

## THE EGGS OF THE NYSSORHYNCHUS GROUP OF ANOPHELES (CULCIDAE) IN PANAMÁ

By

LLOYD E. ROZEBOOM<sup>1</sup>

(Received for publication July 29, 1937)

In Panamá there are seven species of *Anopheles* that belong to the *Nyssorhynchus* group of the subgenus *Nyssorhynchus*, excepting the rare *A. anomalophyllus* recently described by Komp (1936). *Anopheles albitarsis* Lynch Arribálzaga and *A. argyritarsis* Robineau-Desvoidy belong to the *argyritarsis* series, and are separated from the remaining five by the absence of a black ring on the fifth hind tarsal joint, which is present in the *tarsimaculatus* series. *A. albitarsis* females can be readily distinguished from *argyritarsis* by the double row of white scales on the first abdominal sternite (Shannon and Davis, 1930), but it is often difficult, and sometimes impossible, to be sure of one's identifications of females of the *tarsimaculatus* series, as there is a great deal of variation in the size of the markings of the wings, legs, and palpi. The members of the *tarsimaculatus* series in Panamá are *A. albimanus* Wiedmann, *strodei* Root, *bachmanni* Petrocchi, *oswaldoi* Peryassú, and *tarsimaculatus* Goeldi. *A. albimanus* has been considered to be the most dangerous malaria vector in this region ever since Darling's (1910) work, but in recent years the question has been raised as to what part the other anophelines play in the transmission of the disease. Final proof that a mosquito is dangerous or harmless must come through extensive observations on the habits of the adults in nature, and through dissections of "wild" females. But no conclusions can be drawn from results obtained in dissections of females caught in nature when the identification of these females is uncertain. Recognition of such females would be greatly facilitated if it could be shown that the different species deposited eggs readily distinguishable from one another. This is one reason for studying the eggs of the *Nyssorhynchus* group of Panamá. A second reason is that comparison of mosquito eggs from different regions might throw some

<sup>1</sup> Medical Entomologist, Gorgas Memorial Laboratory, Panamá, R. de P.

light on the question of geographical races among the New World anophelines.

#### MATERIALS AND METHODS

The eggs used in this investigation were obtained in three ways. The preferred method was to collect them from the natural breeding places. When larvae are scooped up in a white enameled pan the eggs can be seen with ease on the surface of the water or sticking to the sides of the container, and may be transferred to a vial containing a bit of moist filter paper by means of a small brush. After examination, enough larvae were bred from the eggs to make the identification certain, although this was hardly necessary in the case of *A. strodei* and *argyritarsis*, which breed in restricted localities during the early part of the dry season. *A. bachmanni* is usually the only local anopheline found in patches of *Pistia stratiotes*, and the eggs of this mosquito were collected from these plants. Most of the *A. albimanus* eggs studied were from the writer's laboratory colony, although enough were examined from various natural breeding places to make sure that there was no difference between the eggs of the colony and those in nature. *A. tarsimaculatus* ova were found in a brackish-water ditch near Fort Davis, Canal Zone, while those of *A. albitarsis* were abundant in patches of *Chara*, *Naias*, and *Utricularia* in the Rio Pescado inlet, Gatun Lake; some of *A. albimanus* and *bachmanni* were also found there.

A second method was to rear adult females from larvae, give them blood, and allow them to deposit infertile ova. This procedure was usually unsuccessful, as the infertile females were reluctant to oviposit, but eggs of *albitarsis* and *tarsimaculatus* were obtained in this way.

Finally, fertile *A. oswaldoi* eggs were obtained from wild adult females caught in the Mojinga Swamp, near the mouth of the Chagres River.

For examination the eggs were stranded on a strip of moist filter paper, which was placed on a glass slide. They were illuminated by either reflected or transmitted light, or both, and studied with both dissecting and compound microscopes. In making float ridge counts the writer preferred to place a coverslip over the eggs and use the high, dry magnification of the compound microscope, with both transmitted and reflected light.

