

Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno (Diptera, Culicidae)

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ABSTRACT. Notes on the holotype of *Anopheles marajoara* Galvão & Damasceno, 1942 (Diptera, Culicidae). During studies on the dynamics of malaria transmission in Marajó Island, State of Pará, Brazil, Galvão & Damasceno (1942) collected a single specimen of a new species that they named *Anopheles (Nyssorhynchus) marajoara* Galvão & Damasceno, 1942. Now, examining genitalia slide associated to the holotype, we observed that the ventral claspette of the male genitalia is distinct from those of all other species of the *Argyritarsis* Section and consequently from members of the complex *Anopheles albitarsis* Lynch Arribalzaga, 1878. The male genitalia of the slide belong to a specimen of *Anopheles aquasalis* Curry, 1932, nevertheless, it was originally labeled as *Anopheles marajoara*. To solve this problem, we are setting aside the male genitalia slide associated with the holotype of *Anopheles marajoara* and excluding it from the type material. Illustrations of the male genitalia and adult male are included.

KEYWORDS. *Anopheles albitarsis* Complex; Culicidae; holotype; *Nyssorhynchus*.

RESUMO. Sobre o holótipo de *Anopheles marajoara* Galvão & Damasceno, 1942 (Diptera, Culicidae). Durante estudos sobre a dinâmica de transmissão da malária na Ilha de Marajó, Estado do Pará, Brasil, Galvão & Damasceno (1942) coletaram um espécime de nova espécie de anofelíneo, que foi denominada *Anopheles (Nyssorhynchus) marajoara* Galvão & Damasceno, 1942. Ao examinarmos a lâmina com a genitália, que acompanha o holótipo, observamos que o claspete ventral da genitália masculina difere daqueles apresentados pelas espécies da Seção *Argyritarsis* e, conseqüentemente, de membros do Complexo *Anopheles albitarsis* Lynch Arribalzaga, 1878. Consideramos que a genitália masculina que foi montada na lâmina associada ao holótipo pertence a um espécime de *Anopheles aquasalis* Curry, 1932, embora o adulto e as exúvias de larva e da pupa sejam de *Anopheles marajoara*. Com o intuito de resolver este problema, nós excluímos a lâmina com a genitália de macho do material tipo de *Anopheles marajoara*. A título de elucidação, foram feitas ilustrações da genitália masculina em questão, bem como do adulto de *An. marajoara*.

PALAVRAS-CHAVE. Complexo *Anopheles albitarsis*; Culicidae, holótipo; *Nyssorhynchus*.

Anopheles subgenus *Nyssorhynchus* is a monophyletic Neotropical group (Sallum et al. 2000, 2002) that currently contains 32 named species (Harbach 2004). The most important vectors of human malaria parasites in Central and South America are members of the subgenus and include *An. darlingi* Root, 1926, *An. albimanus* Wiedemann, 1820 and *An. marajoara* Galvão & Damasceno, 1942 (Conn et al. 2002). *Anopheles marajoara* belongs to a group of morphologically similar species, the *Albitarsis* Complex, which also contains *An. albitarsis* Lynch-Arribalzaga, 1878, *An. deaneorum* Rosa-Freitas, 1989 and *An. albitarsis* "B" of Wilkerson et al. (1995). The *An. albitarsis* Complex species are largely indistinguishable morphologically with nearly all characters in all stages either identical or overlapping. *Anopheles albitarsis* s. l. was suspected to contain more than one species by various authors based on epidemiological, behavioral, morphological, cytological, and biochemical evidences (Galvão and Damasceno 1944; Kreutzer et al. 1976; Linthicum 1988; Rosa-Freitas and Deane 1989; Rosa-Freitas et al. 1990; Narang et al. 1993). Wilkerson et al. (1995) using random amplified polymorphic DNA (RAPD-PCR) demonstrated the existence of the above four species. Recently, Lehr et al. (2005) hypothesized a fifth

species (*An. albitarsis* E) in northern Brazil and Venezuela related to *An. marajoara* based on complete sequence of the cytochrome c oxidase I gene (COI).

