

## A dual genetical and taxonomic approach to the resolution of the mosquito taxon, *Anopheles (Cellia) marshallii* (Culicidae)

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**ABSTRACT.** Two largely independent studies, one cytogenetic and another a detailed morphological analysis, have both revealed the existence of four biologically distinct species within populations of *Anopheles marshallii* (Theobald) from South Africa and Zimbabwe. The results of discriminant function analysis using chromosomally identified individuals enabled accurate identification of pinned specimens. Classification of type-specimens using this technique indicates that two of the newly discovered species are undescribed. These new species, *Anopheles letabensis* and *Anopheles hughi*, are described and a redescription of *Anopheles marshallii* is presented.

### Introduction

This work represents the results of a genetical and a morphological study of populations of the African mosquito *Anopheles marshallii* (Theobald). The genetical study examined the polytene chromosomes from ovaries of adult females. The morphological study involved analysis of a correlated range of adults, larvae, pupae and eggs. These two approaches have resulted in identical conclusions and are hence presented here together.

### Taxonomic history

*Anopheles marshallii* was described from Salisbury, Mashonaland, Rhodesia, by Theobald (1903) and placed in the genus *Pyretophorus*. It was described from a single female. Since then a large number of species have been described originally as varieties of *marshallii* but are now considered by most authors to be distinct. Examples of these are *Anopheles gibbinsi*, *hargreavesi*, *mousinhoi*,

*freetownensis* and *kiniensis*. At present there are three synonyms of *A. marshallii*: *A. pitchfordi* Giles, *transvaalensis* Carter and *pseudocostalis* Theobald. Theobald later (1910) seemed uncertain whether *marshallii* should even be considered a distinct species, since he quoted Newstead's remark on six females of *marshallii* he received from the 'Congo Free State':

'They were all associated with *P. costalis* (a synonym of *A. gambiae* s.str.) but may readily be distinguished from the latter by the characteristic banding of the palpi. It is important to notice, however, that among the long series of *P. costalis* there are many intervening forms between typical examples of the two species'.

The taxon *Anopheles marshallii* is common and widespread over East and Southern Africa, being recorded from the Ethiopian highlands through Kenya and Tanzania, and from south-western Sudan through the eastern highlands of the Congo and Katanga to Angola, the Transvaal and Natal. The situation with respect to the West African 'form' of *marshallii* is very unclear. Gillies & de Meillon (1968) reported that specimens in the British Museum

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from Sierra Leone show atypical pale spots on the hind tibia with a variable degree of overlap onto the bases of succeeding segments and are therefore unlike 'typical *marshallii*'.

Adult females of *A. marshallii* are generally considered to be exophilic and zoophilic and the species' occasional presence in houses is presumed to be associated with the presence of animals besides man. However, Bruce-Chwatt & Gockel (1960) showed that 29.5% of individuals collected over a large geographical range were positive for human blood. Larvae of *A. marshallii* are typically found in streams and swampy areas in relatively clear water and generally in areas where there is much vegetation. Larvae are never found in stagnant pools or in heavily forested areas (Gillies & de Meillon, 1968).

Both adult and larval stages show considerable morphological variation. Adult females show great variation in the amount of

pale scaling on the wings. The third and fourth main dark areas of the first vein vary from being much longer to shorter than the adjoining areas. The third vein shows considerable variation in the lengths of the two dark areas and the connecting pale area. There is also great variation in the width of the dark apical palpal band in comparison to the pale bands on either side. Larvae show variation in palmate hairs and the branching of the posterior and outer clypeal hairs.