*Structure of Nyssorhynchus eggs*

The terminology used in this paper is the same as that employed by Christophers and Barraud (1931). *Nyssorhynchus* eggs are boat-shaped, with the upper surface flattened or concave, the ventral surface convex, and with the anterior end more blunt than the posterior end. The shell, or *endochorion*, is black in color and without a pattern. Fitting closely to the endochorion on the ventral surface of the egg is an outer, thin, semi-transparent membrane, the *exochorion*. All of the eggs of the *tarsimaculatus* series in Panamá possess rows of silvery spots on the ventral surface, which Root (1926) called "little white stars"; they are formed by small elevations in the exochorion. In *A. albitarsis* and *A. argyritarsis* the exochorion fits evenly over the endochorion, so that the ventral surface has no pattern, but is of an even black color. For making identifications, the most important structures are the *frill* and the *floats*, which are modifications of the ventral exochorion (see Townsend, 1933<sup>2</sup>); the frill is an extension of the floats, bordering the dorsal surface on the anterior and usually the posterior ends of the egg. The floats are composed of a number of *float ridges*, and it is often difficult to decide where the small subdivisions of the end float ridges stop and the wrinkled frill begins. In most species the floats are placed laterally, in which case the dorsal surface is broad and the frill forms a horseshoe-shaped border at the ends of the egg; in some species the floats cover all of the dorsal surface except for a small, spindle-shaped area centrally, and oblong areas at the ends of the egg. In such cases the small float ridges of each side often meet and fuse on the posterior end, entirely eliminating the small bit of exposed endochorion here, and the frill as well. Such eggs resemble Root's figure of *A. tarsimaculatus* eggs. When the end float ridges do not meet, there is a short frill connecting them and bordering the small exposed area of dorsal surface. Christophers and Barraud separated *Nyssorhynchus* eggs from those of the subgenera *Anopheles* (except *A. pseudopunctipennis*) and *Myzomyia* by the presence of the terminal frill described by Root. The peculiar, crown-like structure pictured by Root for Brazilian *albitarsis* and *darlingi* was never observed among the eggs of Panamanian *Nyssorhynchus* species, and this character cannot be used to separate the

<sup>2</sup> Townsend considers that the dorsal surface is the underside of the egg as it is found in the water. Thus the exochorion would cover the dorsal rather than the ventral surface. Root and Christophers and Barraud called the upper surface of the floating egg the dorsal surface; in the present paper also, the upper surface of the floating egg is referred to as the dorsal surface.

eggs of the subgenus *Nyssorhynchus* from those of other subgenera. Although the rows of white spots are not a characteristic pattern of the eggs of all species of the subgenus *Nyssorhynchus*, they are distinctive for the species belonging to the *tarsimaculatus* series, while the eggs of *A. argyritarsis* and *albitarsis* of Panamá can be distinguished from those of the subgenus *Anopheles* by the structure of the frill and floats, and especially by the complete absence of any ventral pattern.

*Description of the eggs*

The measurements, in microns, of the lengths and widths of the eggs, the float lengths, and the greatest widths of the exposed dorsal

TABLE 1  
*Measurements of eggs in microns; float ridge counts*

		<i>A. albitarsis</i>	<i>A. argyritarsis</i>	<i>A. albitarsis</i>	<i>A. tarsimaculatus</i>	<i>A. oswaldoi</i>	<i>A. bachmanni</i>	<i>A. strodei</i>		
								Type A	Type B	Type C
Egg length	N *	126	115	151	70	93	104	30	83	29
	M †	483	498	482	424	429	425	458	451	454
	$\sigma$ ‡	17	16	24	14	14	16	15	18	15
Float length	N	132	137	144	69	93	123	36	95	36
	M	266	286	371	306	330	354	230	271	261
	$\sigma$	19	17	22	13	18	14	22	16	15
Width (including floats)	N	83	81	95	35	52	120	28	70	27
	M	192	210	211	202	180	177	144	178	167
	$\sigma$	14	11	13	13	14	12	14	12	15
Width of exposed dorsal surface	N	51	79	77	35	54	117	24	73	23
	M	92	82	63	53	22	23	21	50	33
Number of float ridges	N	236	189	200	81	169	233	61	146	52
	M	19	20	25	26	34	28	18	20	20
	$\sigma$	1.3	1.4	2.2	1.9	2.1	2.3	1.9	2.1	1.7

\* N = Number of measurements or float ridge counts.