Anopheles marajoara was demonstrated to be involved in the dynamics of the transmission of human malaria in the Amazon Region. Population studies using molecular markers for species identification showed a significant increase in infected adults female of *An. marajoara* by *Plasmodium* Marchiafava & Celli, 1885, in Macapá, State of Amapá, Brazil. Consequently, the importance of *An. marajoara* in the dynamics of the malaria transmission in Macapá is currently higher than that of *An. darlingi*. More important, the change in the forest environment caused by a continuous, unplanned urbanization and human migration are creating ecological conditions that are adequate for survivorship and dispersion of *An. marajoara* (Conn et al. 2002). Also, *An. deaneorum* was infected by *P. falciparum* and *P. vivax* (Klein et al. 1991) in the laboratory.

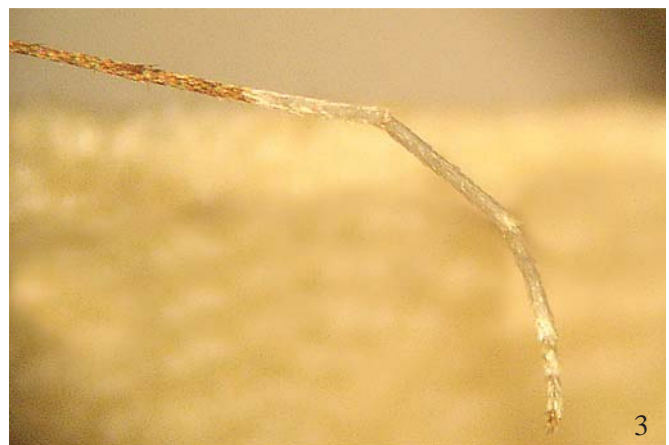
During studies on the dynamics of malaria transmission in Cachoeira do Arari (01°00'36''S 48°57'36''W), Marajó Island, State of Pará, Brazil, Galvão and Damasceno (1942) collected immatures of *An. albitarsis*, *An. aquasalis* Curry, 1932 (identified as *Anopheles tarsimaculatus* var. *aquasalis* Curry,

1932) and a single larva of a new species named *An. (Nyssorhynchus) marajoara*. The collection site was situated in Santa Maria Farm, 400 meters distant from Camará River that is the natural limit between Cachoeira do Arari and Salvaterra municipalities and runs into the Marajó Basin. In the breeding habitat, the water was turbid, polluted, standing, with fishes and immatures of several insect species.

Galvão & Damasceno (1942) named and defined *Anopheles marajoara* based on morphological characters of the fourth-instar larva, pupa and adult male, including those of the male genitalia, and designated the holotype, which was deposited in the Entomological Collection of the Parasitology Department of Faculdade de Medicina, Universidade de São Paulo (FMUSP). The accession number in the collection was FMUSP 619. The FMUSP Entomological Collection was transferred to Faculdade de Saúde Pública, Universidade de São Paulo (FSP-USP), in the early 1970s. Consequently, the holotype of *An. marajoara* was deposited in the FSP-USP where it has the accession number E-2120.

