Systematic Studies on *Anopheles galvaoi* Causey, Deane & Deane from the Subgenus *Nysssorhynchus* Blanchard (Diptera: Culicidae)

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Anopheles galvaoi, *a member of the subgenus* Nyssorhynchus, *is redescribed based on morphological characters of the adults male and female, fourth-instar larva and pupa. Female, male genitalia, larval and pupal stages are illustrated. Data about medical importance, bionomics, and distribution are given based on literature records. Adult female of* An. galvaoi *can be easily misidentified as* An. benarrochi *Gabaldón and* An. aquasalis *Curry. A few characters are indicated for identifying female and immatures of* An. galvaoi. *Phylogenetic relationships among* An. galvaoi *and six other species of the Oswaldoi Subgroup are estimated using COII mtDNA and ITS2 rDNA gene sequences. Lectotype of* An. galvaoi, *an adult female from Rio Branco, State of Acre, is invalidated.*

Key words: Culicidae - Anophelinae - *Nyssorhynchus* - *Anopheles galvaoi* - systematics

Anopheles galvaoi Causey, Deane & Deane was described from specimens collected in Rio Branco, State of Acre, Brazil. Causey et al. (1943) considered the adult stage of *An. galvaoi* to be morphologically similar to that of *An. aquasalis*, however, similar to *An. oswaldoi* (Peryassú) based on characters of male genitalia. Later, Deane et al. (1946a) suggested that females of *An. aquasalis*, *An. benarrochi* and *An. galvaoi* were morphologically similar and that there was no distinctive character to separate them. However, *An. aquasalis* could be separated from *An. galvaoi* and *An. benarrochi* based on its distribution range since the former was restricted to the coastal Region of Latin America (Deane et al. 1946a). Deane et al. (1946b) described the larval stage of *An. galvaoi* which was demonstrated to be similar to those of *An. goeldii* Rozeboom & Gabaldón (= *An. nuneztovari* Gabaldón), *An. noroestensis* Galvão & Lane (= *An. evansae* Brèthes), and *An. rangeli* Gabaldón, Cova-Garcia & Lopez. Forattini (1962) stated that female of *An. galvaoi* could be separated from that of *An. aquasalis* by having the wing basal dark spot as long as 0.5 length of humeral pale spot (B_2), and subbasal pale spot (B_3) shorter than subbasal dark spot. Gorham et al. (1967) suggested that *An. benarrochi* could be distinguished from *An. galvaoi* and *An. aquasalis* by the vein M (= vein 4) which is mainly dark-scaled in *An. benarrochi*, whereas it is all or mostly white-scaled in *An. galvaoi* and *An. aquasalis*. Also, the distinction between *An. galvaoi* and *An. aquasalis* was possible based on relative length of the subbasal dark spot and subbasal white spot (= spot B_3) of vein C, subbasal white spot longer than subbasal dark spot in *An. galvaoi* and shorter in *An. aquasalis* (Gorham et al. 1967). Faran (1980) reports that the character (relative length of basal dark spot and humeral pale spot) used by Forattini (1962) to separate *An. aquasalis* from *An. galvaoi* is not always true for *An. aquasalis*, and that the wing of *An. benarrochi* is similar to that of *An. galvaoi*. Causey et al. (1943) included *An. galvaoi* in the "tarsimaculatus complex" (= *An. oswaldoi*) of the *Nyssorhynchus* subgenus. Similarly, Faran (1980) suggested that *An. galvaoi* belongs to the Oswaldoi Complex of the Oswaldoi Subgroup of the Albimanus Section of *Nyssorhynchus*. The Albimanus Section and also the Oswaldoi Group were demonstrated to be non-monophyletic by Sallum et al. (2000). Based on ultrastructure morphology of the egg, *An. galvaoi* is similar to *An. konderi* Galvão & Damasceno, *An. oswaldoi* and *An. aquasalis* (Sallum et al. 2002a). In contrast, *An. galvaoi* and *An. benarrochi* can be easily distinguished by egg morphology (Lounibos et al. 1997, Sallum et al. 2002a).

An. galvaoi has been reported in several localities of Brazil and Paraguay. Since the separation of adult females from *An. galvaoi*, *An. benarrochi* and *An. aquasalis* is problematic, the distribution range of these species may be either overestimated or underestimated, especially because the focus of most studies was ecology and biology of vector species, and thus the species identification was mainly based on adult female and larva. The goals of the present study are to redescribe male and female adults, fourth-instar larva and pupal stage of *An. galvaoi*, and to compare adults and immatures of *An. galvaoi* with similar stages of morphologically similar species, and to estimate phylogenetic relationships among *An. galvaoi* and those morphologically similar species of the Oswaldoi Subgroup in the sense of Faran (1980).

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MATERIALS AND METHODS

Females of *An. galvaoi* were collected in Brazil, State of São Paulo, Bocaina, Jacaré-Pepira River, Route 255, km 125, Santa Leonor Farm (22º05'00''S 48º26'33''W). Females were blood fed in order to raise progenies as reported by Sallum et al. (2002a). Larvae were reared to the adult stage, and fourth-instar larval and pupal exuviae with their respective adults were used to identify the species. Voucher specimens are deposited in the Entomological Collection at Faculdade de Saúde Pública, Universidade de São Paulo, Brazil (FSP-USP). Female and male specimens deposited in FSP-USP which were collected in other localities in the State of São Paulo, and two males with associated male genitalia on slides collected in the State of Minas Gerais and identified by Causey in 1952 were also used for comparison with specimens from Bocaina. Also, two paratypes, one male and one female deposited in IOC were used for description and comparisons.

DNA sequences for internal transcribed spacer 2 (ITS2) nuclear gene rDNA for seven *Nyssorhynchus* taxa were downloaded from GenBank (Table I). ITS2 sequences consist of 352 base pairs (bp) (*An. galvaoi*) to 379 bp (*An. benarrochi*). For cytochrome c oxidase subunit II (COII) mitochondrial gene, three sequences from a previous analysis using mtDNA (Sallum et al. 2002b) were included. Also, COII sequences (594 bp) for *An. strodei* Root, *An. benarrochi*, *An. galvaoi*, and *An. oswaldoi* were downloaded from GenBank (Table I). The taxa sampled for the present phylogenetic study, source and GenBank accession numbers for each sequence are listed in Table I.

Sequence alignment - Nucleotide sequences of COII and ITS2 were automatically aligned using the multiple alignment program ClustalX 1.8 (Thompson et al. 1997). The nucleotide alignment of COII generated 594 characters. No gaps were necessary to adjust COII sequence alignment. Alignment of ITS2 sequences was performed using default parameters in ClustalX 1.8 and adjusted by visual inspection using MacClade version 4.03 PPC (Maddison & Maddison 2000). The alignment of ITS2 generated 417 positions. Twenty-one nucleotide positions (5% of the positions in ITS2) were hypervariable and excluded from all the analyses. Coincidently, all the positions in this unalignable region were parsimony uninformative. Sequence data for COII and ITS2 were combined into a single data matrix using MacClade version 4.03 PPC (Maddison & Maddison 2000).

Phylogenetic analysis - The data consist of 417 positions of nuclear ITS2 rDNA, and 594 bp of COII mtDNA gene sequences. Parsimony (MP) and maximum likelihood (ML) analyses were performed only on combined dataset (i.e., COII + ITS2) using PAUP version 4.0b8 PPC (Swofford 2001). *An. strodei* was used as outgroup. Gaps for ITS2 sequences were treated as missing data in all the analyses. Twenty-one hypervariable positions of ITS2 were excluded from all analyses.

Parsimony analyses were implemented in PAUP version 4.0b8 PPC (Swofford 2001) using the heuristic search option with Tree Bisection-Reconnection (TBR) branch-swapping and with parsimony-uninformative characters excluded; 10,000 random-taxon-additional replicate analyses were carried out for the unweighted analyses and 200 random-taxon-additional replicate analyses were carried out for the successive approximations weighted analyses. For the successive approximations weighted analyses, character weights were based on the maximum value of the rescaled consistency index and interactive rounds were continued until character weights stabilized (Farris 1969, Carpenter 1988). Bootstrapping (Felsenstein 1985) under parsimony utilized 1000 pseudoreplicates, with 100 random-taxon-additional replicates per pseudoreplicate; parsimony-uninformative characters were excluded.