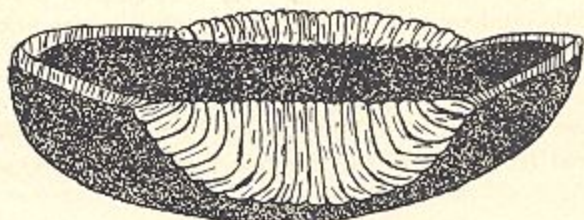
† M = Mean.

‡  $\sigma$  = Standard deviation.

surface, as well as the float ridge counts, are given in table 1 in terms of the mean and standard deviation. The measurements were made with the low power of a calibrated microscope to the nearest unit on

the ocular micrometer. As the width of the exposed dorsal surface was usually covered by two to four ocular units, the standard deviation of these measurements was not determined.

*A. argyritarsis.* No pattern on either dorsal or ventral surfaces; dorsal surface concave. The floats are a little more than half the

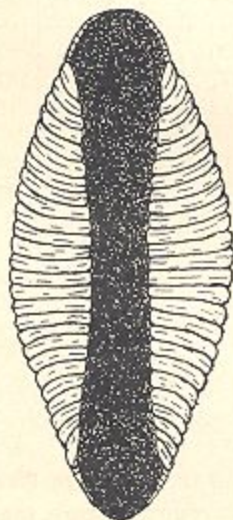


*A. argyritarsis.*

length of the egg. They are placed laterally and their dorsal inner edges are parallel and widely separated from one another. The frill is a broad band extending from the ends of the floats, forming a horse-shoe-shaped border to the dorsal surface on both ends of the egg.

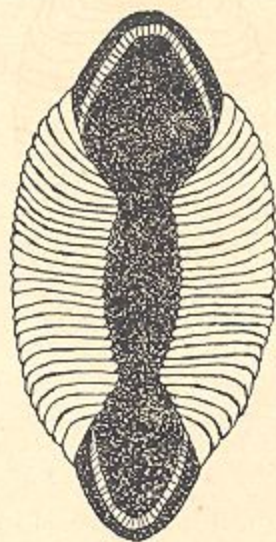
*A. albitarsis.* A description of the eggs of this species has already been published (Rozeboom, 1937). They are indistinguishable from those of *A. argyritarsis*.

*A. albimanus.* The exochorion covering the ventral surface with rows of small silvery spots; the exposed dorsal endochorion of an even



*A. albimanus.*

black color; dorsal surface flattened. The floats are large, about four-fifths the length of the egg, and are placed laterally. The inner dorsal edges of the floats are well separated from one another, and often gradually approach one another at the center of the egg, so that the lateral borders of the exposed dorsal surface are slightly concave. The frill is a very narrow band extending from the end float ridges, bordering the dorsal surface, except at the ends of the egg, where it dips below the rounded tips, so that when viewed dorsally a small portion of the frill cannot be seen.



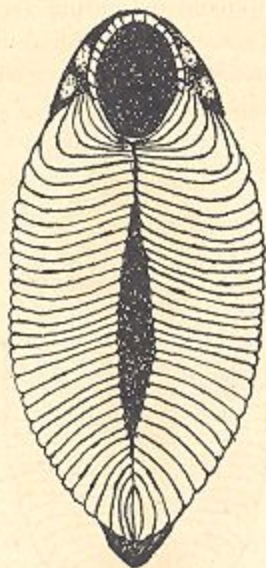
*A. tarsimaculatus.*

*A. tarsimaculatus.* Ventral exochorion with rows of silvery spots; the exposed dorsal surface of an even black color. The dorsal surface is strongly concave. The floats are large, approximately three-fourths the length of the egg, and placed laterally. The margins of the floats may cover a considerable portion of the dorsal surface, and may be quite irregular. The frill is a narrow band extending from the end float ridges, bordering the dorsal surface.

When first seen these eggs may be confused with those of *A. albimanus*, but can be distinguished from them by the following characteristics: usually *tarsimaculatus* eggs are shorter and relatively wider than the eggs of *albimanus*; the dorsal surface is concave instead of flattened; the dorsal inner margins of the floats are irregular instead of straight or slightly convex; the frill, instead of curving below the

tips of the egg, remains on the upper surface, so that when viewed dorsally it can be seen for its entire length.