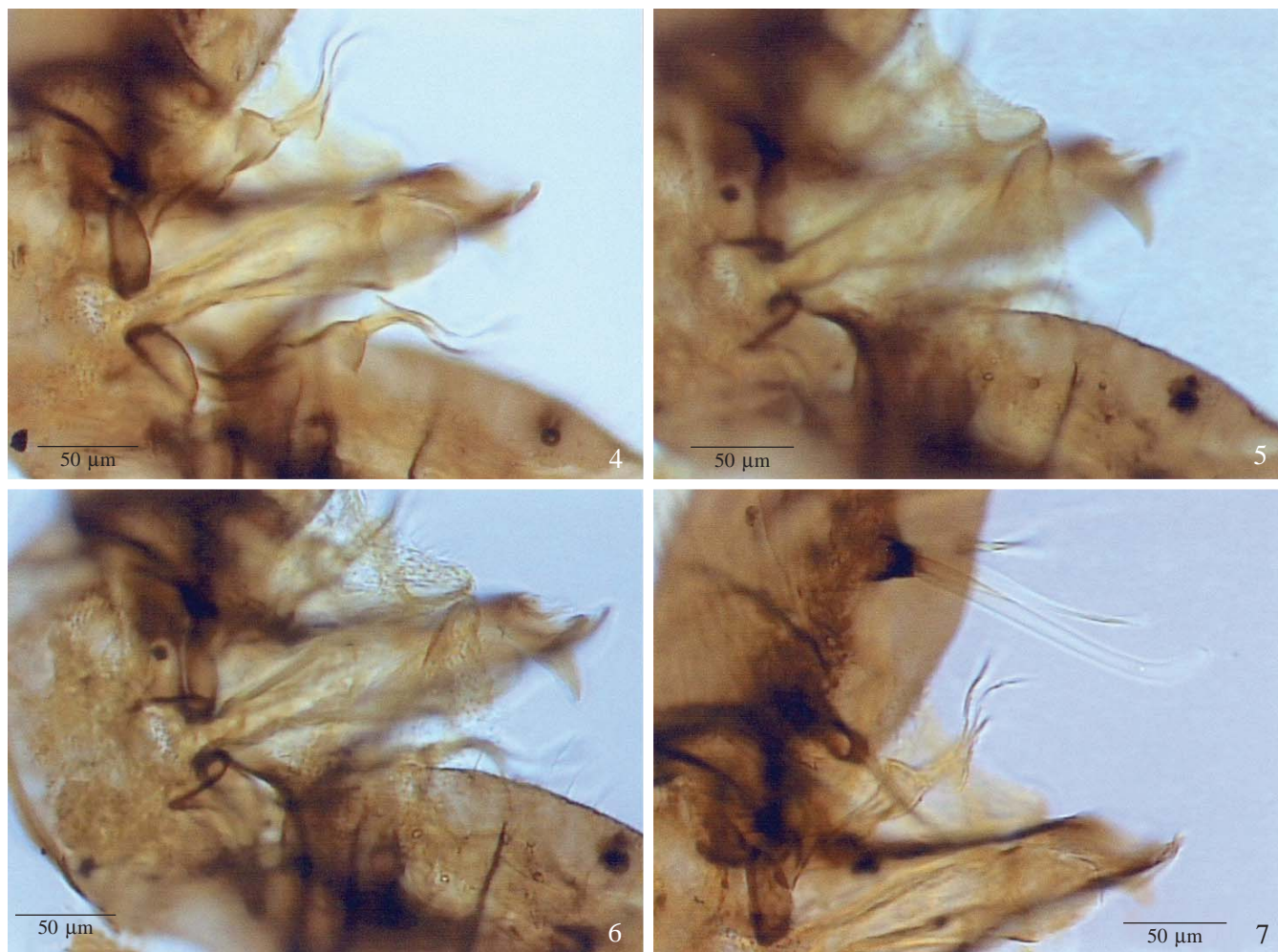
Galvão & Damasceno (1944) compared specimens of *An. albitarsis* collected in the Marajó Island with samples from the State of São Paulo, Brazil, and from Tucuman, north Argentina, thus found morphological and ethological differences between the south and north populations. Although the egg of the specimens of the Marajó Island population was morphologically similar to that of São Paulo population, they differed in the exocorion that in the former population was somewhat a mosaic. Also, the adults of the Marajó Island were smaller when compared to samples from São Paulo. *Anopheles albitarsis* samples collected in Tucuman were morphologically more similar to those of São Paulo than to those of Marajó Island. Additionally, Tucuman samples were exophilic and exophagic, whereas those from Marajó Island were collected in the intradomiciliary environment. Because of those differences, the Marajó Island population was considered to be a subspecies named *Anopheles albitarsis domesticus* Galvão & Damasceno, 1944. Galvão & Lane (1937) described *Anopheles albitarsis limai* Galvão & Lane, 1937 using samples obtained in Pinheiros and Butantan Districts in São Paulo municipality, and Corrêa & Ramos (1943) named *Anopheles albitarsis imperfectus* Corrêa & Ramos, 1943 based on specimens from Vera Cruz, both municipalities of State of São Paulo, Brazil. Lane (1953) considered *An. albitarsis limai*, *An. albitarsis imperfectus* and *An. marajoara* to be morphological variants of *An. albitarsis*, which were transferred to the synonymy of *An. albitarsis albitarsis*.