To obtain an appropriate substitution model and model parameter values, as well as an optimal tree for branch-swapping under ML, the single optimal tree obtained by parsimony analysis was evaluated using the computer program Modeltest 3.06 PPC (Posada & Crandall 1998), which compares 14 basic substitution models. All 14 models were evaluated with and without rate heterogeneity. Rate heterogeneity was accommodated in three ways: using a gamma model, using an invariant sites model, and using a gamma plus invariant sites model (Swofford et al. 1996). Maximum likelihood analysis of combined data was performed under HKY (Hasegawa, Kishino & Yano 1985) + Γ (gamma-distributed rates) model of nucleotide evolution. Employing the adopted model and using the neighbor-joining tree generated for the combined dataset as the starting tree for branch-swapping, three interactive rounds of maximum-likelihood analyses were carried out. The most likely tree identified during each of the first two

TABLE I

Taxa, source and GenBank accession numbers for cytochrome C oxidase subunit II (COII) and internal transcribet spacer 2 (ITS-2) gene sequences used in this study

	GenBank Accession Number / Reference			
Taxon	COII	Reference	ITS2	Reference
An. (*Nys.*) *aquasalis*	AF417733	Sallum et al. 2002b	U92324	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *benarrochi*	U92379	Danoff-Burg & Conn (1997) [a]	U92325	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *galvaoi*	U92339	Danoff-Burg & Conn (1977) [a]	U92328	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *nuneztovari*	AF417736	Sallum et al. (2002b)	U92351	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *oswaldoi*	U92400	Danoff-Burg & Conn (1997) [a]	U92344	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *rondoni*	AF417737	Sallum et al. (2002b)	U92330	Danoff-Burg & Conn (1997) [a]
An. (*Nys.*) *strodei*	U92404	Danoff-Burg & Conn (1997) [a]	U92354	Danoff-Burg & Conn (1997) [a]

a: Danoff-Burg JA, Conn JE 1997. Character congruence in *Anopheles* mosquito phylogenetics (unpublished data).

ML search rounds was used as the starting tree for the next search round, both for the calculation of updated parameter values and for the initiation of TBR branch-swapping. Bootstrapping (Felsenstein 1985) under ML criterion utilized 100 pseudoreplicates, with 10 random-addition starting tree per pseudoreplicates, and TBR branch-swapping. Hypervariable region was excluded from all analyses.

RESULTS

Morphology

Anopheles (*Nyssorhynchus*) *galvaoi* (Figs 1-3)

Causey et al. 1943: 293 (M*, F*, E*). Type locality: Rio Branco, State of Acre, Brazil. Causey et al. 1946: 27 (M*); Deane et al. 1946a, b: 12 (F*); 36, 42 (L*); Coher 1948 (1949): 88 (F); Belkin et al. 1965: 5 (lectotype des.); Faran 1980: 64 (M*, F* redescription); Marchon-Silva et al. 1996: 472 (paratype info.); Sallum et al. 2002a (SEM E*).

Female - Integument light brown with grayish pollinose. Head: interocular space with frontal tuft of long, pale yellow setae, and decumbent, curved, white, spatulate scales along ocular margin (Fig. 1A); vertex immediately posterior to frontal tuft with erect, white spatulate scales and a few long, pale yellow setae, remainder of vertex and occiput with erect black spatulate scales; postgena with tuft of black, spatulate scales and a few semierect, white, spatulate scales at junction of eyes; ocular setae dark brown to black; clypeus bare. Pedicel of antenna dark brown with decumbent, white spatulate scales on dorsal surface; flagellomere 1 with semierect, white scales on medial and lateral surfaces, sometimes a few white scales on ventral surface, and a patch of de-

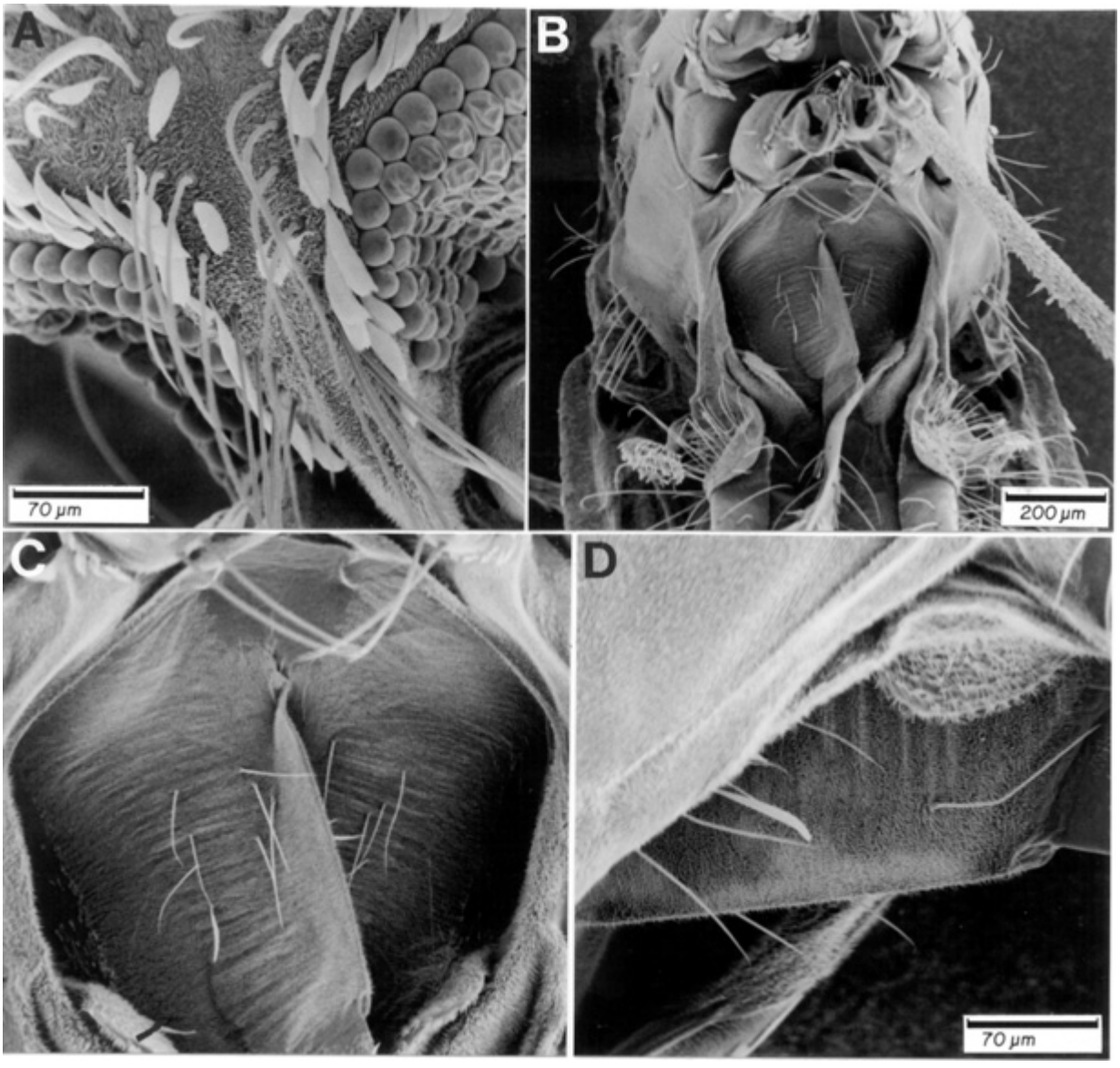

Fig. 1: *Anopheles galvaoi*. Adult female. A: interocular space; B: abdominal sternite I, ventral view; C: detail of abdominal sternite I, ventral view; D: abdominal sternite I, lateral view.