*A. oswaldoi*. Ventral surface with pattern of white spots; exposed areas of dorsal surface evenly black; dorsal surface concave. The



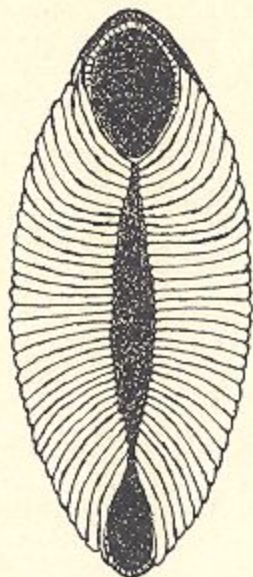
*A. oswaldoi*.

floats are large, covering most of the dorsal surface except for an oblong area at the anterior end and usually a narrow, spindle-shaped strip along the mid-dorsal line. Posteriorly, the end float ridges of one side are fused with those of the other side, eliminating the exposed area, and often the floats meet along the mid-dorsal line for their entire length, thus covering all of the dorsal surface but the anterior portion. The frill is absent posteriorly; anteriorly it borders the small exposed portion of endochorion.

*A. bachmanni*. Ventral surface with pattern of silvery spots; exposed areas of dorsal surface evenly black; dorsal surface concave. The floats cover most of the dorsal surface except for a narrow spindle-shaped strip centrally, an oblong area anteriorly, and a smaller area posteriorly. In about a third of the eggs examined this posterior area was covered with small float ridges that were so irregular that counting them was difficult. When the end float ridges of one side are sepa-

rated from those on the other side, they are connected by a short strip of frill. On the anterior end the frill borders the exposed dorsal surface.

These eggs are almost identical with *oswaldoi* ova, but as a rule they have a few less float ridges, and the majority have a small posterior area of exposed endochorion, bordered by a short strip of frill.



*A. bachmanni.*

*A. strodei.* With the pattern of silvery spots on those parts of the egg covered with exochorion.

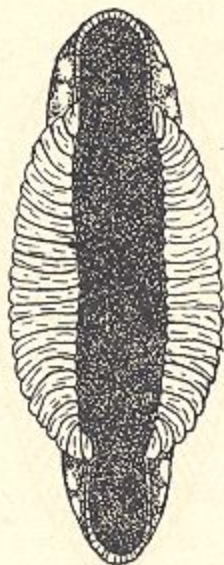
The eggs of *A. strodei* vary a great deal in their structure and, for convenience, were divided into three types. In one extreme, called *type A*, the floats are narrow, small, about one-half the length of the egg, and rest on the dorsal surface. The end float ridges of the two sides meet and fuse both anteriorly and posteriorly; centrally there is only a small spindle-shaped strip of exposed dorsal surface. The floats are separated from the frill, which forms a circle on either end of the egg, enclosing a small portion of exposed endochorion. In some eggs the frill and the floats may approach one another, but do not fuse. The ventral exochorion fills in the space between the floats and the frill; thus the ventral, lateral, and dorsal surfaces all



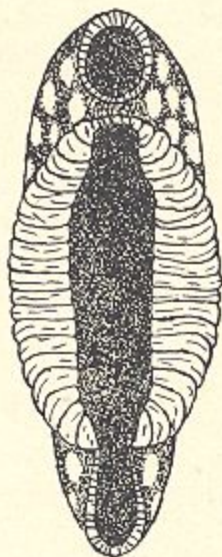
possess the pattern of silvery spots. In the other extreme, *type B*, the floats are larger and bulge past the sides of the egg. Their dorsal inner edges are widely separated from one another, exposing a broad area of endochorion. The small end ridges on one side are widely separated from those on the other side, and are fused with the frill, which forms a horseshoe-shaped border to the dorsal surface. Between



*Type A.*



*Type B.*



*Type C.*

*A. strodei.*

these extremes are all sorts of gradations, which have been lumped into *type C*. The floats and frill may be closed at one end, but open and fused with one another at the other end; the frill may be closed, but the end float ridges of the two sides separated; or the floats and frill may be barely fused with one another. At times, although the end float ridges do not meet, they may be joined by a row of very small corrugations resembling minute float ridges. These were not included in the float ridge counts.