Belkin *et al.* (1971) restricted the type locality of *An. marajoara* to the limits of Cachoeira do Arari, and accepted the synonymy of this species with *An. albitarsis*. Additionally, they considered that there was a type-series "Type: males, females, larvae" (page 4) for *An. marajoara*. However, in the present study, we are considering the type series as an invalid designation for the following fact. Galvão & Damasceno (1942) declared that the holotype of *An. marajoara* was an adult male, which was deposited in FMUSP. The adult male is associated with the fourth-instar larval and pupal exuviae and male genitalia.



Figs. 1-3. *Anopheles marajoara* holotype: 1, Lateral view, habitus. 2, Abdominal sternite I, showing detail of submedial stripes of white scales. 3, Hindtarsomeres, showing detail of the white-scaled hindtarsomere 5.

Linthicum (1988) studied the species of the *Argyritarsis* Section and resurrected *An. marajoara* from the synonymy of *An. albitarsis*. Additionally, Linthicum transferred *An. albitarsis domesticus* to the synonymy of *An. marajoara*. Interestingly, in his revision, Linthicum illustrated the male genitalia of a specimen of *An. marajoara* collected in the Canal Zone, Panama. However, we observed that in the illustration (Figure 14, page 266), both the ventral claspette and the apex of the aedeagus differ from those of *An. albitarsis* (Figure 12, page 264) and



Figs. 4-7. *Anopheles marajoara* holotype, male genitalia: 4, Dorsal view of the aedeagus. 5, Dorsal view of the ventral claspette. 6, Ventral view of the ventral claspette. 7, Dorsal view, showing detail of the accessory setae of the gonocoxite and dorsal claspette.

Anopheles deaneorum Rosa-Freitas, 1989. Both species, *An. albitarsis* and *An. deaneorum* show nearly identical ventral claspette and aedeagus. Consequently, we are considering that the morphological characters described for *An. marajoara* by Linthicum (1988), specially those in Figure 14 should be analyzed carefully because it is possible that they are of an undescribed species of the *An. albitarsis* Complex.

Guimarães (1997) considered “Cachoeira do Colombia” (page 26) to be the type locality of *An. marajoara*. We believe that this information is incorrect because there is none locality named Cachoeira do Colombia in Marajó Island. It is possible that Guimarães was mentioning Cachoeira municipality. Cachoeira do Arari was previously designated as both Cachoeira and Arari (Governo do Pará, 2005). Colombia is the country where *An. marajoara* was also reported in addition to Guyana, French Guyana, Bolivia and Brazil.

While examining the male genitalia slide associated with the holotype of *An. marajoara*, we observed that the ventral claspette is distinct from those of all other species of the *Argyritarsis* Section and consequently from members of the

An. albitarsis Complex. The adult male holotype of *An. marajoara* is in good condition (Figs. 1-7), as well as the associated larval and pupal exuviae, which are both mounted on a microscope slide that has two labels with inscription “n° 619, nota 812-1, D.H. 384, Marajó, Pará, Alfaia col. 1941” and “*Anopheles (N.) marajoara* n. sp. Galvão et Damasceno det. 1941”. The male genitalia (Figs. 4-7) was dissected and mounted on a microscope slide separate from the immatures and brings two labels with inscription “no 819, nota 821-1, D. H. 383, Marajó, Pará, Alfaia col. 1941” and “*Anopheles (N.) marajoara* n. sp. Galvão et Damasceno det. 1941”. The adult male is mounted on the apex of a little white triangle on an entomological pin, and brings two labels with inscription “*Anopheles (N.) marajoara* n. sp. Galvão & Damasceno 1942” and “435-12”. Additionally, two labels were added when the holotype was transferred from FMUSP to FSP-USP collection, one label with the accession number E-2120 and the other is a red holotype label.