cumbent, flat, broad white scales at base of dorsal surface. Proboscis dark-scaled; length 2.13 - 2.18 mm (mean = 2.15 mm ± 0.039), 1.23 - 1.28 length of forefemur (mean = 1.26), 1.10 - 1.11 length of maxillary palpus (mean = 1.10) (*n* = 2). Maxillary palpomere 1 dark-scaled; palpomere 2 mostly dark-scaled with few white scales at apex of dorsal surface; palpomere 3 mostly dark-scaled with white scales at apex of dorsal surface; palpomere 4 mostly white-scaled with dark scales at base, sometimes a few dark scales present at lateral surface and apex; palpomere 5 mostly white-scaled with dark scales at base, scales erect on palpomeres 1 and 2, semierect and decumbent on dorsal surface, and erect on ventral surface of palpomere 3; length 1.93 - 1.98 mm (mean = 1.96 mm), 0.91 length of proboscis, length palpomere 2 / palpus length = 0.19 - 0.23 (mean = 0.21 ± 0.029); length palpomere 3 / palpus length = 0.37 - 0.40 (mean = 0.39 ± 0.022); length palpomere 4 / palpus length = 0.16 - 0.19 (mean = 0.17 + 0.02); length palpomere 5 / palpus length = 0.13 - 0.14 (mean = 0.14 ± 0.004) (*n* = 2). 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; pale yellow, spatulate, decumbent scales on acrostichal and dorsocentral areas, scutal fossa, anteriorly on prescutellar area; supraalar and antealar areas with white, spatulate, decumbent scales; elongate, narrow, erect, white, spatulate scales along lateral margin of antealar area extending posteriorly onto supraalar area; scutum bare anteriorly between acrostichal and dorsocentral areas, posteriorly to scutal fossa, and posteriorly on prescutellar area; anterior promontory with erect piliform, white scales; lateral anterior end of dorsocentral area with erect, piliform scales; anterior lateral margin of scutum with erect, broad spatulate, white scales dorsally, black scales ventrally. Scutellum with few, spatulate, yellowish scales, posterior margin with long and short pale yellow setae. Mesopostnotum bare. Antepronotum with dark setae and a patch of spatulate scales, these scales pale yellow to white dorsally, dark brown ventrally on upper area, remainder of antepronotum without scales, with scattered pale yellow setae. Pleura with small patches of pale yellow, spatulate scales on upper mesokatepisternum, posterior border of middle mesokatepisternum and prealar knob; dark brown setae on upper proepisternum, pale yellow setae on upper mesokatepisternum, middle posterior border of mesokatepisternum, prealar knob, upper mesepimeron and prespiracular area. Wing: length 3.17 - 3.32 mm (mean = 3.25 mm ± 0.07) (*n* = 3); wing spots measurements in Table II; veins dark-scaled with spots of pale yellow scales as follows: costa always with basal pale, pre-humeral dark, humeral pale, humeral dark, presector pale, subcostal pale, preapical dark, preapical pale and apical dark spots; sector pale and proximal sector dark present in 50% of wings examined; presector dark, accessory sector pale and distal sector dark frequently present, rarely absent; remigium white-scaled; vein R base pale about 0.7 distance to sector pale spot; R_1 with accessory sector pale, subcostal pale, preapical pale spots; R_2 mostly dark-scaled with preapical pale spot and a pale spot at proximal end at furcation with vein R_3; R_S with a patch of pale scale at junction of R_{4+5}, and a few pale scales on basal 0.5; R_{2+3} with a patch of pale scales along middle region and a few dark scales at apex just before furcation of R_2 and R_3; R_{4+5} mostly pale-scaled, with small patch of dark scales at proximal 0.2 and distal end; vein M variable, mostly dark-scaled, or with pale scales at basal 0.3 - 0.5, or mostly pale-scaled with dark spot at middle region and at distal end at furcation of M_{1+2} and M_{3+4}; CuA mostly pale-scaled with small dark spot at distal 0.2 before furcation of CuA_1 and CuA_2; CuA_1 mostly pale-scaled with 2 separate dark spots at basal 0.5 and a small dark spot at distal end; pale fringe spots at apices of veins R_2, R_{4+5}, M_{1+2}, M_{3+4}, CuA_1, CuA_2 and 1A. Halter: scabellum and pedicel with pale integument; capitellum dark-scaled with patch of white scales at base. Legs: anterior surface of forecoxa

TABLE II

Wing spot measurements (in mm) for male and female of *Anopheles galvaoi*

	Female				Male			
Spot	Range	Mean	S D	N	Range	Mean	S D	N
Basal pale	0,17 - 0,21	0,19	0,02	3	0,13 - 0,19	0,17	0,02	7
Prehumeral dark	0,05 - 0,07	0,05	0,01	3	0,02 - 0,09	0,05	0,02	7
Humeral pale	0,21 - 0,24	0,22	0,01	3	0,16 - 0,28	0,23	0,04	7
Humeral dark	0,08 - 0,11	0,09	0,01	3	0,04 - 0,19	0,09	0,05	7
Presector pale	0,12 - 0,13	0,12	0,01	3	0,07 - 0,16	0,13	0,03	7
Presector dark	0,34 - 0,51	0,4	0,09	3	0,35 - 0,56	0,45	0,09	6
Sector pale	0,08 - 0,09	0,08	0,003	3	0,04 - 0,06	0,05	0,01	3
Proximal setor dark	0,06 - 0,06	0,06	0,004	3	0,07 - 0,15	0,12	0,04	3
Accessory setor pale	0,08 - 0,10	0,09	0,01	3	0,13- 0,16	0,14	0,02	6
Distal setor dark	0,71 - 0,73	0,72	0,01	3	0,50 - 0,61	0,56	0,04	6
Subcostal pale	0,21 - 0,25	0,24	0,02	3	0,20 - 0,34	0,3	0,05	7
Preapical dark	0,68 - 0,80	0,75	0,06	3	0,46 - 0,56	0,53	0,04	7
Preapical pale	0,16 - 0,25	0,2	0,05	3	0,15 - 0,25	0,2	0,03	7
Apical dark	0,05 - 0,09	0,07	0,02	3	0,07 - 0,11	0,09	0,01	7
Total length	3,17 - 3,32	3,25	0,07	3	2,77 - 3,14	3,03	0,14	7

SD: standard deviation; N: number of specimens analyzed.

with a patch of spatulate scales distally, these scales white laterally and dark medially, and a few white, spatulate scales proximally, posterior surface of forecoxa with a patch of ventrally directed, black, spatulate scales, these scales white at posterolateral side; outer surface of midcoxa with patches of white, spatulate scales, proximal patch with semierect scales, posterior surface with patch of white, spatulate scales at apex, anterior surface with patch of white scales at apex; anterior surface of hindcoxa with small patch of white, spatulate scales, posterior surface with few scales at apex, small patch of white, spatulate scales at base of lateral surface. Fore-, mid- and hindtrochanters pale and dark-scaled. Foretarsomeres 1 - 3 with apical, pale yellow to white scales; foretarsomeres 4, 5 totally dark-scaled, sometimes tarsomere 5 pale-scaled at about apical 0.5; midtarsomere 2 with an apical ring of pale yellow to white scales, midtarsomere 3 with a few apical pale yellow to white scales, segment 4 entirely dark-scaled, segment 5 with pale yellow scales at about apical 0.5; hindtarsomere 2 dark-scaled in basal 0.48 - 0.52; hindtarsomere 5 dark-scaled in about basal 0.56 - 0.64; remainder of hindtarsomeres 2 and 5 and tarsomeres 3 and 4 white-scaled. Abdomen: integument light to dark-brown; terga II - VII covered with goldenish, narrow, curved scales, mostly scales disposed in a subtriangular pattern on segment II - V, segments VI - VII more equally covered with scales; dark caudolateral scales tufts large, present on tergites II - VII. Sternite I with few short, pale yellow submedian setae (Fig. 1B-D), and a few white, broad, spatulate, submedian scales.

Male (Fig. 2) - Similar to female except for sexual differences. Maxillary palpus pale and dark-scaled, scales semierect on basal 0.5 of palpomere 2, decumbent on remaining of palpomere 2 and palpomeres 3 - 5; medial surface of palpomeres without scales, palpomere 3 with long, strong, dark brown setae at apex of medial surface, palpomere 4 with long, dark brown setae along dorsal and ventral margins of medial surface; palpomere 2 with sparse, pale scales at basal 0.5, white scales mostly on dorsal surface, white band at junction of palpomere 3; palpomere 3 white-scaled at base and at apex; palpomere 4 mostly white-scaled with subbasal 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. Male genitalia: in general similar to that described by Faran (1980) who should be seen for details.