*Pyretophorus pitchfordi* was described by Giles (1904) who did not mention how many specimens he examined. The British Museum (Natural History) has, at present, a single type-specimen which fits the original description, and corresponds with respect to collecting locality, etc. The type locality is given as 80 miles north of Eshowe, Zululand, South Africa. After being described as a distinct species it was later reduced to a variety

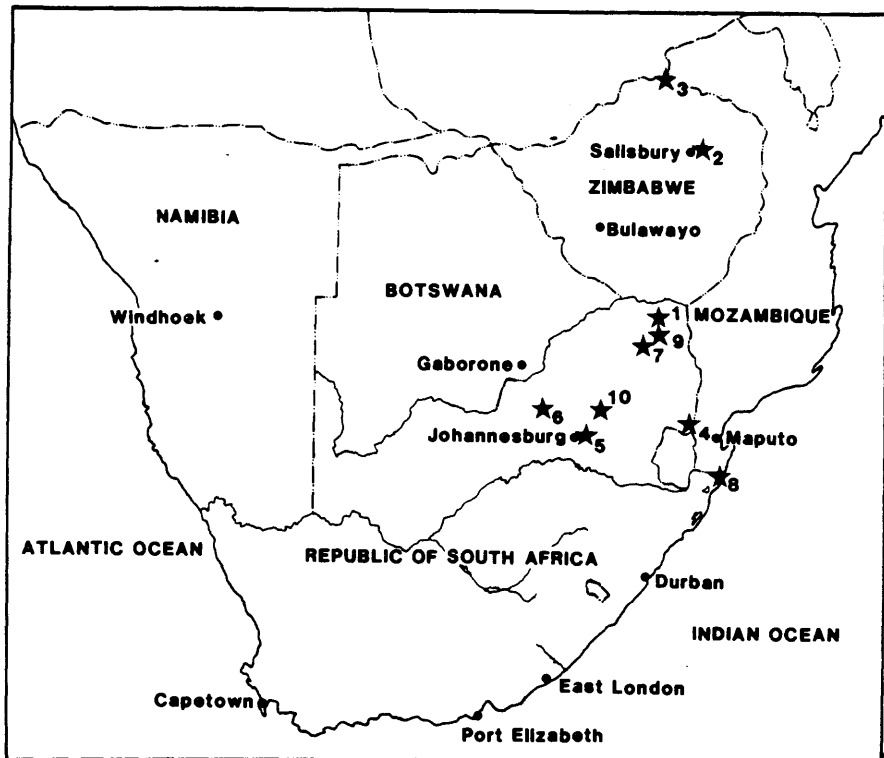


FIG. 1. Collection localities of material used for chromosome and morphological analyses: Locality 1, Makonde Study Area ( $30^{\circ} 31' E$ ,  $22^{\circ} 46' S$ ); 2, Salisbury; 3, Kanyemba ( $30^{\circ} 20' E$ ,  $15^{\circ} 40' S$ ); 4, Komati-poort; 5, Johannesburg; 6, Rustenburg; 7, Tzaneen; 8, Kosi Bay ( $32^{\circ} 49' E$ ,  $26^{\circ} 57' S$ ); 9, Kwa-Nkuzane ( $30^{\circ} 20' E$ ,  $23^{\circ} 15' S$ ); 10, Pretoria.

(Evans, 1927) and, in the latest revision (Gillies & de Meillon, 1968), the authors argue that *pitchfordi* should be placed in synonymy with *marshallii*. This view is based mainly on the evidence presented by Vincke *et al.* (1957), who showed that, in material collected from Kivu Province, Congo, a complete range of variation could be found in the characters used to separate *pitchfordi* and *marshallii* adults.

*Pyretophorus pseudocostalis* was described by Theobald (1910) from three females. The British Museum (Natural History) has currently two females in its possession which belong to the type-series. One of these corresponds to the description of the type. The other specimen does not belong to the *marshallii* group (it lacks an interruption in the third main dark area of the first vein) and it is labelled as

'variety'. Both specimens were collected from Bihe, Angola, on 24 February 1905.

*Pyretophorus transvaalensis* Carter (1910), was described from two females collected from Leydsdorp, Transvaal, South Africa. Only one specimen remains and corresponds with the type description.