By examining the adult holotype (Figs. 1-7), we observed that it can be easily recognized as a species of the *An. albitarsis*

Complex of the Argyritarsis Section by having the sternum I with a submedial stripe of white scales (Fig. 2); the hindtarsomere V entirely covered with white scales (Fig. 3); and caudolateral scale tufts absent on abdominal segment II. Regarding to the fourth-instar larval and pupal exuviae associated with the adult male, we can also recognize both as members of the *An. albitarsis* Complex. The pupa by having seta 9-V short, less than or equal to 3.0 length of 9-IV; seta 3-C triple; and pinna of trumpet moderately long. The fourth-instar larva shares the following characters with other members of the *An. albitarsis* Complex: seta 1-P always palmate with lanceolate branches; 1,2-P inserted on a common sclerotized tubercle; setae 3-T and 1-I palmate, with well-developed lanceolate branches; seta 2-C widely spaced; and pecten with median teeth mostly subequal. In contrast, the male genitalia associated with the holotype of *An. marajoara* can be recognized as of a specimen of the Albimanus Section. Obviously, it is the same individual described and illustrated by Galvão & Damasceno (1942). Arguing in favor of our hypothesis there are the following facts: according to Linthicum (1988) members of the Argyritarsis Section can be distinguished from those of the Albimanus Section, except *Anopheles albimanus*, by the lack of spicules on the ventral claspette and the preapical plate and the refringent structure are at most weakly developed and are often absent. Additionally, members of the Albimanus Subgroup are distinct from those of the Albimanus Section by possessing the aedeagus without subapical leaflets, apex of ventral claspette somewhat rounded, with a moderately shallow median sulcus, dorsal claspette narrow, apical setae without a well-developed basomesal projection, and preapical plate absent. The male genitalia associated with the holotype of *An. marajoara* (Figs. 4-7) can be easily identified as *An. aquasalis* by having the following characters, listed in Faran (1980): ventral claspette spiculate (Figs. 5, 6); setae along basal margin of basal lobule of ventral claspette moderately short, about equal to or slightly longer than width of aedeagus, setae usually not reflexed; and preapical plate moderately small, circular to oval and weakly sclerotized (Figs. 5, 6). In agreement with our hypothesis that the male genitalia is not derived from the adult male designated as holotype, it is important to say that Galvão & Damasceno (1942) collected specimens of *An. aquasalis* (as *An. tarsimaculatus*) and *An. albitarsis* in association with *An. marajoara*. It is possible that either during the preparation process or by labeling mistake, the male genitalia of an *An. aquasalis* specimen had been labeled as the male genitalia of the holotype of *An. marajoara*. Unfortunately, except for the holotype of *An. marajoara* there is no other specimen either of *An. aquasalis* or *An. albitarsis* collected in Ilha do Marajó by Galvão & Damasceno (1942) that has been deposited in the FSP-USP collection. Also, in favor of our hypothesis, new recent collections carried out in the type locality in Marajó Island allowed us to examine adults male and female associated with immature stages and male genitalia. All male genitalia which we have examined are distinct from that associated with the holotype of *An. marajoara*, but similar to those of members of

the *An. albitarsis* Complex. The International Code of Zoological Nomenclature (1999), article 73.1.5 says "if a subsequent author finds that a holotype which consists of a set of components (e.g. disarticulated body parts) is not derived from an individual animal, the extraneous components may, by appropriate citation, be excluded from the holotype." Consequently, with the International Code of Zoological Nomenclature endorsement and considering the epidemiological importance of *An. marajoara*, the slide with male genitalia associated with the holotype of *An. marajoara* was set aside and excluded from the type material, because it belongs actually to another species as evidences pointed out.

Acknowledgments. We are thanks to FAPESP grant number 05/53973-0. Professor Albino Sakakibara is acknowledged for providing critical comments. W. Senise and D. Alonso helped in elaborating the illustrations.

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