Pupa (Fig. 2) - Position and development of setae as figured; range and modal number of branches in Table III. All measurements were made in 9 to 10 specimens unless otherwise indicated. Cephalothorax: integument weakly pigmented; leg cases darker; trumpet angusticorn with meatal cleft; pinna moderately pigmented; meatal cleft basally pointed. Abdomen: length 2.18 - 3.02 mm (mean = 2.63 mm ± 0.24); seta 2 - I with 3 - 7 branches; 3 - I single as long as 2-I; 4-I with 2 - 7 branches; 5, 6-I single, long; 7-I with 2 - 5 branches, as long as or shorter than 6-I; 9-I single as long as 7-I; 0-II - VII moderately developed, 0-II with 2 - 6 branches, 0-III with 3 - 7 branches, 0 - IV with 2 - 5 branches, 0-V - VII often triple; 1-II, III well developed, with 3 - 9 and 2 - 8 branches, respectively, 1-IV- VII always single, strong, long, extending beyond the following segment; 3-IV with 2 - 5 branches, extending beyond caudal margin of segment, 3-V often double, extending slightly beyond caudal margin of segment; 5-III with 2 - 10 branches, slightly shorter than length of following segment, 5-IV with 3 - 5 branches, 5-V- VII normally single; 6-II most often single (1 - 4 branches), 1 - 1.56 length of 7-II (mean = 1.32 ± 0.25) (n = 6), 6-III single to triple, 6-IV- VII single; 7-II with 2 - 4 branches, 7-III, V normally double, 7-IV often triple, 7-VI, VII single; 8-III with 2 - 4 branches, 8-IV- VII normally double; 9-II minute, unpigmented, 9-III short, stout, 1.10 - 1.86 (mean = 1.44 ± 0.255) length of 9-II, 9-IV tick, 1.23 - 3.36 (mean = 2.04 ± 0.601) length of 9-III, dark, 9-V strong, 1.50 - 2.75 (mean = 2.06 ± 0.425) length of 9-IV, 9-VI strong, weakly curved, 1.09 - 1.50 (mean = 1.27 ± 0.143) length of 9-V, 9-VII strong, weakly curved, sharply pointed, 0.96 - 1.89 (mean = 1.27 ± 0.259) length of 9-VI, 9-VIII straight, 0.92 - 1.26 (mean = 1.07 ± 0.097) length of 9-VII, 9-II - IV less than 0.10 length of segment, 9-VI - VIII less than 0.40 length of segment; 10-III normally triple (1 - 3 branches), 10-IV, V always single, long; 10-VI present in 4 out of 10 specimens examined for this study; one extra seta, possible seta 12-VI present in 6 out of 10 specimens examined, this seta is uncommon within the genus *Anopheles*; 4-VIII with 1 - 4 branches. Genital lobe: tick at base, with sides sloping toward apex, apex with mamiliform protuberance. Paddle: obovate, 1.14 - 1.34 (mean = 1.24 ± 0.055) more long than wide, length 0.64 - 0.78 mm (mean = 0.73mm ± 0.04), width 0.50 - 0.64 mm (mean = 0.59mm ± 0.045); refractile index 0.66 - 0.76 (mean = 0.70 ± 0.03), outer margin distal of buttress with very fine, minute spicules, extending around apex and becoming sparse along caudal 0.5 of inner margin; seta 1-P stronger than 2-P, 2-P single or double.

Fourth-instar larva (Fig. 3) - Position and development of setae as figured; range and modal number of branches in Table IV. Measurements were made in 8 or 9 specimens unless otherwise indicated. Head: length 0.60 - 0.67 mm (mean = 0.63 mm ± 0.23); width = 0.60 - 0.64 mm (mean = 0.61 mm ± 0.17) (n = 6). Integument weakly pigmented with dark spots on posterior region of dorsal apotome and lateralia; mental plate 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 aciculae on 0.5 distal, 1.03 - 1.58 length of 3-C (mean = 1.32 ± 0.168), seta 2-C close to mate of opposite side, distance between bases 2.40 - 4.50 (mean = 3.45 ± 0.75) width base of single seta; 3-C single, weakly aciculate, 0.63 - 0.98 length of 2-C (mean = 0.77 ± 0.10); clypeal index 1.83 - 2.92 (mean = 2.36 ± 0.34) (distance between bases of 2-C and 3-C on one side / distance between the bases of 2-C). Seta 4-C with 2 - 5 branches, short, extending half way to anterior margin of head; 8-C with 3 - 7 branches; 9-C with 4 - 10 branches; 10, 12-C with 2 - 4 and 2 - 6 branches, respectively; 13-C with 4 - 6 branches. Collar dark brown, moderately wide dorsolaterally. Antenna: length 0.28 - 0.33 mm (mean = 0.30 mm ± 0.016) enlarged toward base, 5.80 - 6.71 (mean = 6.31 ± 0.308) more long than wide; with long and thin spicules on mesal margin, spicules shorter and fewer on

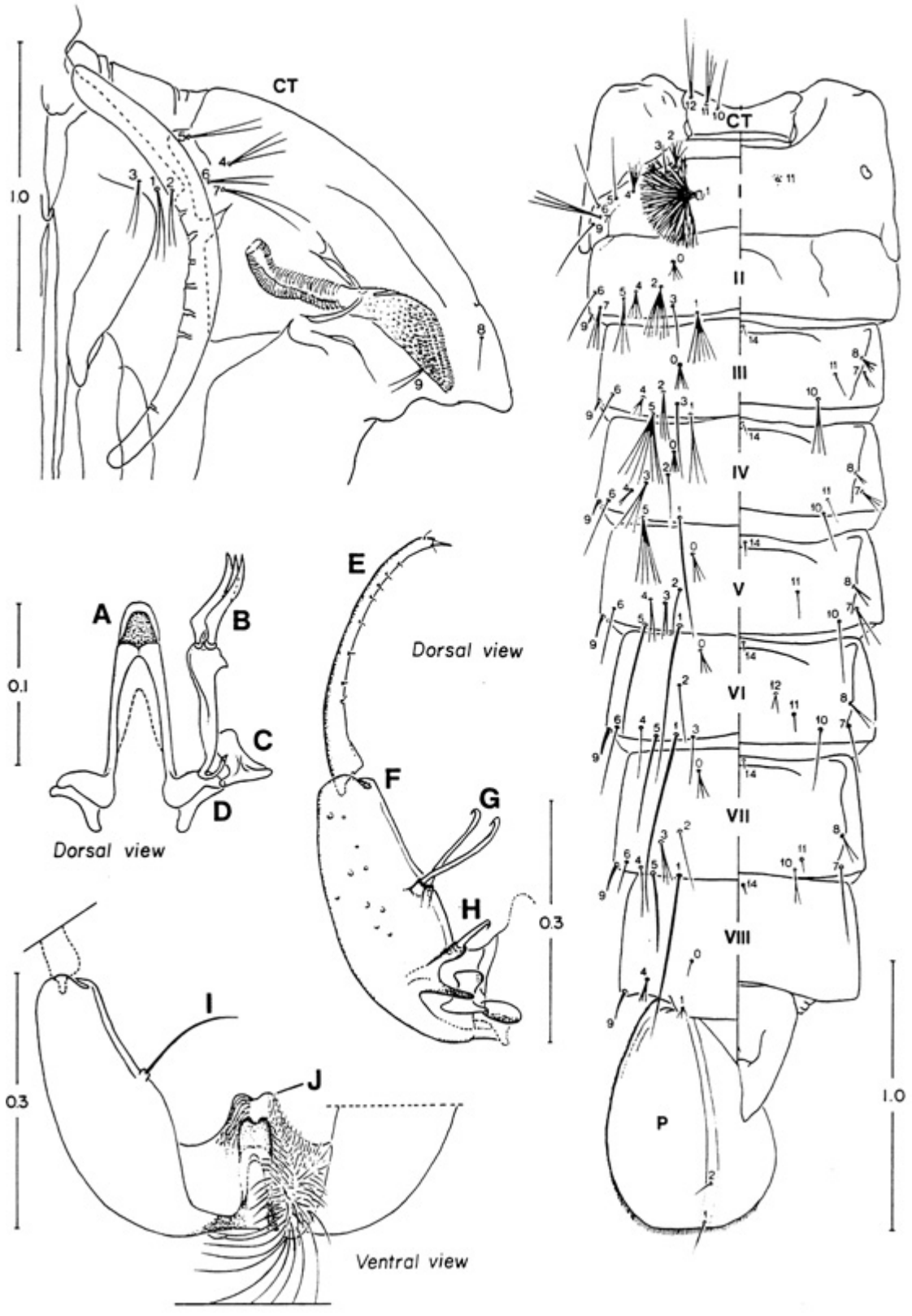

Fig. 2: *Anopheles galvaoi.* Male genitalia and pupa. A: aedeagus; B: dorsal claspette; C: gonocoxal apodeme; D; parameter; E: gonostylus; F: gonocoxite; G: accessory seta; H: parabasal seta; I: internal seta; J: ventral claspette; CT: cephalothorax; P: paddle; I - IX: abdominal segments, left side dorsal, right side ventral; scales in mm.

