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A new species of the Nuneztovari Complex of *Nyssorhynchus* (Diptera: Culicidae) from the western Brazilian Amazon

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Abstract

Nyssorhynchus (*Nyssorhynchus*) *jamariensis*, a new species of the Nuneztovari Complex, previously known as *Anopheles* (*Nyssorhynchus*) *nuneztovari* A, is described and validated using morphological characters of the adult male and female, male genitalia and immature stages. The species is recorded from the western Brazilian Amazon, where it was collected in pastures in the vicinity of the Jamari River, municipality of Monte Negro, Rondônia State, Brazil. Illustrations of the male genitalia, fourth-instar larva and pupa are provided. *Nyssorhynchus jamariensis* may be involved in malaria transmission, but its vector status needs further investigation.

Key words: Anophelinae, description, morphology, *jamariensis* n. sp., taxonomy

Introduction

The Nuneztovari Complex (Harbach 2004; Foster *et al*. 2013) of the genus *Nyssorhynchus* Blanchard, 1902 (generic status in accord with Foster *et al*. 2017) includes *Ny.* (*Nyssorhynchus*) *nuneztovari s.s.* (Gabaldon, 1940)*, Ny.* (*Nys.*) *goeldii* (Rozeboom & Gabaldon, 1941), *Ny.* (*Nys.*) *dunhami* (Causey, 1945) and *Ny.* (*Nys.*) *nuneztovari* A. All formerly known as species of the genus *Anopheles* Meigen, 1818, subgenus *Nyssorhynchus*.

Studies focused on behavioral, morphological, cytogenetics and molecular polymorphisms showed that *Ny. nuneztovari s.l.* is a species complex (Elliotti 1972; Kitzmiller *et al*. 1973; Fritz *et al*. 1994, Hribar 1994, Linley *et al*. 1996; Conn 1990; Conn *et al*. 1993; Scarpassa *et al*. 1999; Mirabello & Conn 2008; Ramos *et al*. 2008). Kitzmiller *et al*. (1973) found geographic differences in the fixed inversion of the X-chromosome and an autosomal inversion in Brazilian and Venezuelan populations of *Ny. nuneztovari s.l.* In addition, Conn (1990) and Conn *et al*. (1993) discovered three chromosomal forms of *Ny. nuneztovari s.l.*: chromosome type A comprised specimens from the Amazon River basin, chromosome type B found in Venezuela and chromosome type C, which occurs in Colombia and Venezuela. The results of phylogenetic analyses using the ITS2 region of ribosomal DNA revealed the presence of two genetic groups of *Ny. nuneztovari s.l.* in Brazil (Fritz *et al*. 1994), and Conn *et al*. (1998), employing restriction fragment length polymorphism (RFLP) of mitochondrial DNA, recovered one genetic group in Venezuela and Colombia and two genetic groups in the Amazon River basin. Recently, the genetic structure of *Ny. nuneztovari s.l.* was investigated using DNA sequences of the nuclear single copy *white* gene (Mirabello & Conn 2008), which showed the presence of three sympatric lineages in the Brazilian Amazon and Suriname and two lineages in Colombia/Venezuela. Recently, Calado *et al*. (2008) showed that DNA sequences of the *COI* mitochondrial gene, ITS2 rDNA and the nuclear *white* gene of several specimens of *Ny. nuneztovari s.l.* from Brazil are distinct from those of specimens from Colombia and Venezuela. Also, Scarpassa & Conn (2011), employing a fragment of the *COI* mitochondrial gene, showed that specimens from Bolivia/Colombia/Venezuela grouped in a lineage that included *Ny. nuneztovari s.s.*, whereas those from the Brazilian Amazon/Suriname were grouped in three lineages. Recently, Scarpassa *et al*. (2016) employed the barcode region of the *COI* gene and 12 microsatellite *loci* to reveal three genetic lineages, including lineage III that may represent another new species of the Nuneztovari Complex.

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The purpose of this study is to formally name and describe species A of the Nuneztovari Complex using morphological characters of the adult male and female, male genitalia and immature stages, and provide characters to distinguish it from the other species of the complex. In addition, information on the bionomics, distribution and molecular characterization of the species is also provided.

Material and methods

Specimens of the new species were captured in a pasture in the vicinity of the Jamari River, municipality of Monte Negro, Rondônia State, Brazilian Amazon: Fazenda Boa Sorte, BR421 (10º 17′ 02.7″ S, 63º 18′ 14.8″ W, Datum SAD69), Sítio João Careca (10º 18′ 03.5″ S, 63º 14′ 09.1″ W, Datum SAD69), Jamari River (10º 17′ 56.1″ S, 63º 14′ 22.5″ W, Datum SAD69). Females were collected using a Shannon trap, and larvae and pupae were taken from several aquatic habitats. The immature specimens were raised in the laboratory to obtain adult males and females with associated larval and pupal exuviae. In addition, larvae, pupae and adults were obtained from link-reared offspring of blood-fed field-collected females. Freshly emerged mosquitoes were euthanized with ethyl acetate vapor and individual adults were kept in separate plastic vials with silica gel. Larval and pupal exuviae were collected and preserved in 80% ethanol prior to slide-mounting and male genitalia were dissected and slide-mounted in Canada balsam. Larval and pupal chaetotaxy was examined, setae measured and the branches of each were counted. Morphological characters of the female, 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. Terminology for morphological descriptions follows Harbach & Knight (1980, 1982), except for wing veins and spots, which follow Wilkerson & Peyton (1990).

Nyssorhynchus (*Nyssorhynchus*) *jamariensis* n. sp.