## KEY TO SPECIES

1. Eggs with an even black color on both dorsal and ventral surfaces; no ventral pattern . . . . . *A. albitarsis*.  
*A. argyritarsis*.  
Eggs with a pattern of rows of small silvery spots on the ventral surface . . . . . 2.
2. Floats large, covering almost all of the dorsal surface . . . . . 3.  
Floats smaller, placed laterally, usually covering only a small portion of the dorsal surface . . . . . 4.  
Floats narrow and small, placed dorsally, and separated from the frill, which forms a small circle at each tip of the egg . . . . . *A. strodei*, type A.
3. With approximately 28 float ridges; often with a small bit of posterior frill . . . . . *A. bachmanni*.  
With approximately 34 float ridges; without frill on posterior end of egg . . . . . *A. oswaldoi*.
4. Narrower eggs, with smaller floats; approximately 20 float ridges . . . . . 5.  
Broader eggs, with larger floats; approximately 25 float ridges . . . . . 6.
5. Small end float ridges of one side widely separated from those on the opposite side; the frill fused with the ends of the floats and forming a horseshoe-shaped border to the exposed dorsal endochorion at the ends of the egg . . . . . *A. strodei*, type B.  
Intermediate between type A and type B eggs of *A. strodei* . . . . . *A. strodei*, type C.
6. Dorsal surface flattened. Dorsal inner margins of the floats even and parallel or slightly convex. The narrow, horseshoe-shaped frill extending from the ends of the floats and curving below the tips of the egg. Longer eggs . . . . . *A. albimanus*.  
Dorsal surface concave. Dorsal inner margins of the floats irregular. The horseshoe-shaped frill extending from the ends of the floats, and lying for its entire length on the upper surface of the egg. Shorter and relatively broader eggs . . . . . *A. tarsinaculatus*.

## DISCUSSION

The eggs of *A. albitarsis* and *A. argyritarsis* cannot be separated from one another on morphological characters, but so far as identification is concerned this is of no importance, as the adult females can be distinguished from all the other *Nyssorhynchus* species of Panamá by the hind tarsal markings, and from one another by the double row of white scales on the first abdominal sternite of *A. albitarsis*. Furthermore, in Panamá these two species breed in different locations, and it can be assumed that all eggs with *albitarsis* or *argyritarsis* structure collected in patches of *Chara*, *Naias*, or *Utricularia* in Gatun Lake belong to the former species, while those found in sunny or partially shaded ground pools belong to the latter species.

The key given above is not entirely satisfactory, as it is difficult to

stress any character aside from general appearance to distinguish *type B* and *C strodei* eggs from *albimanus* and *tarsimaculatus* eggs, while *bachmanni* and *oswaldoi* ova are almost identical with one another. However, examination of the ova will be of value in making specific identifications if the location in which the eggs or pregnant females were found is taken into consideration, especially if these observations can be supplemented by adult characters.

Root (1926) decided that the Brazilian mosquito called *Cellia oswaldoi* Peryassú really was *A. tarsimaculatus*, and that there was "no justification for the retention of *oswaldoi* even as a varietal name." In 1932 Curry reported the presence in Panamá of a mosquito which was closer to *A. oswaldoi* than it was to the Isthmian form of *A. tarsimaculatus*; he named this new, fresh-water-breeding mosquito *A. tarsimaculatus* var. *aquacaelestis*, and the old brackish-water breeder *A. tarsimaculatus* var. *aquasalis*. Since then malariologists and entomologists in Panamá have considered these mosquitoes to be different species: *A. oswaldoi* and *A. tarsimaculatus* respectively. By a comparison of Root's figure of an *A. tarsimaculatus* egg with the eggs of *A. tarsimaculatus* and *A. oswaldoi* of Panamá, and by the small amount of black on the second hind tarsal joint of the female, it can be concluded that Root's Brazilian fresh-water-breeding *A. tarsimaculatus* is much closer to the Isthmian *A. oswaldoi* than it is to the local *A. tarsimaculatus*. Townsend (1934) stated that Root's drawing of an *A. tarsimaculatus* egg must have been made from a race of *A. oswaldoi*, but gives an entirely different description of *oswaldoi* eggs from the Rio Tapajós. The eggs described by Townsend appear to be quite similar to either *tarsimaculatus* or *albimanus* eggs of Panamá, as "the smooth chorion is exposed in a wide even median space on ventral surface between the floats; the exposure thence widening toward each extremity of the egg." Before the true relationship between the various *Nyssorhynchus* species and races can be understood, as Townsend said, these variations in egg structure need clarification. The differences in the eggs of Isthmian *oswaldoi* and *tarsimaculatus* also demonstrate that workers in Panamá are justified in considering these mosquitoes to be distinct species, or at least separate races.

