Diptera)*. CABI, Wallingford, Oxfordshire, xviii + 378 pp.  
<https://doi.org/10.1079/9781786399052.0000>
- Harbach, R.E. & Knight, K.L. (1980) *Taxonomists' glossary of mosquito anatomy*. Plexus Publishing, Inc., Marlton, New Jersey, xi, 413 pp.
- Harbach, R.E. & Knight, K.L. (1982) Corrections and additions to *Taxonomists' glossary of mosquito anatomy*. *Mosquito Systematics*, 13 (2), 201–217.
- Lamas, C.J.E., Nihei, S.S., Foster, P.G., Conn, J.E. & Sallum, M.A.M. (2021) *Anopheles darlingi* versus *Nyssorhynchus darlingi*, the discussion continues. *Trends in Parasitology*, 37 (10), 847–848.  
<https://doi.org/10.1016/j.pt.2021.07.014>
- Lounibos, L.P., Duzak, D. & Linley, J.R. (1997) Comparative egg morphology of six species of the Albimanus Section of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Journal of Medical Entomology*, 34 (2), 136–155.  
<https://doi.org/10.1093/jmedent/34.2.136>
- Marinotti, O. (2021) What is in a name? *Anopheles darlingi* versus *Nyssorhynchus darlingi*. *Trends in Parasitology*, 37 (10), 856–858.  
<https://doi.org/10.1016/j.pt.2021.06.003>
- Meigen, J.W. (1818) *Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten. Vol. 1*. Aachen, xxxvi + 332 pp., errata (1 p.), 11 pls.  
<https://doi.org/10.5962/bhl.title.12464>
- Neiva, A. & Pinto, C. (1922) Contribuição para o conhecimento dos anophelinas do estado de Matto Grosso com a descrição de uma nova especie. *Brazil-Medico*, 36 (2), 321–322.
- Oliveira-Ferreira, J. de, Lourenço-de-Oliveira, R., Teva, A., Deane, L.M. & Daniel-Ribeiro, C.T. (1990) Natural malaria infections in anophelines in Rondônia State, Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene*, 43 (1), 6–10.  
<https://doi.org/10.4269/ajtmh.1990.43.1.TM0430010006>
- Peryassú, A. (1922) Duas novas especies de mosquitos do Brasil. *Folha medica*, 3, 179.
- Root, F.M. (1926) Studies on Brazilian mosquitoes. I. The anophelines of the *Nyssorhynchus* group. *American Journal of Hygiene*, 6 (5), 684–717, 9 pls.
- Sallum, M.A., Foster, P.G., Santos, C.L.S. dos, Flores, D.C., Motoki, M.T. & Bergo, E.S. (2010) Resurrection of two species from synonymy of *Anopheles* (*Nyssorhynchus*) *strode* Root, and characterization of a distinct morphological form from the Strodei Complex (Diptera: Culicidae). *Journal of Medical Entomology*, 47 (4), 504–526.  
<https://doi.org/10.1093/jmedent/47.4.504>
- Sallum, M.A.M., Obando, R.G., Carrejo, N. & Wilkerson, R.C. (2020) Identification key to the *Anopheles* mosquitoes of South America (Diptera: Culicidae). III. Male genitalia. *Parasites & Vectors*, 13, 542.  
<https://doi.org/10.1186/s13071-020-04300-1>
- Sallum, M.A.M., Schultz, T.R. & Wilkerson, R.C. (2000) Phylogeny of Anophelinae (Diptera Culicidae) based on morphological characters. *Annals of the Entomological Society of America*, 93 (4), 745–775.  
[https://doi.org/10.1603/0013-8746\(2000\)093\[0745:POADCB\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2000)093[0745:POADCB]2.0.CO;2)
- Sant'Ana, D.C. & Sallum, M.A.M. (2017) *Anopheles* (*Nyssorhynchus*) *striatus*, a new species of the Strodei Subgroup (Diptera, Culicidae). *Revista Brasileira de Entomologia*, 61 (2), 136–145.  
<https://doi.org/10.1016/j.rbe.2016.12.005>
- Theobald, F.V. (1902) The classification of the Anophelina. *Journal of Tropical Medicine*, 5, 181–183.
- Theobald, F.V. (1905) A catalogue of the Culicidae in the Hungarian National Museum, with descriptions of new genera and

- species. *Annales Musei Nationalis Hungarici*, 3 (1), 61–119, figure legends, 4 pls.
- Theobald, F.V. (1907) *A monograph of the Culicidae or mosquitoes*. Volume 4. British Museum (Natural History), London, xix + 639 pp., 16 pls.  
<https://doi.org/10.5962/bhl.title.58067>
- Unti, O. (1940) Anofelinos do Vale do Paraíba. Nota III.—Biologia do *Anófeles* (*Nyssorhynchus*) *strodei* Root, 1926 [sic] com a descrição de uma variedade nova. *Anófeles* (*Nyssorhynchus*) *strodei ramosi* var. n. *Anais Paulistas de Medicina e Cirurgia*, 40 (12), 489–505.
- Unti, O. (1941) Anofelinos do vale do Rio Paraíba, *Anopheles* (*Nyssorhynchus*) *strodei* Root, 1926 com a descrição de tres variedades novas. *Publico Serviço de Profilaxia em Malária*, 33, 3–18.
- Wilkerson, R.C. & Peyton, E.L. (1990) Standardized nomenclature for the costal wing spots of the genus *Anopheles* and other spotted-wing mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, 27 (2), 207–224.  
<https://doi.org/10.1093/jmedent/27.2.207>
- Wilkerson, R.C., Linton, Y.-M. & Strickman, D. (2021) *Mosquitoes of the world*. Vols. 1 & 2. Johns Hopkins University Press, Baltimore, Maryland, 1332 pp.  
<https://doi.org/10.1353/book.79680>