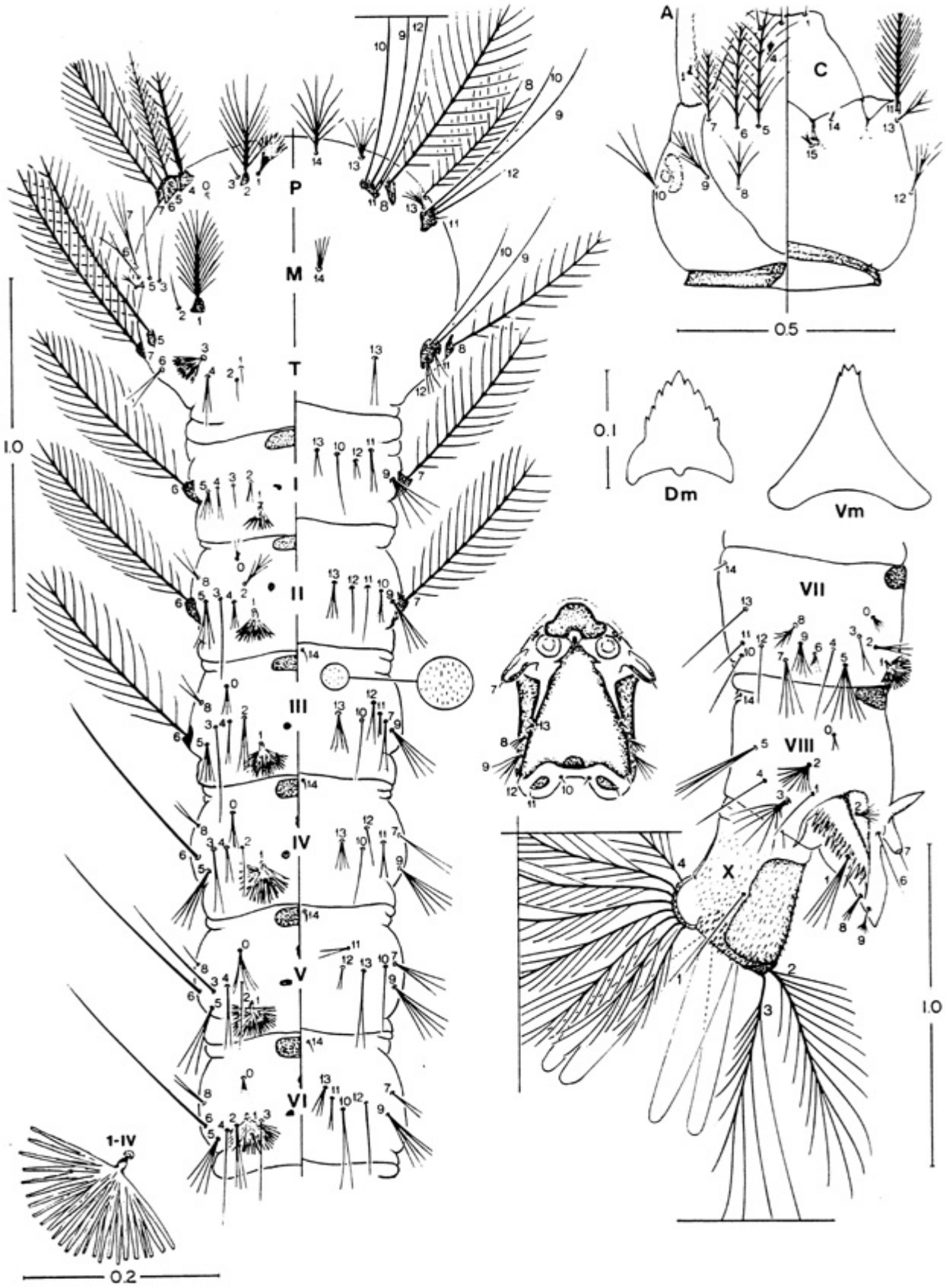

Fig. 3: *Anopheles galvaoi*. Larva. A: antenna; C: head; Dm: dorsomentum; P: prothorax; M: mesothorax; T: metathorax; Vm: ventromentum; I – VIII: abdominal segments; X: anal lobe; scales in mm.

dorsal and ventral surfaces; seta 1-A with 6 - 12 branches, about as long as width of antenna at point of insertion, 0.13 - 0.26 (mean = 0.16 ± 0.045) distance from base; seta 2-A with minute spicules on mesal margin. Thorax: seta 1, 2-P not sharing a common tubercle; 1-P palmate, with 8 - 14 moderately narrow, lanceolate leaflets; leaflets truncate at apex, 2-P with 11 - 19 branches; 14-P with 7 - 12 branches, large, branches arising from a short shaft, median branches longer than lateral branches, extending beyond anterior margin of thorax; 1-M strongly plumose, 21 - 34 branches; 2-T long, extending beyond caudal margin of thorax; 3-T palmate, with 7 - 14 moderately long, narrow leaflets. Abdomen: integument with minute spicules on ventral surface of segments II - VIII; seta 0-II - VII moderately large, often with 5 branches; 1-I - VII palmate, 1-I with 8 - 15 narrow leaflets, 1-II - VII with dark, moderately narrow, truncate leaflets; 2-II with 3 - 7 branches, strongly developed, large, 2-III with 3 - 6 branches, stronger than 2-II; 2-IV single, 2-V long, single or double; 5-I with 3 - 5 branches, inserted about its length from lateral margin of abdomen; 5-II with 6 - 10 branches; 11-I with 3 - 5 branches, large; 13-I, II, III with 5 - 9, 8 - 12, 7 - 12 branches, respectively, 13-IV with 4 - 6 branches, large, subequal to 2-V, extending beyond caudal margin of segment, 13-VI with 6 - 12 branches, moderately developed. Pecten with 3 - 5 long, 12 - 16 short spines, long spines 1.64 - 2.32 (mean = 1.88 ± 0.22) length of short spines; lateral arm of median plate of spiracular apparatus short, direct dorsolaterally; setae 8, 9-S with 1 - 6 and 3 - 6 branches, respectively. Segment X: most of segment covered with fine spicules, spicules stronger on posterior margin; seta 1-X as long as length of saddle, inserted outside saddle; anal gills longer than saddle.

Distribution - Since distinction among *An. galvaoi*, *An. benarrochi* and *An. aquasalis* by adult female is problematic, perhaps these three taxa have been largely misidentified throughout their distribution range. Consequently, it would be important to collect and raise immatures to adult in order to have male and female with associated larval and pupal exuviae for accurate identification and to have a better understanding of the distribution of those species. Distribution data for *An. galvaoi* listed in the present study are from literature records, and thus may be either underestimated or overestimated.

An. galvaoi is known from Brazil and Paraguay. In Brazil it was found in the States of Acre, Amazonas, Rondônia, Mato Grosso, Pará and São Paulo (Faran 1980). Literature records also report *An. galvaoi* in the States of Rio de Janeiro, Goiás and Paraná, however, these records were questioned by Faran (1980). More recently, *An. galvaoi* was found in the Ribeira Valley, southeast of the State of São Paulo (Dutra et al. 1996), in the vicinities of the States of São Paulo and Paraná, more specifically, in the Paranapanema River basin (Tubaki et al. 1999), in the Itaipu Dam area, west of the State of Paraná near border of Brazil and Paraguay (Guimarães et al. 1997), north of the State of Paraná (Lopes & Lozovei 1995), in the State of Maranhão in a malaria endemic area (Xavier & Rebêlo 1999, Oliveira-Pereira & Rebêlo 2000), in the State of Amazonas (Lourenço-de-Oliveira & Luz 1996), and in the States of Rondônia and Amapá (Tadei et al. 1998). Finally, *An. galvaoi* occurs in the State of Minas Gerais. This new record in Brazil is based on two adult male which were collected and identified by Causey in 1952 and are deposited in FSP-USP Entomological Collection.

Medical importance - Not known but Tadei et al. (1998) found females of *An. galvaoi* positive for *Plasmodium vivax* in an immunoenzymatic test using monoclonal antibodies against circumsporozoites.