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Anopheles nuneztovari in part of Kitzmiller *et al*. 1973: 435–455 (polytene chromosome polymorphism); of Conn 1990: 400, 403, 404 (chromosomes); Scarpassa *et al*. 1999: 1010–1017 (phylogenetics); Trindade & Scarpassa 2002: 613–618 (genetics); Mirabello & Conn 2008: 109–117 (phylogeography).

Anopheles nuneztovari s.l. in part of Scarpassa *et al.* 2016: 1–16 (phylogenetics).

Anopheles nuneztovari A in part of Conn 1990: 401 (chromosomes); Conn *et al*. 1993: 294 (chromosome); Calado *et al*. 2008: 791–799 (molecular taxonomy); Foster *et al*. 2013: 1–7; Foster *et al*. 2017: 5 (phylogenetics); Santos *et al.* 2019: 235–244 (morphology).

Anopheles nuneztovari cytotype A of Conn *et al*. 1993: 300 (chromosome); Conn *et al*. 1998: 314, 317, 320, 321 (phylogenetics); Perera *et al.* 1998: 673, 675, 676, (polytene chromosomes); Scarpassa *et al.* 1999: 1016, (phylogenetics); Trindade & Scarpassa 2002: 618 (genetics); Mirabello & Conn 2008: 109, 110, 113, 115 (phylogeography).

Female. Integument light to dark brown. *Head*. Vertex with white erect forked scales and a few long white setae along ocular line, dark brown to black erect forked scales posteriorly on vertex and occiput. Proboscis dark-scaled, length 1.90–2.15 mm (mean 2.03 mm ± 0.10; *n* = 5), length of maxillary palpus 1.85–2.13 mm (mean 1.94 ± 0.13; *n* = 5). Maxillary palpomere 1 with dark semi-erect scales, palpomere 2 similar to palpomere 1, with narrow apical white band, palpomere 3 mostly dark-scaled with white scales at apex, palpomere 4 mostly white-scaled on dorsal and outer surfaces, with dark scales basally, palpomere 5 predominantly white-scaled with 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, not extending onto acrostichal area; acrostichal setae strong; dorsocentral setae long; scutellum with long dark setae along posterior margin, with white spatulate scales anterior to setae. Antepronotum with dark setae; prespiracular area without setae and scales; upper mesokatepisternum without setae and scales, lower mesokatepisternum with white spatulate scales and brownish setae. *Wing* (Fig. 1a). Length 3.06–3.36 mm (mean 3.22 ± 0.13; *n* = 5). Veins covered with dark and pale scales. Dorsal spots as follow: Costa with basal and basal pale (BP), prehumeral dark (PHD), humeral pale (HP), humeral dark (HD), presector pale (PSP), presector dark (PSD), sector pale (SP), subcostal dark (SCD), subcostal pale (SCP), preapical dark (PD), preapical pale (PP) apical dark (AD) and apical pale (AP); vein R_s mostly

dark-scaled; R_{2+3}, R_{4+5} and M predominantly pale-scaled. *Legs*. Foretarsomeres 1–3 and 5 with white scales at apices, foretarsomeres 4 dark-scaled. Midtarsomeres 1, 2 and 5 dark-scaled with apical white bands, midtarsomeres 3 and 4 dark-scaled. Hindleg (Fig. 1b): Hindtarsomere 1 ($Ta\text{-}III_1$) predominantly dark-scaled, with narrow pale-scaled spot at apex, hindtarsomere 2 ($Ta\text{-}III_2$) white-scaled on apical 0.25, hindtarsomeres 3 ($Ta\text{-}III_3$) and 4 ($Ta\text{-}III_4$) entirely white-scaled, hindtarsomere 5 ($Ta\text{-}III_5$) white-scaled apically. *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; dark posterolateral scale-tufts on terga II–VII. Sternum I bare; sterna II–VII with a few pale scales; sternum VIII covered with pale scales and a few dark scales.

