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DESCRIPTION OF THE LARVA, PUPA AND EGG OF *ANOPHELES (LOPHOPODOMYIA) SQUAMIFEMUR* ANTUNES WITH NOTES ON DEVELOPMENT (DIPTERA: CULICIDAE)[1]

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Abstract: Females of *Anopheles squamifemur* were collected by horse-baited trap in the Panama Canal Zone, and oviposition obtained. The larva, pupa and egg are described and illustrated for the first time, and the male genitalia are refigured from associated specimens. Taxonomic comparisons are made with other species of the subgenus *Lophopodomyia*. Data are presented on the bionomics of *A. squamifemur*, with observations on the laboratory development of the immature stages.

Anopheles squamifemur, a sylvan mosquito of Panama, Colombia, Venezuela, Brazil and French Guiana, until now has been known only from the adult stage (Stone et al. 1959). Antunes (1937) described this species from a female captured at Vega Grande, municipio de Restrepo, Intendencia del Meta, Colombia. The description of the male was later published by Deane et al. (1949) in Brazil.

During investigations on anophelines in Panama, the 4th-instar larva, pupa and egg of *A. squamifemur* were obtained for the first time as laboratory-reared progeny of wild-caught females. Descriptions of these stages, and our laboratory observations and field collection data follow.

DESCRIPTION OF IMMATURE STAGES

FOURTH-INSTAR LARVA FIG. 1–3

Chaetotaxy as figured, based on 14 specimens (8 whole larvae and 6 exuviae). Head: 0.44 mm long, 0.40 mm wide. Anal saddle: 0.18 mm long. *Head:* Pigmentation light to moderate, collar dark. Inner clypeals (2-C) separated by approximately 1 diameter of basal tubercle, their length more than 2 × that of outer clypeals (3-C). Hairs 2–3-C simple, rather stout. Hair 4-C short and inconspicuous. Frontal hairs (5–7-C) branched as shown. Hair 11-C well-developed and plumose. *Antenna:* Lightly pigmented, shaft with prominent spicules on medial surface beyond hair 1-A, hair 1-A short, 2–4 branched. Hairs 2–3-A sharply pointed, subequal in length. Hair 4-A single. *Thorax:* Epidermis bare, unpigmented. Prothoracic submedian group (1–3-P) with only 2-P having distinct basal tubercle; 1-P with 10–11 branches; 3-P single, about 1/2 length of 1-P. Hairs 4–6-P moderately developed, reaching base of antenna. Prothoracic pleural group (9–12-P) arising from common tubercle; 9-P and 11-P branched; 10-P and 12-P single, subequal in length. Mesothoracic hair 1-M well-developed, deeply pigmented. Metathoracic hair 3-T palmate, with 10–16 smooth lanceolate leaflets. *Abdomen:* Epidermis lightly pigmented, tan to brownish. Abdominal hair 0 minute, single on segments II–VIII; hair 1-I palmate, having 9–12 lightly pigmented leaflets with shallow notches on distal margins; 1-II–VII with fully developed palmate hairs showing distinct notches on distal margins, tips sharply pointed, in some specimens an overlap of leaflets giving appearance of bifid grouping; hair 6-IV–V with 3–4 branches. Anterior mesal sclerites light brown, triangular on segments I and III, moderately developed; sclerite very small, circular on segment II; on segments IV–VII sclerites somewhat quadrangular, moderately developed. *Spiracular Lobe:* Pecten teeth as shown, with spinules evident on external edges of some teeth. Posterior spiracular plate without tail-like processes. Hair 1-S with about 6 branches. Hair 8-S double to triple. *Anal Segment:* Hair 4a-X with 3–4 branches usually shorter than anal saddle. Anal saddle with numerous fine spicules on distal 1/2 of sclerite.

PUPA FIG. 4–5

Chaetotaxy as figured, based on 6 associated exuviae. Abdomen: About 1.9 mm. Trumpet: 0.27 mm. Paddle: 0.5 mm long, 0.27 mm wide. *Cephalothorax:* Very light yellowish tan pigmentation. *Trumpet:* Light yellowish tan, shape as shown in FIG. 4–4a. *Abdomen:* Hair 0 minute, single on II–VIII; hair 9-V–VII well-developed, branched apically; hair 9-VIII strongly developed, usually 10 branches. *Paddle:* As figured, lightly pigmented, with dense fringe of filamentous spicules; bases of spicules give margins a minutely serrated appearance. Hair 1-P well developed, single; hair 2-P single.