Bionomics - It is important to have in mind that literature records on biology of *An. galvaoi* could refer to either one of those morphologically similar species that could be easily misidentified with *An. galvaoi* or perhaps to several mixed species**.** In the present work, we summa-

TABLE III

Number, range (mode), of branches for setae of the pupa *Anopheles galvaoi* [a]

Seta	Cephalothorax	Abdominal segments									Paddle
no.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	-	-	2 - 6 (4)	3 - 7 (5)	2 - 5 (4)	2 - 5 (3)	2 - 4 (3)	2 - 5 (3)	1 - 3 (1)	-	-
1	1 - 3 (2)	nc	3 - 9 (6)	2 - 8 (3)	1	1	1	1	-	2 - 3 (3)	1
2	2	3 - 7 (5)	4 - 9 (7)	3 - 5 (3)	1 - 3 (1)	1	1	1 - 2 (1)	-	-	1 - 2 (1)
3	1 - 3 (2)	1	1	1 - 2 (1)	2 - 5 (3)	1 - 3 (2)	1 - 5 (1)	1 - 3 (3)	-	-	-
4	2 - 3 (3)	2 - 7 (5)	2 - 7 (4)	2 - 6 (3)	1 - 4 (2)	2 - 3 (2)	1 - 3 (1)	1 - 2 (1)	1 - 4 (3)	-	-
5	1 - 3 (2)	1	2 - 4 (3)	2 - 10 (7)	3 - 5 (5)	1 - 3 (1)	1 - 2 (1)	1 - 2 (1)	-	-	-
6	1 - 3 (2)	1	1 - 4 (1)	1 - 3 (1)	1	1	1	1	-	-	-
7	1 - 3 (2)	2 - 5 (3)	2 - 4 (3)	1 - 4 (2)	1 - 4 (3)	1 - 3 (2)	1	1	-	-	-
8	1	-	-	2 - 4 (3)	1 - 3 (2)	1 - 2 (2)	1 - 2 (2)	2 - 3 (2)	-	-	-
9	2 - 4 (2)	1	1	1	1	1	1	1	1 - 2 (1)	-	-
10	1	-	-	1 - 3 (3)	1	1	1 - 2 (1)	1 - 4 (2)	-	-	-
11	2 - 4 (3)	-	-	1	1	1	1	1	-	-	-
12	1 - 3 (2)	-	-	-	-	-	1 - 3 (2)	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	1	1	1	1 - 2 (1)	1 - 3 (1)	1	-	-

a: based on counts made on 9 - 20 setae of 20 individuals; nc: not counted.

TABLE IV

Number, range (mode), of branches for setae of the fourthinstar larva of *An. galvaoi* [a]

Seta	Head	Thorax			Abdominal segments								
no.	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	-	1	-	-	-	4 - 7 (5)	3 - 7 (5)	4 - 7 (5)	4 - 7 (5)	3 - 8 (5)	2 - 6 (5)	2 - 4 (3)	-
1	1	8 - 14 (11)	21 - 34 (30)	1	8 - 15 (8)	18 - 28 (21)	17 - 29 (27)	20 - 31 (25)	21 - 32 (24)	22 - 30 (25)	17 - 27 (24)	1	1
2	1	11 - 19 (14)	1 - 3 (2)	1	2 - 5 (3)	3 - 7 (4)	3 - 6 (3)	1	1 - 2 (1)	2 - 4 (3)	4 - 8 (6)	6 - 13 (10)	14 - 21 (19)
3	1	1	1	7 - 14 (12)	1	1	1	1 - 3 (2)	1 - 2 (1)	1	2 - 3 (3)	6 - 14 (8)	7 - 10 (8)
4	2 - 5 (3)	15 - 22 (20)	3 - 4 (3)	3 - 6 (4)	4 - 8 (5)	3 - 8 (5)	2 - 4 (3)	2 - 4 (3)	2 - 3 (2)	1	1	1	8
5	11 - 19 (15)	26 - 41 (36)	1	21 - 38 (28)	3 - 5 (4)	6 - 10 (8)	5 - 12 (8)	2 - 7 (3)	3 - 6 (5)	5 - 7 (6)	5 - 9 (7)	3 - 7 (5)	-
6	10 - 15 (13)	1	1 - 3 (2)	1 - 3 (2)	25 - 31 (30)	27 - 37 (34)	19 - 29 (22)	1	1	1	4 - 7 (6)	1-S	3 - 6 (4)
7	10 - 18 (13)	25 - 39 (-)	2 - 4 (3)	20 - 33 (31)	22 - 29 (23)	22 - 30 (27)	2 - 3 (3)	2 - 3 (3)	2 - 3 (3)	1 - 3 (2)	4 - 6 (5)	2-S	5 - 8 (6)
8	3 - 7 (5)	23 - 32 (32)	19 - 28 (19)	25 - 39 (29)	-	3 - 4 (3)	2 - 4 (3)	3 - 5 (3)	2 - 3 (3)	2 - 4 (3)	4 - 7 (5)	6-S	1 - 2 (1)
9	4 - 10 (7)	1	1	1	3 - 6 (5)	5 - 10 (7)	6 - 9 (7)	4 - 10 (6)	5 - 9 (8)	5 - 9 (7)	4 - 9 (7)	7-S	1
10	2 - 4 (3)	1	1	1	1 - 2 (1)	2 - 3 (2)	1	1	1	1 - 3 (2)	4 - 6 (5)	8-S	1 - 6 (4)
11	39 - 48 (-)	1 - 2 (2)	1	1 - 2 (1)	3 - 5 (3)	1	2 - 4 (2)	2 - 3 (2)	2 - 3 (2)	1 - 2 (1)	1 - 2 (1)	9-S	3 - 6 (3)
12	2 - 6 (4)	1	1	1 - 2 (2)	1 - 2 (2)	1	2 - 3 (3)	2 - 3 (3)	2 - 3 (2)	1	1	-	-
13	4 - 6 (5)	4 - 7 (5)	4 - 7 (6)	2 - 3 (2)	5 - 9 (6)	8 - 12 (9)	7 - 12 (8)	4 - 6 (5)	3 - 5 (3)	6 - 12 (9)	3 - 7 (4)	-	-
14	1 - 4 (1)	7 - 12 (9)	6 - 12 (9)	-	-	-	1	1	1	1	1	1	-
15	2 - 6 (3)	-	-	-	-	-	-	-	-	-	-	-	-

a: based on counts made on 9 - 24 setae of 20 individuals; S: spiracular setae.

rized biological data from literature records for individuals identified as *An. galvaoi*.

Little is known about bionomics of *An. galvaoi*. Deane et al. (1946b) observed immatures of *An. galvaoi* in a puddle with grass and algae in full sun. Similarly, Lopes and Lozovei (1995) found immatures of *An. galvaoi*, which they did not separate from those of *An. evansae* and *An. oswaldoi*, in streamlet containing floating and emergent vegetation in full sun. The breeding site was in highly devastated anthropic environment. *An. galvaoi* was observed to be a zoophilic species, blood feeding on cattle and pigs but it was also attracted to humans (Tadei et al. 1998). This species was collected inside primary tropical forest in Balbina, State of Amazonas (Lourenço-de-Oliveira & Luz 1996), in rural zone in Ariquemes, State of Rondônia (Tadei et al. 1998), in Itaipu hydroelectric reservoir area (Guimarães et al. 1997) and Taquaruçu dam area in the Paranapanema River basin (Tubaki et al. 1999) the latter two dams in Southern Brazil. In the Ribeira Valley area, State of São Paulo, *An. galvaoi* was collected in an open area and in peridomiciliary environment (Dutra et al. 1996). Females of *An. galvaoi* were also collected inside and outside houses, feeding on humans all night (from 6:00 pm to 6:00 am) in endemic area of malaria in the State of Maranhão, North of Brazil (Oliveira-Pereira & Rebêlo 2000). It was also collected in Shannon trap, with activity peaks between 6:45 pm to 7:30 pm (Guimarães et al. 1997). Tubaki et al. (1999) found that *An. galvaoi* population activity peaked either in winter or early spring in Taquaruçu dam area, Southern Brazil. Also, generally *An. galvaoi* frequency in Shannon trap collections was higher before filling up the reservoir and then this taxon was replaced by *An. albitarsis* l.s. after the formation of the lake. Tadei et al. (1998) observed increase in *An. galvaoi* population density during dry season when *An. darlingi* decreased. Contrary, it was more frequent during rainy season in Pinheiro, State of Maranhão (Oliveira-Pereira & Rebêlo 2000) but it was collected during dry and rainy seasons, however, more frequent in the latter in São Luis, State of Maranhão (Xavier & Rebêlo 1999).

Material examined - PARATYPE: *An. galvaoi*, Rio Branco City, State of Acre, Brazil, Causey et al. coll., 1 male, 1 female reared from eggs, larval and pupal exuviae and male genitalia were not examined (IOC). OTHER MATERIAL: 1 female labeled as *An. capanemai* by Causey from the same locality of paratypes and designated lectotype of *An. galvaoi* by Belkin et al. (1971) (FSP-USP nº E-2140). One hundred ten specimens of *An. galvaoi* were examined, as follows. BRAZIL, State of São Paulo, Bocaina, Jacaré-Pepira River, Route 255, km 125, Santa Leonor Farm, Jacaré Pepira River (22º05'00"S 48º26'33"W), ES Bergo coll., 3-VII-1995, progeny broods from females collected upon human bait, 29 Le, 33 Pe, 22 males, 9 females and 5 male genitalia; Pariquera-Açu, Experimental Station, OP Forattini coll., 18-VIII-1992, Shannon trap, open area, 6 females; OP Forattini coll., 21-VII-1992, CDC light trap, 1 female; Cananéia, Galiléia Reserve, OP Forattini coll., 19-VIII 1992, Shannon trap, 1 female; State of Minas Gerais, Passos, Serviço de Febre Amarela coll., Causey det., 1952, 2 males, 2 male genitalia.