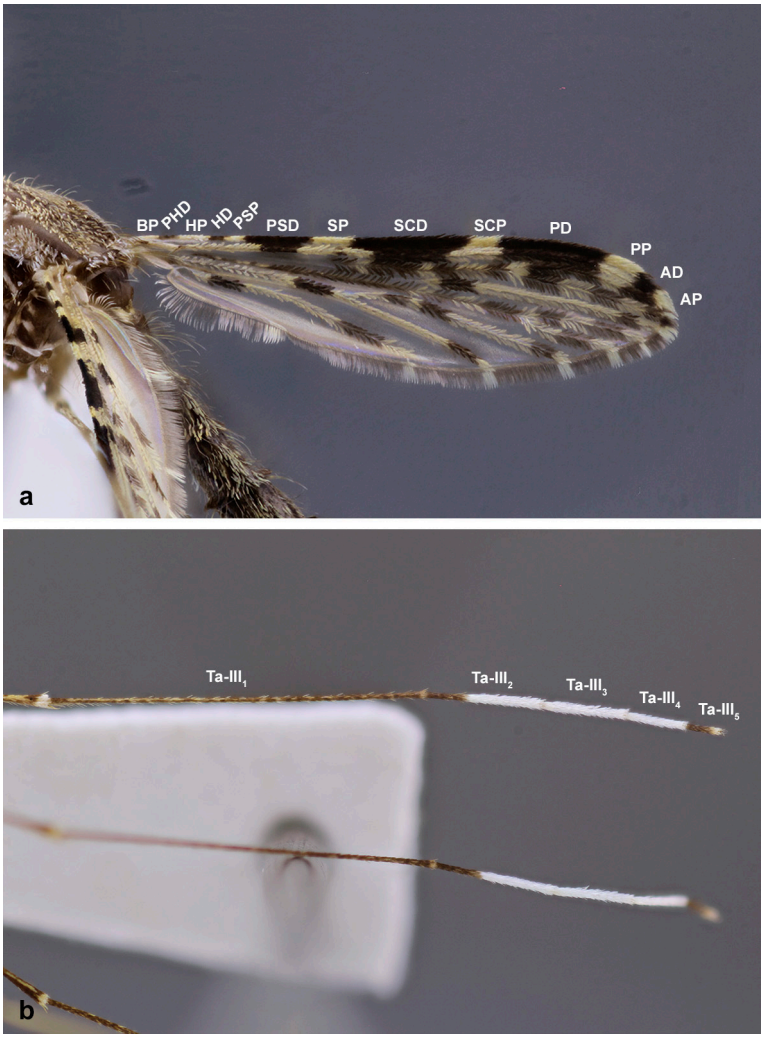

FIGURE 1. Female of *Nyssorhynchus jamariensis* n. sp. a, Wing; b, hindtarsi. Costal wing spots: AD, apical dark; AP, apical pale; BP, basal pale; HD, humeral dark; HP, humeral pale; PD, preapical dark; PHD, prehumeral dark; PP, preapical pale; PSD, presector dark; PSP, presector pale; SCD, subcostal dark; SCP, subcostal pale; SP, sector pale; $Ta\text{-}III_1$– $Ta\text{-}III_5$, hindtarsomeres 1–5.

Male. Similar to female except for sexual characters. *Head.* Proboscis length 2.56–2.65 mm (mean 2.60 mm ± 0.04; *n* = 5), maxillary palpus mostly dark-scaled, with pale spots; palpomere 2 with dark erect scales and a few pale scales; palpomere 3 with dark erect scales basally, with pale apical band; palpomere 4 dark-scaled, with pale scales at base and apex. *Genitalia* (Fig. 2c). Tergum and sternum VIII with spatulate scales and long setae. Sternum IX moderately long, sub-trapezoidal. Dorsal claspette: Pedicel long, moderately broad to moderately narrow, base rounded, leaflets broad and curved. Ventral claspette: Relatively short, broad, apex flat without median sulcus; short spicules on lateral margins, ventral and lateral surfaces, including basal lobule, extending to or nearly to apex; basal lobule moderately expanded laterally, rounded distally, with sparse small spicules similar in size and development, medium spicules abundant on inner margin; preapical plate moderately developed and weakly sclerotized, circu-

lar, well defined. Aedeagus with apex moderately rounded, somewhat triangular; subapical leaflets present, small, membranous, weakly sclerotized, non-serrate.

Pupa (Fig. 2). Position and development of setae as figured. All measurements, 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 2.48–2.65 mm (mean 2.58 ± 0.09); seta 1-I dendritic, number of branches not counted; seta 2,4-I normally triple; 3-I single; 5,6-I single, long; 7-I usually with 2 branches, shorter than 6-I; 9-I single, as long as 7-I; 0-II–IV normally with 4 branches, 0-V–VII with 2–4 branches; 1-II,III well developed, usually with 9 and 7 branches respectively, 1-IV–VII always single, strong, long, extending beyond following segment; 3-II,III,VI single, 3-III long, 3-IV with 4–6 branches, never reaching caudal margin of segment, 3-V normally triple; 5-III,IV well developed, usually with 6 and 4 branches respectively, 5-V–VII single, long; 6-II most often single, 6-III–VII single; 7-II,V double or triple, 7-III,IV with 2–4 branches, 7-VI,VII single; 8-III normally with 4 branches, 8-IV,VII with 2–4 branches, 8-V,VI frequently double; 9-II minute, lightly pigmented, 9-III,IV short, 9-V–VIII well developed, strong, pigmented, sharply pointed; 10-III normally triple, 10-IV,V,VII single, long; 4-VIII usually with 2 branches. *Genital lobe*: Thick at base, with sides sloping toward apex, apex with mammiliform protuberance. *Paddle*: Length 0.72–0.75 mm (mean 0.74 ± 0.01), width 0.44–0.51 mm (mean 0.48 ± 0.03); obovate, outer margin distad 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 normally single.