EGG FIG. 7–7a

Measurements based on 10 specimens. Egg dorsum convex, surface with silvery (apparently air-filled) vesicles, venter slightly concave with similar but much smaller vesicles. Egg length 400–437 μ ($\bar{x}$ = 420 μ); width, including lateral floats, 175–200 μ ($\bar{x}$ = 189 μ), without floats 100–125 μ ($\bar{x}$ = 108 μ). Floats long, 325–363 μ ($\bar{x}$ = 345 μ), 0.8 of egg length; ventral separation of floats 75 μ at anterior pole and 50 μ at posterior pole; numerous transverse float ridges as shown. Black endochorion protruding from exochorion at each pole. Black oval micropyle dorsal to tip of egg at anterior pole. Exochorion generally dark gray in fresh specimens.

MALE GENITALIA

The description of the adult male of *Anopheles squamifemur* was published in Portuguese, and provided a lateral view of the whole adult plus drawings of the terminalia including the lateral aspect of phallosome leaflets (Deane et al. 1949); we give below an English translation of the section describing these structures.

Genitalia: Side piece elongate, covered with microtrichia, provided with spatula-shaped scales and fine hair, some very long distal hairs almost length of claspers. Basal spine thin,

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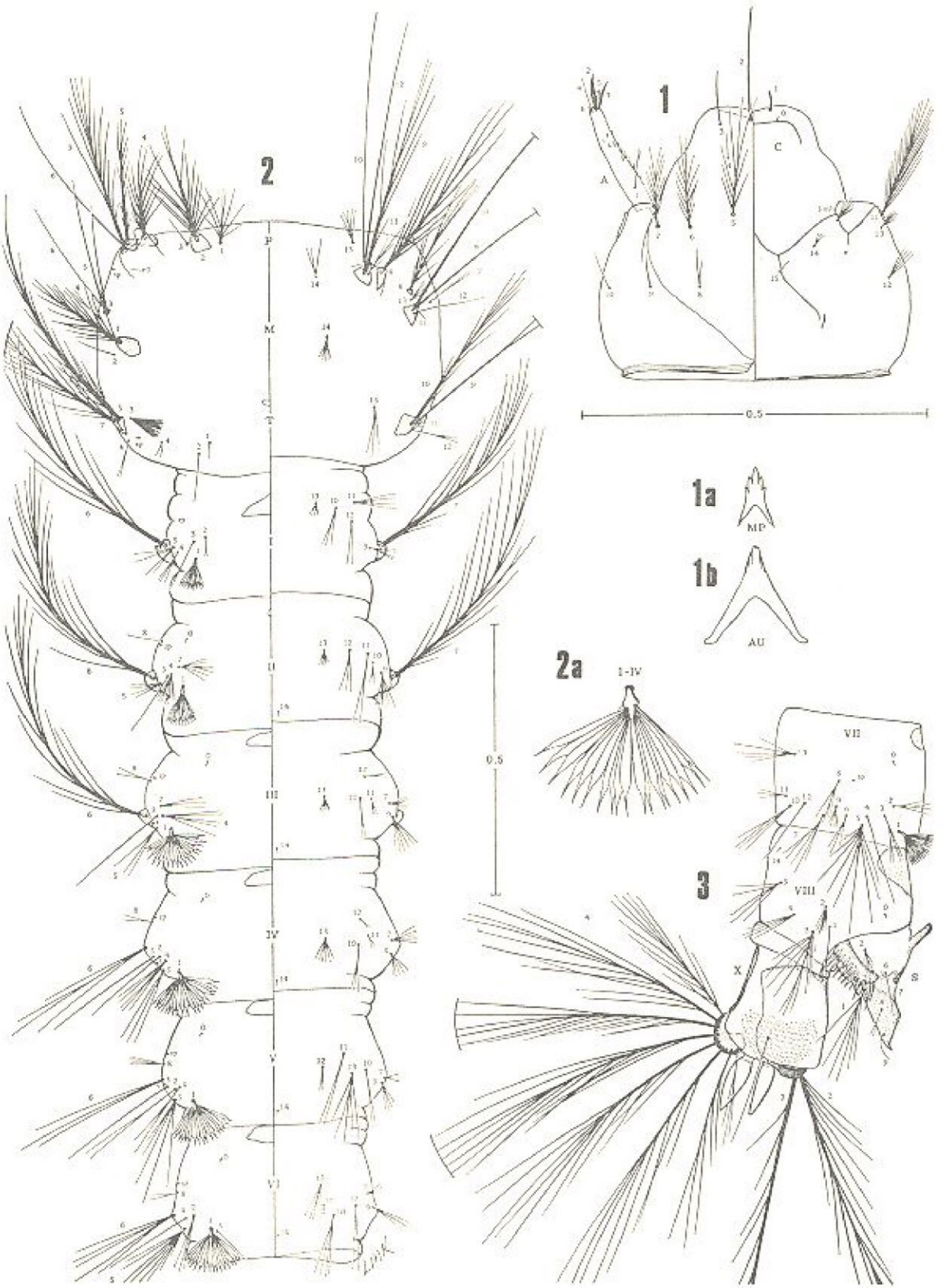
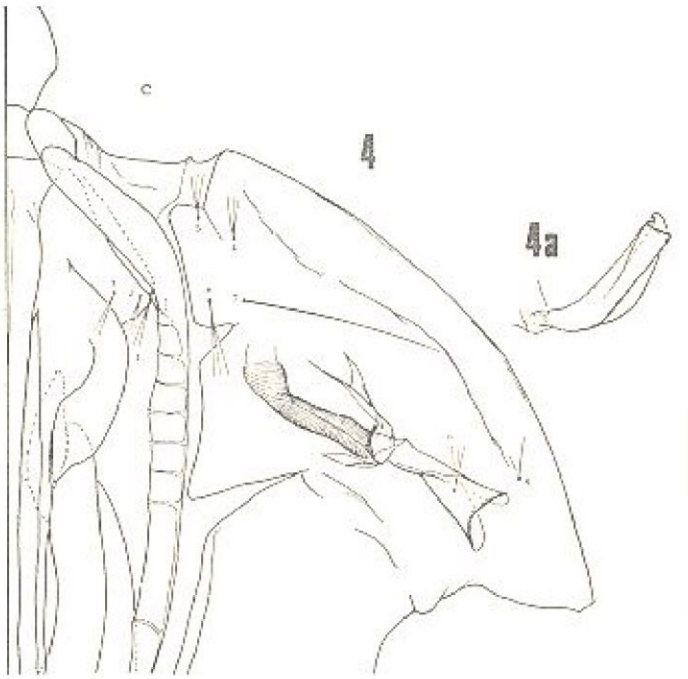
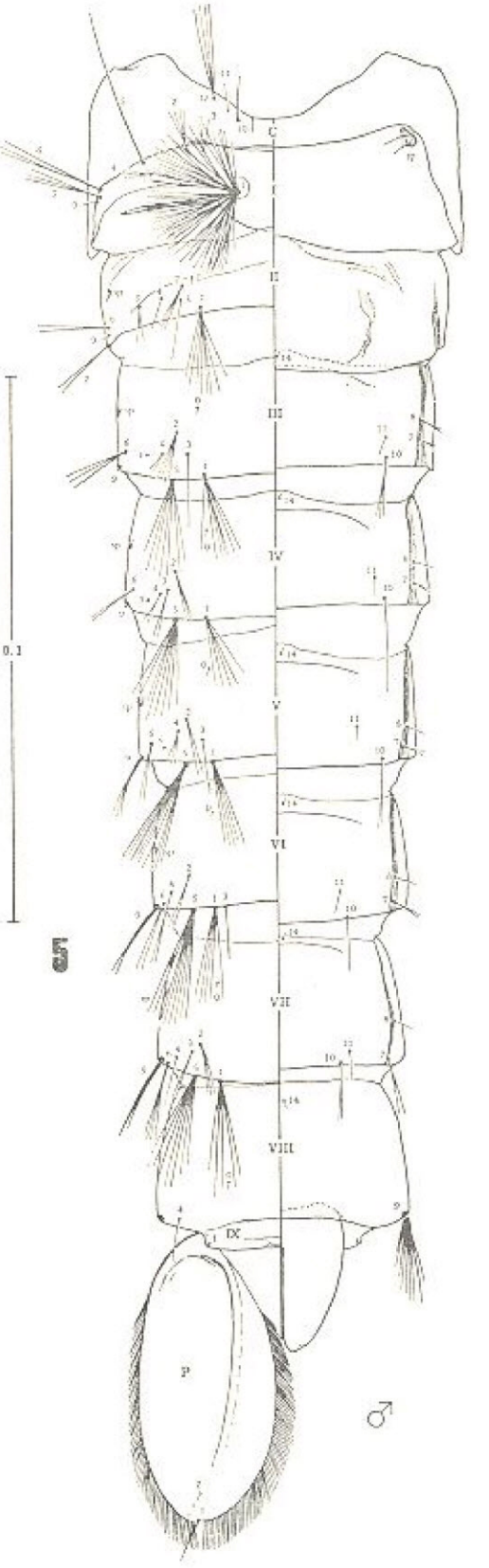