The writer (1937) has recently suggested that *A. albitarsis* of Panamá may belong to a race different from that of this species in Brazil, because of dissimilarities in the habits of adults and in the morphology of the eggs from the two regions. Since Matheson and Hurlbut (1937) have shown that there are marked seasonal variations in the

eggs deposited by *A. walkeri*, it should be stated that no seasonal variations have been noted in Isthmian *albitalarsis* ova.

Matheson's and Hurlbut's observations, as well as the work of Gibbins (1933) with *A. marshalli*, demonstrate that variations in egg structure do not necessarily mean the presence of separate races. The eggs of *A. strodei* also emphasize that care must be exercised in establishing Anopheline races without proper study of the habits and physiology of the mosquitoes. It seems unlikely that the different types of *strodei* eggs were deposited by separate races, as they were all collected from the same breeding place at the same time, and there were all kinds of gradations between the extremes.

#### SUMMARY

1. Descriptions are given of the eggs of the *Nyssorhynchus* group of *Anopheles* in Panamá. In most cases specific identification can be made by egg structure, and in those cases where the eggs of two species are similar, identification can be made when the location in which the eggs or adults were collected is taken into consideration.

2. Comparison of Root's figure of *A. tarsimaculatus* eggs of Brazil with Isthmian *A. tarsimaculatus* and *A. oswaldoi* eggs shows that Root's mosquito is more closely related to the *A. oswaldoi* than to the *A. tarsimaculatus* of Panamá. The differences in the eggs of these two mosquitoes lend support to the belief that *A. oswaldoi* and *A. tarsimaculatus* are separate species.

3. No seasonal variation has been observed in the eggs of *A. albitalarsis* in Panamá.

4. The presence of three types of *A. strodei* eggs in Panamá indicates that egg structure alone is not a dependable criterion of the existence of distinct races among the species in the *Nyssorhynchus* group.

#### BIBLIOGRAPHY

- CHRISTOPHERS, S. R., AND BARRAUD, P. J.  
1931. The eggs of Indian Anopheles, with descriptions of the hitherto undescribed eggs of a number of species. *Rec. Mal. Surv. India*, 2, 1, 161-192.
- CURRY, D. P.  
1932. Some observations on the *Nyssorhynchus* group of the *Anopheles* (Culicidae) of Panamá. *Amer. Jour. Hyg.*, 15, 566-572.
- DARLING, SAMUEL T.  
1910. Studies in relation to malaria. I. C. C. Rept., U. S. Govt. Printer, Washington, D. C.

GIBBINS, E. G.

1933. Eggs of some Ethiopian *Anopheles* mosquitoes. Bull. Ent. Res., 24, 257-262.

KOMP, W. H. W.

1936. *Anopheles (Nyssorhynchus) anomalophyllus*, a new species of *Anopheles* from Panamá and Costa Rica (Dipt., Culicidae). Proc. Ent. Soc. Wash., 38, 160-164.

MATHESON, R., AND HURLBUT, H. S.

1937. Notes on *Anopheles walkeri* Theobald. Amer. Jour. Trop. Med., 17, 237-243.

ROOT, F. M.

1926. Studies on Brazilian mosquitoes. I. The Anophelines of the *Nyssorhynchus* group. Amer. Jour. Hyg., 6, 684-717.

ROZEBOOM, L. E.

1937. On *anopheles albitarsis* Lynch-Arribalzága in Panamá. South. Med. Jour. (in press).

SHANNON, R. C., AND DAVIS, N. C.

1930. Observations on the Anophelini (Culicidae) of Bahia, Brazil. Ann. Ent. Soc. Amer., 23, 467-505.

TOWNSEND, CHARLES H. T.

1933. On *Nyssorhynchus tarsimaculatus* Goeldi and the races of *Nyssorhynchus*. Rev. de Entomol., 3, 7-12.
1934. Mosquitoes of the Rio Tapajós. Ibid., 4, 486-499.