Fourth-instar larva (Fig. 3). Position and development of setae as figured; all measurements, number and mode of setal branches are based on 5 specimens. *Head*: Length 0.61–0.69 mm (mean 0.65 ± 0.03), width 0.61–0.67 mm (mean 0.63 ± 0.03). Integument weakly pigmented, yellowish to light brown, with dark spots, not forming distinct pattern. Seta 2-C single with few moderately developed spicules distally; 3-C shorter than 2-C, single, distance between bases of 2-C 0.04–0.05 mm (mean 0.04 ± 0.002); distance between bases of 2-C and 3-C 0.05–0.06 mm (mean 0.05 ± 0.002); seta 4-C frequently single, extending approximately to base of 3-C; 5-C with 13–19 branches, extending well beyond base of 2-C, reaching almost 0.5 length of 2-C; 6,7-C normally with 14 and 21 branches respectively, extending approximately to base of 3-C; 8-C frequently triple; 9-C normally with 6 branches; 10-C frequently triple; 12,13-C usually with 4 branches. Collar strongly pigmented, dark brown. *Antenna*: Length 0.25–0.27 mm (mean 0.26 ± 0.01), enlarged toward base, longer than wide; with long and thin spicules on mesal margin; seta 1-A normally with 4 branches, small, inserted 0.06–0.07 mm (mean 0.06 mm ± 0.005) distant from base. *Thorax*: Setae 1,2-P arising separately, 1-P palmate, normally with 12 narrow lanceolate leaflets, leaflets pointed apically, 2-P long with 13–16 branches; 3-P single; 14-P frequently with 6 branches, long, extending beyond anterior margin of thorax; 1-M strongly plumose, usually with 24 branches, extending beyond base of 0-P; 2,3,5-M single, simple; 4,6,7-M normally triple; 8-M plumose; 14-M usually with 8 branches; 1,2-T single, simple; 3-T palmate, with narrow semi-transparent leaflets, usually with 9 leaflets; 4-T small usually with 3 branches; 13-T normally triple. *Abdomen*: Seta 0-II-VII moderately long; 1-I–VII palmate, 1-I usually with 13 leaflets, 1-II–VII with moderately narrow truncate leaflets; 2-I normally triple, 2-II with 5–7 branches, strongly developed, large, 2-III frequently triple, stronger than 2-II, 2-IV,V simple, single, 2-VI,VII normally with 4 and 6 branches respectively; 5-I usually with 4 branches, 5-II,III,VII frequently with 7 branches, 5-III–VII well developed, 5-IV with 3–6 branches, 5-V,VI normally with 6 branches; 6-I-III plumose, inserted on tubercle, 6-IV–VI simple, single, large, 6-VII with 4 or 5 branches; 7-I,II plumose, inserted on tubercle, 7-III–VI frequently triple, 7-VII normally with 6 branches; 8-II–VI usually triple, 8-VII with 3–5 branches; 9-I–V frequently with 6 branches, 9-VI with 4 or 5 branches, 9-VII with 6 or 7 branches; 10-I,III–V simple, single, 10-II double or triple, 10-VI normally triple, 10-VII usually with 8 branches; 11-I with 2–4 branches, large, 11-II,VI,VII single, 11-III,IV with 3 or 4 branches, 11-V normally with 6 branches; 13-I-III,VI,VII normally with 6 or 7 branches, 13-IV with 3 or 4 branches, 13-V well developed, normally with 4 branches; 1-VIII single; 2-VIII normally with 5 branches; 3-VIII with 13–15 branches; 4-VIII single; 5-VIII usually with 7 branches. *Pecten*: With 3–5 long and 9–11 short spines; median plate of spiracular apparatus moderately pigmented with lateral arms minute. *Segment X*: Seta 1-X inserted on margin of saddle; anal papillae narrow, longer than saddle.

Etymology. The name, *jamariensis*, is derived from the name of the geographical locality where the specimens were first collected, the Jamari River. The Jamari is a tributary of the Madeira River in the western Brazilian Amazon of Rondônia State.