FIG. 1–3. *Anopheles squamifemur*. (1) Dorsal and ventral chaetotaxy of larval head, (1a) Mental plate, (1b) Aulaueum, (2) Dorsal and ventral thoracic and abdominal chaetotaxy of larva, (2a) Enlarged view of palmate hair I-IV, (3) Lateral abdominal chaetotaxy of segments VII and VIII of larva.





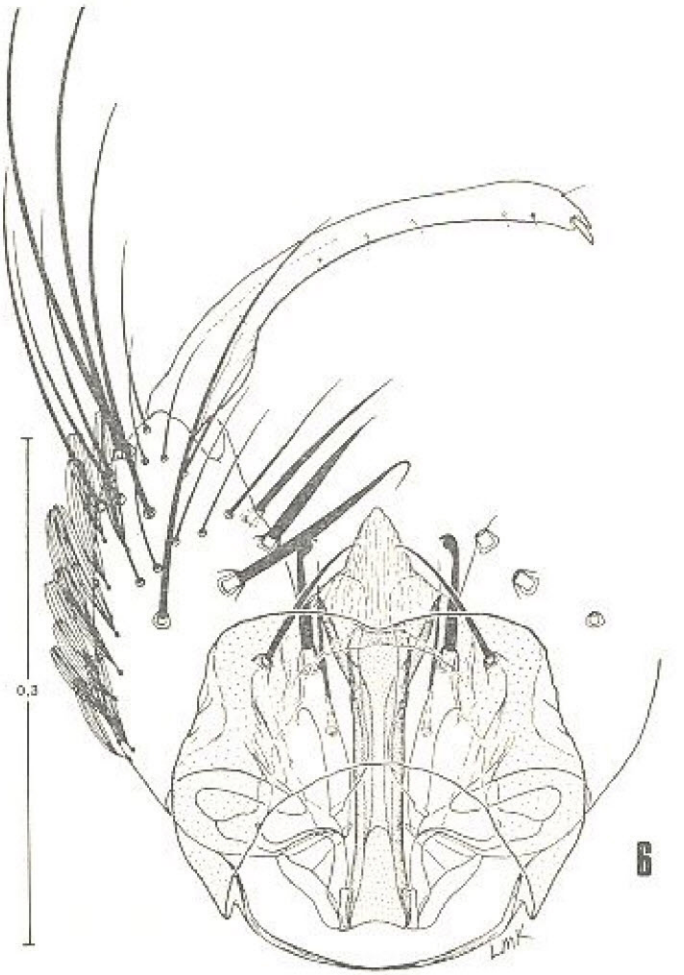


FIG. 4–6. *Anopheles squamifemur*. (4) Cephalothorax of pupa, (4a) Lateral view of trumpet, (5) Dorsal and ventral abdominal chaetotaxy of pupa, (6) ♂ genitalia from associated adult.