Phylogenetic analysis - The congruence of the separate ITS2 and COII datasets was tested using the Incongruence Length Difference test of Farris et al. (1995), implemented as the "Partition Homogeneity Test" in PAUP version 4.0b8 PPC (Swofford 2001), with 1000 replicates and 10 random additional tree searches per replicate and with invariant sites excluded (Cunningham 1997). The results of this test indicate that congruence cannot be rejected for ITS2 and COII datasets (P = 1.000000).

The COII alignment produced 594 positions of which 493 were constant, 101 were variable and 43 were parsimony informative. The ITS2 alignment generated 417 positions, of which 277 were constant, 140 were variable and 55 were parsimony informative. The average nucleotide composition for COII was 36% A, 38% T, 14% C, and 12% G. For ITS2 the average nucleotide composition was 25% A, 20% T, 27% C, and 28% G. For ITS2, uncorrect (p) sequence distance ranged from 0.06165 between *An. strodei* and *An. rondoni* (Neiva & Pinto) to 0.20189 between *An. aquasalis* and *An. benarrochi*. For the COII mtDNA, uncorrect (p) sequence distance ranged from 0.02140 between *An. strodei* and *An. rondoni* to 0.09806 between *An. nuneztovari* and *An. benarrochi*.

Parsimony analysis of combined ITS2 (rDNA) and COII (mtDNA) generated a single most parsimonious tree (MPT) with parsimony informative L = 195, CI = 0.692, and RI = 0.496 (Fig. 4). Analysis using successive approximations character weighting identified the same tree that was found in the unweighted analysis. The identical trees recovered in the unweighted and successive approximations character weighting analyses define one major clade containing two clades: a clade consisting of (*An. benarrochi* + *An. galvaoi*), and a clade corresponding to [(*An. aquasalis* + (*An. oswaldoi* + *An. nuneztovari*)]. Bootstrap support for the major clade is strong (100% bootstrap support value). In contrast, relationship between *An. benarrochi* and *An. galvaoi* is only weakly supported (59% bootstrap value). Bootstrap support for the grouping (*An. aquasalis* + (*An. oswaldoi* + *An. nuneztovari*)) is moderate (79 %) and for (*An. oswaldoi* + *An. nuneztovari*) is weak (68%) (Fig. 4).

The single unweighted MPT was evaluated in the program Modeltest 3.06 PPC (Posada & Crandall 1998). The likelihood ratio test found the HKY + Γ model to be significantly better fitting than the next less complex model (P ≤ 0.000001), whereas the Akaike Information Criterion (AIC) found the GTR + I (Rodríguez et al. 1990; General Time Reversible with a proportion of sites Invariant) model to be the best fitting. The model HKY + Γ was used to conduct likelihood analysis. As a result, likelihood analysis yielded a single tree with a log likelihood of - Ln likelihood = 2976.53740. The single ML tree topology (Fig. 5) is similar to that of MPT (Fig. 4). However, a comparison of the ML and MP trees indicates that disagreement between optimal MP and ML trees is restricted to the sister group relationship between *An. galvaoi* and *An. benarrochi* in the MP tree (Fig. 4), in contrast to the basal position of *An. benarrochi* relative to *An. galvaoi* in the ML tree (Fig. 5). Also, *An. galvaoi* is recovered in basal position within the clade [*An. aquasalis* + (*An. oswaldoi* + *An. nuneztovari*)]. This basal placement of *An. galvaoi*

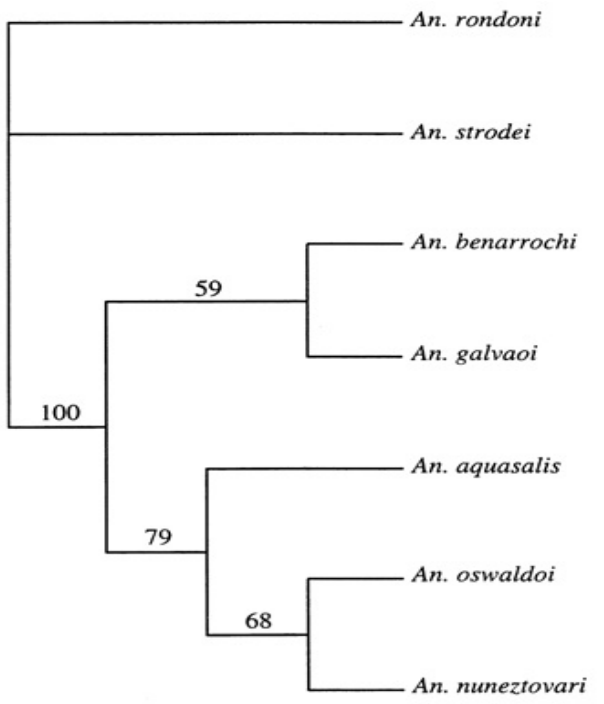

Fig. 4: the single tree identified by unweighted parsimony analysis of the combined rDNA (ITS2) and mtDNA (COII) data. Numbers above branches indicate parsimony bootstrap proportions. *An*: *Anopheles*.

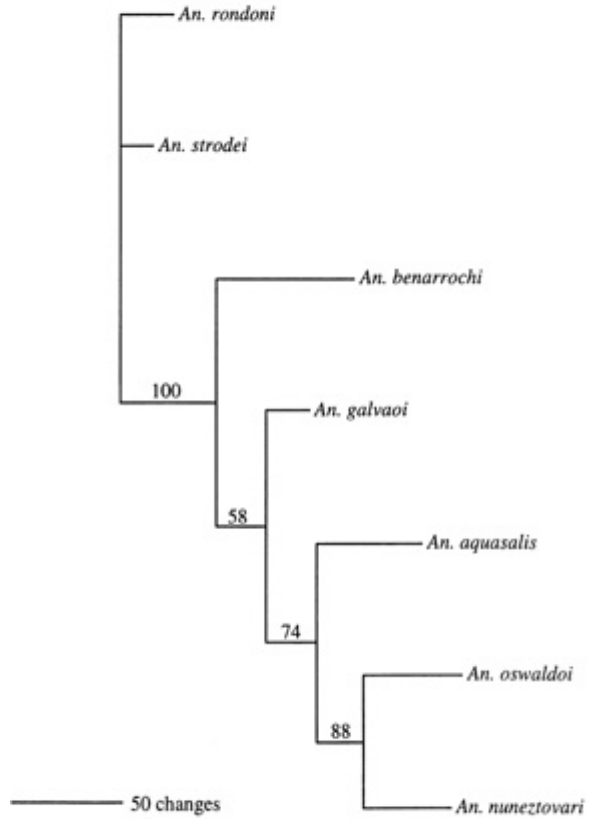

Fig. 5: the single tree identified by maximum likelihood analyses of the combined rDNA (ITS2) and mtDNA (COII) data under HKY + G model of nucleotide evolution. Numbers above branches indicate maximum likelihood bootstrap proportions. *An*: *Anopheles*.

is only weakly supported (58 % bootstrap proportion). Similarly to MP topology, ML topology defines a strongly supported clade consisting of [*An. benarrochi* + (*An. galvaoi* + *An. aquasalis* + *An. oswaldoi* + *An. nuneztovari*)] (100% bootstrap proportion). The clade formed by [(*An. aquasalis* + (*An. oswaldoi* + *An. nuneztovari*)] is moderately supported (74 % bootstrap proportion), and the sister-group relationship between *An. oswaldoi* and *An. nuneztovari* is moderately supported (88% bootstrap proportion) (Fig. 5). Additionally, in all MP and ML analyses, *An. rondoni* is placed outside to the major clade leading to (*An. benarrochi* + *An. galvaoi* + *An. aquasalis* + *An. oswaldoi* + *An. nuneztovari*) as is *An. strodei* in unrooted trees.