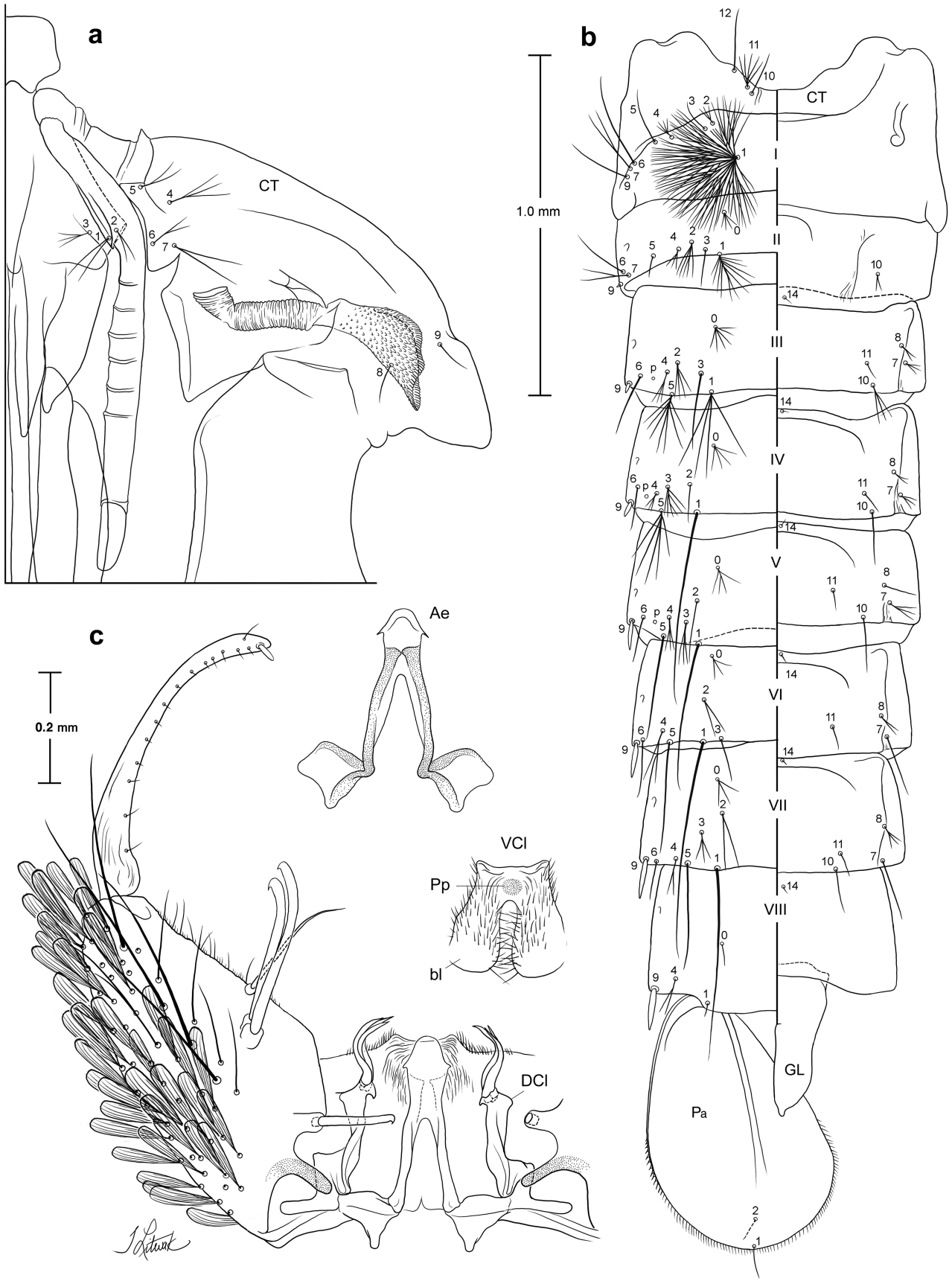

FIGURE 2. Pupa and male genitalia of *Nyssorhynchus jamariensis* n. sp. a, Cephalothorax; b, abdomen; c, male genitalia. Pupa: CT, cephalothorax; GL, genital lobe; Pa, paddle; I–VIII, abdominal segments. Male genitalia: Ae, aedeagus; bl, basal lobule; DCI, dorsal claspette; Pp, preapical plate; VCI,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

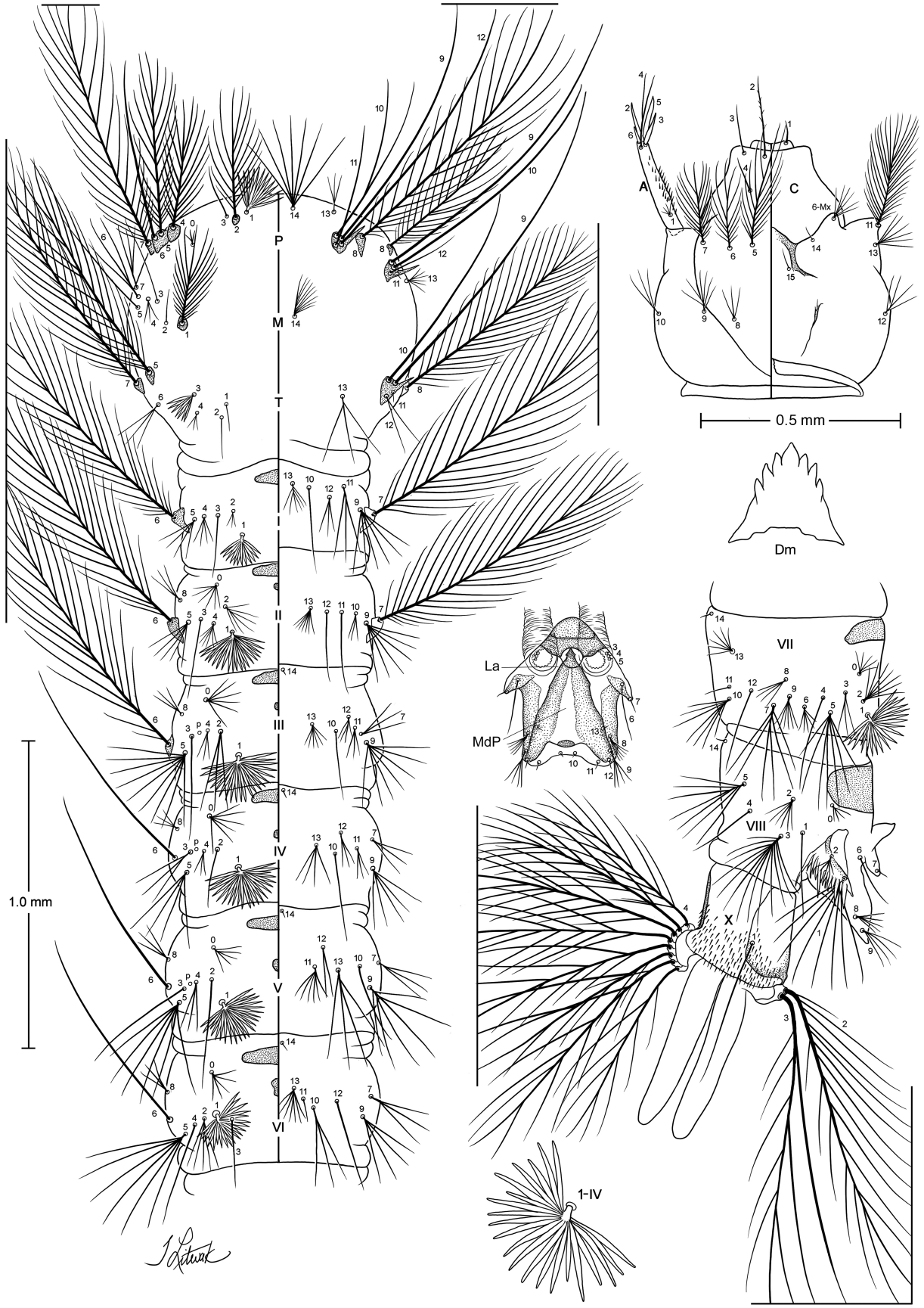

FIGURE 3. Fourth-instar larva of *Nyssorhynchus jamariensis* n. sp. A, antenna; C, cranium; Dm, dorsomentum; La, lateral arms, M, mesothorax; MdP, median plate; P, prothorax; T, metathorax; I–VIII,X, abdominal segments. Scales in mm.