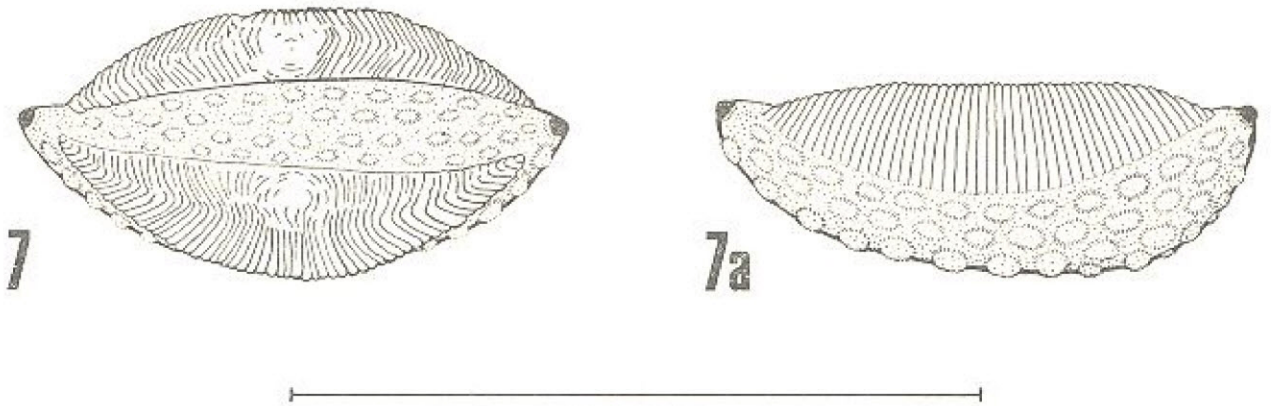


FIG. 7–7a. *Anopheles squamifemur*. (7) Oblique view of egg. (7a) Lateral view of egg.

sharp, weakly curved, implanted on small, lightly chitinized tubercle located medially on side piece. Two clearly differentiated parabasal spines, strong, pointed, implanted on conspicuous, well-chitinized, slightly raised tubercles located medially on side piece, one away from other; external one long, markedly recurved apically, while internal spine smaller and straight. Internal spine anterior to parabasal spines intersects distal 1/3 of side piece, thin and straight to barely curved, slightly larger than basal spine. *Claspers* somewhat longer than side piece, narrow medially, with short, thick spiniform and minute subapical hairs. *Ninth tergite* bare. *Phallosome* tubular, curved, moderately chitinized with membranous convex apex; 2 chitinized leaflets apically on each side, internal leaflet smaller, more transparent than external leaflet, frequently hidden by latter. *Ventral lobes of claspettes* conical, tip slightly curved inward; near base of ventral lobes a narrow conical formation ending in long, thin, transparent spine almost parallel to apical leaflet. *Dorsal lobes of claspettes* fused, transparent, with medial excavation, microtrichia principally on medial portion. *Proctiger* conical, transparent, membranous with fine hairs at base, glabrous for remainder and wrinkled at apex.

FIG. 6 illustrates the male genitalia from our associated reared specimens, additionally showing the long accessory spine of the side piece and spatula-tipped spine of the claspette on the dorsal lobe. Comparison of the terminalia of these males from Panama with the above description shows close agreement in all significant characteristics.

TAXONOMIC DISCUSSION

After Antunes established the monotypic subgenus *Lophopodomyia* in 1937 with *squamifemur* as the type, Galvão (1941) described the subgenus *Arthuromyia* with *Myzorynchella gilesi* Peryassú, 1908 taken as its type-species. As summarized by Deane et al. (1949), the following species later were placed under *Arthuromyia*: *pseudotibiamaculatus* Galvão & Barretto, 1941; *vargasi* Gabaldón, Cova-García & López, 1941; and *oiketorakras* Osorno-Mesa, 1947. With their description of the male of *squamifemur*, Deane et al. (1949) synonomized *Arthuromyia* under *Lophopodomyia* based on characters of the male terminalia. A 6th species, *gomezdelatorrei* Levi-Castillo, 1955, has been described under the subgenus *Lophopodomyia*. For further taxonomic review, see Lane (1953) and Stone et al. (1959).

Although we have not seen larval material of other members of the subgenus *Lophopodomyia*, characteristics from published descriptions and keys were compared and showed that most 4th instars of these species could be readily distinguished. *Anopheles gomezdelatorrei* and *oiketorakras* both have inner and outer clypeal hairs of nearly equal length as compared to the other 4 members of the subgenus, which have much shorter outer clypeals. Of these 2 species, *oiketorakras* has branched clypeal hairs and is the only member to have smooth margins on its palmate tuft leaflets, while *gomezdelatorrei* has leaflets with serrate margins and simple clypeals. Among the other 4 species, only *gilesi* possesses long, pointed filamentous tips on its palmate tuft leaflets, while *vargasi* is unique in having bifid outer clypeal hairs. Both *squamifemur* and *pseudotibiamaculatus* have simple clypeal hairs and serrate palmate tuft leaflets. Although the original description of the larva of *pseudotibiamaculatus* was not available to us, it is noted in Lane (1953) that the intersegmental plates (anterior mesal sclerites) on segments II and III were described as being smaller than those on the other segments. In *squamifemur*, only the sclerite on the 2nd abdominal segment is decidedly smaller than the rest. Other than this, no adequate characteristics for the separation of the 2 species could be found.