## Results

Collections of *marshallii* females from ten localities in South Africa and Zimbabwe were made from March 1976 to June 1979 for ovarian polytene chromosome analysis and for the study of eggs, larvae, pupae and adults from family series. The sites of collection of material are shown in Fig. 1.

Both the genetical and morphological

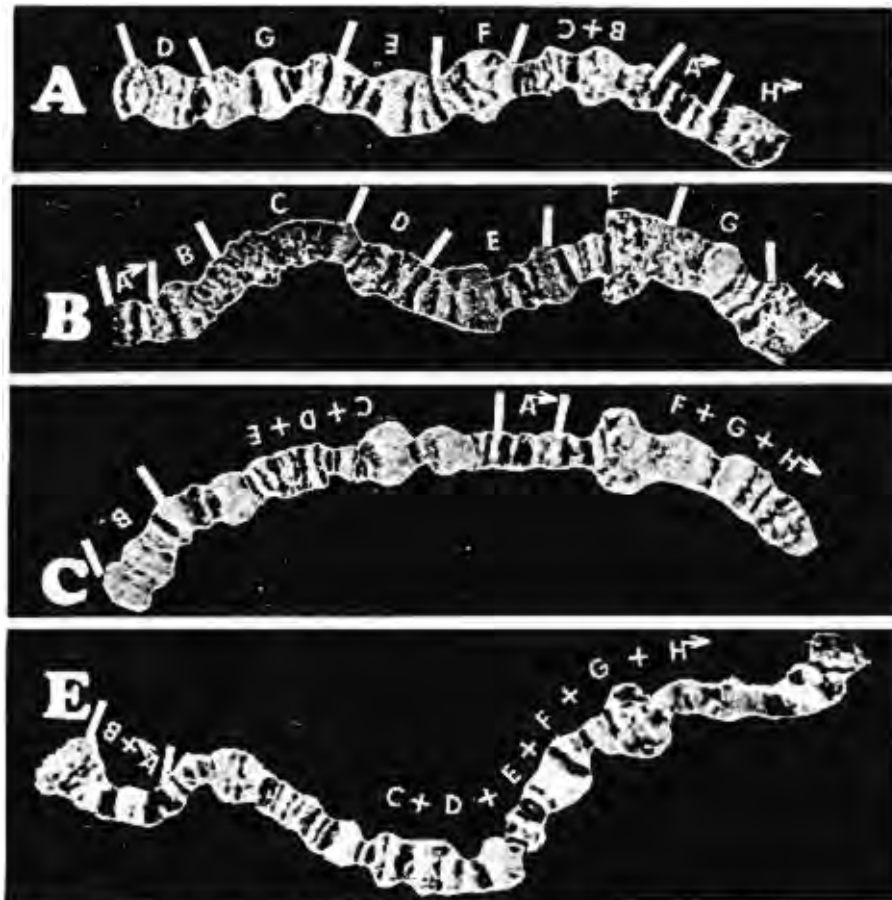


FIG. 2. Sex chromosomes of species A, B, C and E of the *Anopheles marshallii* complex.

studies, carried out independently, arrived at the same basic conclusion. Within the sample of material, four biologically distinct groups were apparent. Some of the differences between the sex chromosomes of the species have previously been presented (Lambert, 1979, 1980, 1981) and Fig. 2 shows the sex chromosomes of all four species, which were arbitrarily designated species A, B, C and E. These polytene chromosomes came from the nurse cells of the ovary and were obtained using the technique of Green (1972), Hunt (1973) and Green & Hunt (1980).

As previously pointed out (Lambert, 1979), there can be no doubt as to the specific status of these forms. Any hybrid between them would be unmistakable since, in sex chromosome banding patterns alone, the four species differ greatly. Typical heterozygous loops (e.g. Dobzhansky, 1970) would be present in the offspring of the mating between a male of one species and a female of another. During the chromosome analysis 1393 wild-caught females were examined and not a single hybrid individual was recorded. The specific status of the forms is therefore beyond question. The only possible exception is species E which was not found sympatric with any of the other species of the complex (Lambert, 1981).