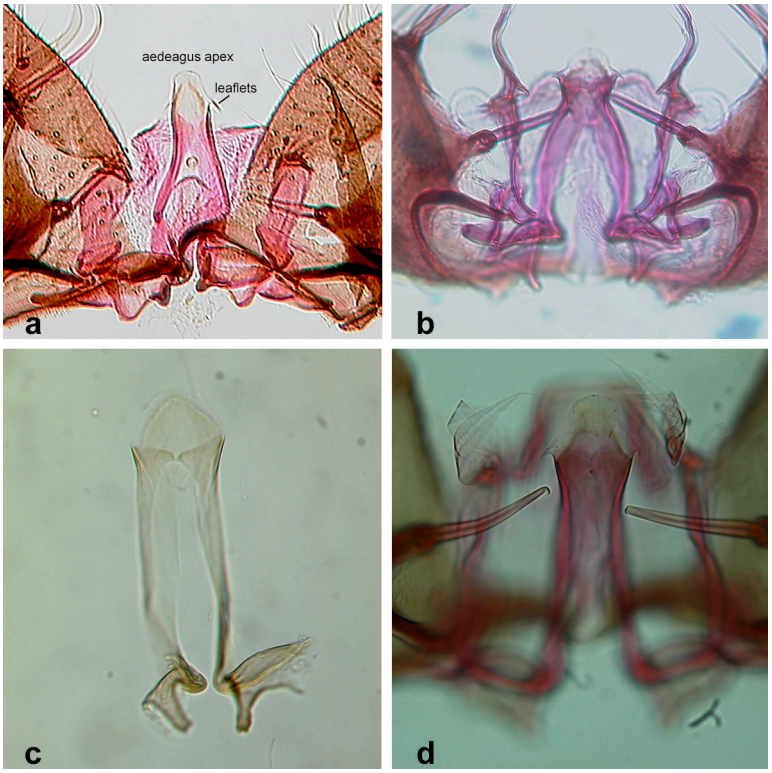

FIGURE 4. Details of the aedeagus of male genitalia. a, *Nyssorhynchus nuneztovari s*.s (Venezuela, image preparation #1353 WRBU); b, *Ny. goeldii* (holotype); c, *Ny. dunhami* (lectotype number #58031WRBU); d, *Ny. jamariensis* n. sp. (Rôndonia, Brazil).

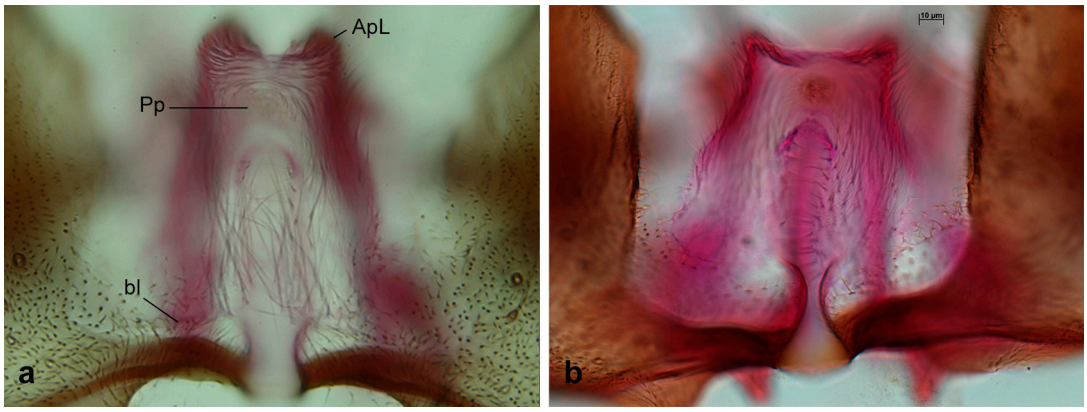

FIGURE 5. Details of the ventral claspette of male genitalia. a, *Ny. goeldii* (Pará, Brazil); b, *Ny. jamariensis* n. sp. (Rôndonia, Brazil). ApL, apicolateral lobes; bl, basal lobule; Pp, preapical plate.

Bionomics. Larvae and pupae of *Ny. jamariensis* were taken from partially shaded lake margins, ground pools and flooded areas. The water was stagnant, fresh, clear or turbid, with abundant floating and emergent vegetation. The temperature of the larval habitats was around 31°C. Females were collected in a Shannon trap from 18:00 to 21:00 h in pasture. It is unknown whether *Ny. jamariensis* is a local vector of malaria because it has been misidentified as both *Ny. goeldii* and *Ny. nuneztovari s.s*. Further studies are necessary to verify the potential association of *Ny. jamariensis* with malaria transmission in the Amazon River basin.

Distribution. *Nyssorhynchus jamariensis* occurs in Rondônia State in the western Brazilian Amazon. Because of morphological similarities shared with *Ny. nuneztovari s.s*., *Ny. goeldii* and *Ny. dunhami*, the geographical range of *Ny. jamariensis* needs further investigation, but most records of *Ny. nuneztovari* A refer to this species.