DISCUSSION

As previously mentioned, Belkin et al. (1971) designated a female specimen (no. E-2140) deposited in the FSP-USP, as a lectotype of *An.* (*Nys.*) *galvaoi*. However, we are considering it, in the present study, as an invalid lectotype designation due to the following facts: (1) There is no evidence that such a specimen actually belonged to the type material. The specimen brings two labels: a) "R426, sp nova, Rio Branco" [handwritten in red ink]; b) newly typed, with accession number for FSP-USP collection. Attached to the glass vial, where the specimen is preserved, there is also a label with inscription "*Anopheles capanemai*". This name had never been published. In fact, this latter label was added when the material was deposited in the FSP-USP based on the register book of Faculdade de Medicina, Universidade de São Paulo (FMUSP): a) specimen female no. 694, *An.* (*Nys.*) *capanemai* Causey; b) Det. OR Causey; c) Rio Branco, Acre, Dr OR Causey, leg, Ad; d) 16-303; e) R426 (Causey). (2) Causey et al. (1943), when described *An.* (*Nys.*) *galvaoi*, declared that the "types", reared from eggs, would be deposited in the Universidade de São Paulo [Faculdade de Medicina, later, in 1969, transferred to FSP,], and the paratypes in the United States National Museum, Washington-DC, and in the Instituto Oswaldo Cruz, Rio de Janeiro. So, we are considering the "types" as holotype female and alotype male (according to the original description). Unfortunately, it was neither possible to find them in the USP collection nor the paratypes in the USNM. Therefore, the holotype is considered lost. Marchon-Silva et al. (1996) found two paratypes in the collection of the Instituto Oswaldo Cruz, Rio de Janeiro: 1 female, with 4 labels – "*Anopheles* (*Nyssorhynchus*) *galvaoi* Causey, Deane & Deane", "Paratype", "paratipos criados de ovos postos por femea capturada em Rio Branco, Acre, Brasil" [paratypes reared from eggs laid by female captured in Rio Branco, Acre, Brazil], and "col. Inst O. Cruz no. 392". 1 male, with 5 labels – "*Anopheles* (*Nyss.*) *galvaoi* Causey, Deane & Deane, paratype male", "criado de ovos postos por 1 femea de Rio Branco, Acre, Brasil", "col. Inst. O. Cruz no. 392, "lamina de gen.", and "Paratype".

Female of *An. galvaoi* is morphologically similar to that of *An. benarrochi*, *An. aquasalis* and *An. strodei*. The correct separation of females of these four species is not an easy task because except for *An. aquasalis*, the other three species are sympatric in most of their distribution range. Sallum et al. (1997) found *An. galvaoi*, *An. benarrochi*, and *An. strodei* sympatric in the State of São Paulo, Southern Brazil. Separation of *An. aquasalis* from *An. benarrochi* is possible based on their distinct geographical distribution range, and also by a few characters suggested by Faran (1980). However, distinction of *An. galvaoi* from *An. benarrochi*, *An. aquasalis* and *An. strodei* by morphological characters of adult female is more problematic. Tentatively, female of *An. strodei* can be separated from those of *An. aquasalis*, *An. benarrochi* and *An. galvaoi* based on the hindtarsal segment 2 which is dark-scaled in less than basal 0.40 in *An. strodei* but extends to more than basal 0.4 in *An. benarrochi*, *An. galvaoi*, and *An. aquasalis*. Wing pale and dark spot characters used to separate *An. benarrochi* from *An. galvaoi* and *An. aquasalis*, and *An. galvaoi* from *An. aquasalis* seem to be variable. For example, the wing pattern of dark and pale spots was demonstrated to vary depending on environmental conditions (Hribar 1995, 1997). The character scale color on vein M proposed by Gorham et al. (1967) to distinguish *An. benarrochi* from both *An. galvaoi* and *An. aquasalis* was observed to be variable. Specimens of *An. benarrochi* and *An. galvaoi* used for comparison in the current study were found to have vein M either mainly dark-scaled or pale-scaled. Distinction between *An. galvaoi* and *An. aquasalis* is not always possible by using the relative length of the dark basal spot and humeral pale spot, and subbasal pale spot and subbasal dark spot as proposed by Forattini (1962). Male of *An. galvaoi* can be easily distinguished from those of *An. benarrochi*, *An.strodei* and *An. aquasalis* by genitalic characters (see Faran 1980, for details). Distinction among pupal stages of *An. galvaoi* and the remaining members of the Albimanus Section (Faran 1980) seems to be problematic because it is mostly based on characters that are polymorphic. For example, using Faran's (1980) key, it is impossible to key out some specimens of *An. galvaoi* even in the first couplet because seta 9 - V I I length/segment VII length ranges from 0.21 - 0.37 for this species. Consequently, specimens of *An. galvaoi* can be keyed out in both couplets. Pupal stage of *An. galvaoi* is very similar to those of *An. benarrochi* and *An. nuneztovari*. Generally, pupa of *An. galvaoi* differs from that of *An. benarrochi* in having pinna moderately long, about 3.5 of meatus length, whereas in *An. benarrochi* it is 4.5 - 5.1 of meatus length. However, *An. galvaoi* seems to be indistinguishable from *An. nuneztovari* in the pupal stage. The presence of the pupal seta 12-VI was considered an anomaly for *An. freeborni* (Belkin 1953). Also, it is rarely present in members of Sabethini as either a short, reduced seta or only its alveolus (Harbach & Knight 1980). In *An. galvaoi*, seta 12-VI, when present is short, reduced or moderately developed, 1-3-branched. It is interesting to note that the pupal seta 10-VI is also present in 40 % of the specimens examined for the present study. Similarly to seta 12-VI, seta 10-VI is not commonly observed in pupal stage of species of the genus *Anopheles*. Fourth-instar larva of *An. galvaoi* is very similar to those of *An. benarrochi*, *An. aquasalis* and *An. evansae*. However, *An. galvaoi* can be distinguished from *An. benarrochi*

and *An. aquasalis* by having both setae 2-C and 3-C barbed. In *An. benarrochi* and *An. aquasalis* both 2-C and 3-C are plumose in about apical 0.5, having moderately long to long branches. *An. galvaoi* can be separated from *An. evansae* based on few characters: (1) seta 1-X inserted outside saddle; (2) lateral arms of median spiracular plate, small but evident and distinct from the main plate; (3) seta 1-P with branches moderately broad, never narrow; and (4) seta 1-I with 8-15 long, broad leaflets. *An. evansae*: (1) seta 1-X inserted within saddle; (2) lateral arms of median spiracular plate minute; (3) seta 1-P with narrow, long leaflets; and (4) seta 1-I with 13-18 long, narrow leaflets.

Phylogenetic relationship - Faran (1980) divided the subgenus *Nyssorhynchus* into two sections: the Albimanus and Argyritarsis Sections based on morphological evidence, and the Albimanus Section was subdivided into two groups: the Albimanus and the Oswaldoi Groups, the former is monotypic and the latter includes 13 species. All species included in the current phylogenetic analysis belong to the Oswaldoi Subgroup of the Oswaldoi Group in the sense of Faran (1980). *An. benarrochi*, *An. strodei* and *An. rondoni* belong to the Strodei Complex, and *An. galvaoi*, *An. aquasalis*, *An. oswaldoi* and *An. nuneztovari* to the Oswaldoi Complex. The results of the current ML analysis place *An. galvaoi* within the clade composed by members of the Oswaldoi Complex, and *An. benarrochi* in the most basal position within a larger clade consisting of the Oswaldoi Complex plus *An. benarrochi* (Fig. 5). Contrary to Faran's (1980) hypothesis of phylogenetic relationships within the Oswaldoi Complex, *An. galvaoi* is placed as outgroup of the clade [(*An. aquasalis* + *An. oswaldoi* + *An. nuneztovari*)], and *An. oswaldoi* is sister to *An. nuneztovari*. According to Faran (1980), *An. galvaoi* is member of a clade, which includes *An. aquasalis* and *An. oswaldoi*, and *An. nuneztovari* belong to the clade (*An. nuneztovari* + *An. trinkae* Faran + *An. rangeli*). The results of all MP analyses found *An. benarrochi* sister to *An. galvaoi*, although with weak bootstrap support (59 %), and the clade consisting of these two taxa is the sister group of the clade (*An. aquasalis* + *An. oswaldoi* + *An. nuneztovari*) (Fig. 4). MP bootstrap support for the (*An. benarrochi* + *An. galvaoi*) and (*An. galvaoi* + *An. aquasalis* + *An. oswaldoi* + *An. nuneztovari*) clades is strong (100%). Finally, in both ML and MP topologies, relationship between *An. galvaoi* and *An. benarrochi* is ambiguous. In contrast, results of ML and MP bootstrap analyses strongly support monophyly of the clade corresponding to members of the Oswaldoi Complex + *An. benarrochi* (100 % bootstrap proportion) (Figs 4, 5).

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