Using the keys by Stojanovich et al. (1966) and Gorham et al. (1973), the 4th-instar larva of *A. squamifemur* would be identified as *A.* (*Anopheles*) *eiseni*. However, *squamifemur* can be easily differentiated by the presence of a palmate tuft (hair 1) on the 1st abdominal segment in addition to its

smaller size. The average length of the 4th instar of *squamifemur* is 3.3 mm compared to 4.5 mm for *eiseni*. Furthermore, the anterior mesal sclerite on the 2nd abdominal segment of *squamifemur* is small and circular, while this sclerite is well-developed and triangular in *eiseni*.

LABORATORY REARING

Adult *A. squamifemur* were collected during the period from July through September 1972 in a horse-baited trap operated at Mojinga Swamp on the Caribbean side of the Isthmus of Panama. This locality is a fresh-water habitat which lies on the margin of dense tropical rainforest, and is adjacent to a coastal brackish water mangrove swamp. Upon capture, blood-engorged females were held in 200-ml bottles lined with plaster of Paris, or were transferred to screened 0.5-liter (pint) cartons. These individuals were wholly gravid by the 4th day after feeding. Although at times eggs were deposited voluntarily on moistened surfaces of the containers, oviposition was best when induced by dealation and placement of the mosquito on water. As many as 132 eggs were obtained from a single gravid female. After oviposition, none would attempt to take blood from a human source.

At room temperature (23 °C), eggs required about 4 days to complete embryonic development, with a hatch success rate of 50–95%. Hatching was stimulated by water agitation, but also occurred spontaneously. It was noted that some eggs stranded several mm above the water surface on moist filter paper readily hatched, and the larvae, with caterpillar-like locomotion, moved down into the water. Larvae were reared in aged tap water or stream water from Mojinga Swamp. Both enameled pans and glass bowls were used, and small clumps of filamentous green algae and aquatic grass plants were added to provide feeding surfaces. To help reduce surface pellicle formation, early instar larvae of *A. albimanus* were added on an ad hoc basis. These larvae were removed upon molting to the 3rd instar as they would then be large enough to consume the very small, slow-developing *squamifemur* immatures. Small amounts of a specially prepared anopheline larval food were given daily (Baerg 1971); water was changed as needed. Constant incandescent light was maintained throughout larval development via a 60 W lamp positioned 0.3 m (1 ft) from the water surface, with a partial cover provided to produce some shade.

Mortality of *A. squamifemur* larvae was especially high (50–75%) prior to molt to the 3rd instar, whereupon almost all survivors lived to pupate and emerge as adults. The developmental periods for the larvae ranged as follows: 1st instar, 5–8 days; 2nd instar, 3–5 days; 3rd instar, 3–11 days; 4th instar, 7–12 days. Eclosion of adults occurred 2 days after pupation. Among the broods reared to adults, 23–36 days were required for completion of the aquatic cycle (egg deposition to emergence).

BIONOMICS

Other species of *Lophopodomyia* are known to breed in shaded side pools of streams rich in organic matter. After determining the identity of the aquatic stages of *A. squamifemur* through associated rearings, a search for them centered on the fresh-water streams and pools within Mojinga Swamp. To this date, immatures have not been found in the field.

For the calendar year 1972, 98 blood-engorged female *A. squamifemur* were captured in the Mojinga Swamp horse-baited trap station. Sixty of these were taken from May through August, with a high of 33 in June. Twenty-six of the total were collected during the peak of the rainy season in October and November. In Panama, adults of *A. squamifemur* have been taken previously in light traps at the Pequeni River (Galindo et al. 1949), and at Mojinga Swamp and Bocas del Toro (Blanton & Peyton 1956). Belkin et al. (1965) stated that this species has been captured at the borders of forests (1830–1930 hr) using animal bait. Galindo (pers. commun.) collected *squamifemur* from human bait and has observed that it is widespread and locally common in the Atlantic coastal forests of the Isthmus.

Nothing is known of the importance of *A. squamifemur* as a disease vector with the exception that it is known to bite man and horses.

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