Material examined. *Holotype*: Adult male with associated Le and Pe and dissected genitalia mounted on microscope slides, specimen code RO02(13)-1, bearing the following collection data: Brazil, Rondônia State, municipality of Monte Negro, Sítio João Careca, Jamari River, 10º 18′ 03.5″ S, 63º 14′ 09.1″ W, coll. 08-Jan-2008, Bergo

et al., in Shannon Trap, in a rural area, altitude 150 m. The holotype is a sibling of a progeny of a female bearing the field code RO02(13). *Paratypes*: 25 specimens with the following information: Rondônia State, municipality of Monte Negro, Sítio João Careca, Jamari River, 10º 18′ 03.5″ S, 63º 14′ 09.1″ W, coll. 08-Jan-2008, Bergo et al., RO02(13)-2, FSP-USP no. E-15949 [MLePe]; RO02(13)-7, FSP-USP no. E-15950 [FLePe]; RO02(13)-9, FSP-USP no. E-15951 [FLePe]; RO02(13)-10, FSP-USP no. E-15952 [MLePe]; RO02(13)-13, FSP-USP no. E-15953 [MLePe]; RO02(13)-14, FSP-USP no. E-15954 [FLePe]; RO02(13)-19, FSP-USP no. E-15955 [FLePe]; RO02(13)-27, FSP-USP no. E-15956 [MLePe]; RO02(13)-100, FSP-USP no. E-15957 [MPeG], RO06-3, FSP-USP no. E-15958 [MLePe]. Rondônia State, municipality of Monte Negro, Fazenda Boa Sorte, BR421, 10º 17′ 02.7″ S, 63º 18′ 14.8″ W, coll. 15-Jan-2008, Bergo & Sallum, RO20(1)-1, FSP-USP no. E-15959 [MLePeG]; RO20(1)-5, FSP-USP no. E-15960 [FLePe]; RO20(1)-7, FSP-USP no. E-15961 [FPe], RO20(2)-1, FSP-USP no. E-15962 [MLePeG]; RO20(2)-8, FSP-USP no. E-15963 [FLePe]; RO20(2)-11, FSP-USP no. E-15964 [FLePe]; RO20(2)-13, FSP-USP no. E-15965 [MLePe]; RO20(2)-14, FSP-USP no. E-15966 [MLePe]; RO20(2)-15, FSP-USP no. E-15967 [MLePe]; RO20(2)-19, FSP-USP no. E-15968 [FLePe]. Rondônia State, municipality of Monte Negro, Jamari River, 10º 17′ 50.9″ S, 63º 16′ 05.9″ W, coll. 8-Jan-2008, Bergo, RO01-17, FSP-USP no. E-15969 [MLePeG], RO01-103, FSP-USP no. E-15970 [MLePeG]. Rondônia State, municipality of Monte Negro, Jamari River, 10º 17′ 56.1″ S, 63º 14′2 2.5″ W, coll. 9-Jan-2008, Bergo, RO04-4, FSP-USP no. E-15971 [FLePe], RO04-103, FSP-USP no. E-15972 [FPe], RO04-106, FSP-USP no. E-15973 [MPeG]. *Other specimens examined*: Specimens of *Ny. jamariensis* with the following data: Brazil, Rondônia State, municipality of Monte Negro, Fazenda Boa Sorte, BR421, 10º 17′ 02.7″ S, 63º 18′ 14.8″ W, coll. 15-Jan-2008, Bergo & Sallum, 10 specimens with associated Le and Pe (RO20(1)-8, -9, -10, -13, -16, -17; RO20(2)-2,-4, -10, -23). Specimens of *Ny. dunhami* with the following data: Brazil, Amazonas State, Parintins, Vila Amazonia settlement, 2º 38′ 66.2″ S, 56º 38′ 29.6″ W, coll. 6-June-2005, Sallum et al., FSP-USP no. E-12999 [G], BRAM013-105 [G], BRAM013-106 [G]. Specimens of *Ny. goeldii* with the following data: Brazil, Pará State, Óbidos, 2° 32′ 19.1″ S 57° 45′ 21.6″ W, coll. 6-July-2005, Sallum et al., FSP-USP no. E-13000 [G], FSP-USP no. E-13001 [G]. Pará State, Santarém, Urumanduba, 2° 28′ 56.2″ S 54° 39′ 39.3″ W, coll. 8-Oct-2008, Bergo et al., PA3(9)-1 [MLePeG], PA3(11) [M], PA3(15)-3 [F]. Pará State, Santarém, Bom Jardim, 2° 33′ 07.9″ S 54° 35′ 38.7″ W, coll. 9-Oct-2008, Bergo et al., PA5(4)-1 [MLePeG]. Pará State, Belterra, São Domingos, 2° 45′ 07.2″ S 55° 01′ 04.9″ W, coll. 10-Oct-2008, Bergo et al., PA7(2)-1 [MLePe], PA7(2)-8 [FLePe], PA7(3)-1 [MLePeG], PA7(3)-3 [M], PA7(6)-5 [M], PA7(7)-2 [F], PA7(7)-8 [F], PA7(17)-6 [M]. The holotype, paratypes and additional specimens are deposited in the Coleção Entomológica de Referência (FSP-USP), Faculdade de Saúde Pública, Universidade de São Paulo, Brazil.

Discussion

The Nuneztovari Complex includes *Ny. nuneztovari s.s*., *Ny. goeldii*, *Ny. dunhami* and *Ny. jamariensis* (formerly *Ny. nuneztovari* A). Based on polythene chromosomal differences, Kitzmiller *et al*. (1973) suggested that specimens of *Ny. nuneztovari* they studied comprised populations that were recognized by distinct chromosomal banding patterns. The cytotypes of Kitzmiller *et al*. (1973) were informally designated cytotype B, found in Venezuela east of the Andes, and cytotype C, identified in populations from Venezuela west of the Andes and Colombia (Conn 1990). Conn *et al*. (1993) recognized cytotype A to delimit the Brazilian Amazon form of *Ny. nuneztovari*. The chromosomal forms, cytotypes B and C found in populations of *Ny. nuneztovari* in Colombia, were shown to be conspecific (Sierra *et al*. 2004). More recently, Jaramillo *et al*. (2011) employed the *COI* mitochondrial gene and the *white* single copy nuclear gene to show that *Ny. nuneztovari* comprises a single taxon in the areas studied in Colombia. Employing *COI* barcode sequence and 12 microsatellites, Scarpassa *et al*. (2016) found evidence of genetic lineages in *Ny. goeldii*, with lineage III as a new species of the Nuneztovari Complex. Despite Scarpassa and collaborators having identified lineage III in *Ny. goeldii*, it was possible that the specimens belonged to *Ny. nuneztovari* cytotype A, and thus to *Ny. jamariensis*.

Despite sharing morphological similarities in the female, fourth-instar larva and pupa, species of the Nuneztovari Complex can be identified based on morphological characteristics of the male genitalia (Table 1). Specimens of *Ny. jamariensis* differ from those of *Ny. nuneztovari s.s*., *Ny. dunhami* and *Ny. goeldii* by having the aedeagus with the apex moderately rounded, somewhat triangular, and subapical leaflets present, membranous and weakly sclerotized (Fig. 4), while in *Ny. nuneztovari s.s*. the apex of the aedeagus is triangular and has well-developed

leaflets (Fig. 4), in *Ny. dunhami* the apex of the aedeagus is rounded, broad and lacks leaflets and in *Ny. goeldii* the apex of the aedeagus is moderately rounded, somewhat quadrangular and subapical leaflets are present or absent (Fig. 4). In addition, *Ny. jamariensis* differs from *Ny. goeldii* by characteristics of the ventral claspette and aedeagus, such as having the apex of the ventral claspette broad and flat apically, the basal lobule moderately expanded laterally, rounded distally, with sparse small spicules similar in size and development, evenly distributed along the distal margin, and the spicules are more abundant on the inner margin of the basal lobule (Fig. 4). In *Ny. goeldii*, the apex of the ventral claspette is broad with an evident wrinkled and striated appearance, the basal lobule is moderately expanded laterally, rounded distally, with short spicules along its basal margin, and the spicules are evenly distributed (Fig. 4).

TABLE 1. Comparison of morphological characters of the male genitalia of *Ny. dunhami*, *Ny. goeldii*, *Ny. jamariensis* n. sp. and *Ny. nuneztovari s.s.*

Species	Ventral claspette	Aedeagus
Ny. dunhami	Broad, apex flat, without median groove, quadrangular in outline, forming an angle of about 90° with lateral margin, with small spicules on lateral surface; basal lobule with spicules along basal margin short and evenly distributed over basal surface, spicules long, more abundant on basomesal margin and inner margin (Peyton 1993; Sallum *et al.* 2020)	Apex rounded and broad, no leaflets (Peyton 1993; Calado *et al*. 2008)
Ny. goeldii	Apex broad with abruptly angled, rounded, sclerotized lateral margins, ventral and lateral surfaces, including basal lobule, with short spicules, extending to or nearly to apex; basal lobule moderately expanded laterally, rounded distally, spicules along basal margin short, evenly distributed over basal surface, spicules long, more abundant on basomesal margin (Sant'Ana *et al*. 2015)	Apex moderately rounded, quadrangular; subapical leaflets variable, present or absent (Sant'Ana *et al*. 2015; Sallum *et al*. 2020)
Ny. jamariensis n. sp.	Broad, apex flat, without median sulcus; short spicules on lateral margins, ventral and lateral surfaces, including basal lobule, extending to or nearly to apex; basal lobule moderately expanded laterally, rounded distally, with sparse small spicules similar in size and development, median spicules abundant on inner margin	Apex moderately rounded, somewhat triangular, subapical leaflets present, membranous, weakly sclerotized
Ny. nuneztovari s.s.	Apex broad, without median sulcus, with rounded, striated, sclerotized lateral margins; ventral and lateral surfaces, including basal lobule, with short spicules, extending to or nearly to apex; basal lobule moderately expanded laterally; basomesal portion with short sparse spicules (Savage 1986; Sallum *et al*. 2020)	Apex triangular, characterized by well-developed leaflets (Gabaldon, 1940; Savage 1986; Sallum *et al*. 2020)

Furthermore, *Ny. jamariensis* is easily identified based on the following morphological characters: Female with dark caudolateral tufts of semi-erect scales on abdominal terga II–VII; hindtarsomere 2 mostly white-scaled, but with dark scales on the basal 0.25–0.27 mm (mean 0.26 mm ± 0.014); the pale wing scales yellowish to cream-colored, at least on the anterior veins, the prehumeral dark spot is smaller than the basal pale and humeral pale spots, and the humeral pale spot is 1.3–1.5 times as long as the prehumeral dark spot. The fourth-instar larva of *Ny. jamariensis* is easily identified by having seta 2-C single with a few moderately developed spicules distally, seta 3-C shorter than seta 2-C, single, seta 4-C usually single and usually extending approximately to the base of seta 3-C, seta 1-P palmate, seta 1-I–VII palmate with smooth leaflets, the leaflets of 1-II–VII moderately narrow and truncate apically; spiracular lobe with the lateral arm of the median plate minute and directed dorsolaterally.

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