#### *Discriminant function analysis and the identity of type-specimens*

Through our chromosomal and morphological approaches we have found species A, B, C and E at the type locality, Salisbury. This presents a problem in deciding which of the

species represents *A.marshallii* sensu stricto. However, it should be emphasized that the existence of only one of our species in Salisbury would not be conclusive evidence that this was the species to which the type-specimen belonged.

Since the type-specimens are all adult females, we decided to attempt to distinguish between females of our four newly discovered species using morphological characters. Various attempts to find simple, easily distinguishable differences between females of the different species failed. We therefore decided to employ the technique of canonical discriminant function analysis.

Discriminant function analysis is a multivariate technique which is being increasingly used in biology (see Blackith & Reyment, 1971, and Sneath & Sokal, 1973, for details).

An analysis of adult pinned females belonging to species of the *marshallii* complex revealed variation in a number of wing and palp characters which appeared to be of possible use in distinguishing these morphologically similar species. The characters measured are given in Fig. 3. These twelve characters were measured for a total of 239 individual wild-caught females of the four species (collection details are given in Table 1).

The program used to execute the discriminant function analysis was SPSS (Nie *et al.*, 1975). Using the stepwise method, good separation of the species based on these twelve measured characters was obtained. Linear discriminant analysis (i.e. considering only two species at any one time)

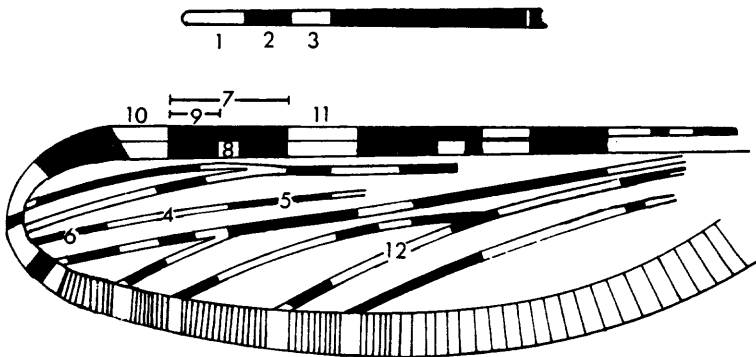


FIG. 3. Wing and palp characters of the *Anopheles marshallii* complex measured for discriminant function analysis.

TABLE 1. Details of samples used in multivariate analysis.

| Species | Collection locality | Sample size |
|---------|---------------------|-------------|
| B       | Salisbury           | 64          |
| C       | Salisbury           | 27          |
| A       | Makonde             | 136         |
| E       | Kosi Bay            | 7           |
| A       | Kwa-Nkuzane         | 5           |
| Total   |                     | 239         |

was shown to have increased power of correct identification in comparison to multiple discriminant analysis which considers more than two groups at any time.

The general ability to discriminate unknown individuals of the four species from these localities was 94%. Specimens belonging to the type-series of *marshallii*, *transvaalensis*, *pseudocostalis* and *pitchfordi*, were then obtained from the British Museum (Natural History) collection. These four specimens were measured for the twelve characters shown in Fig. 3. These data were introduced into the

TABLE 2. Results of identification of type-specimens of *A. marshallii* complex.

| Type-specimens                  | Chromosomally identified species to which the specimen belongs at a probability > 95% |
|---------------------------------|---|
| <i>marshallii</i> (holotype)    | B   |
| <i>transvaalensis</i> (syntype) | B   |
| <i>pseudocostalis</i> (syntype) | None  |
| <i>pitchfordi</i> (syntype)     | None  |

discriminant function analysis using the 239 'chromosomally known' individuals as a basis. The probability that the specimens belonged to any of the four known groups was established with a probability of greater than 95%. These results are presented in Table 2 and an example of discrimination between two species showing the within-group variation is given in Fig. 4.

It appears that species B, the common *A. marshallii* species from Salisbury, is *A. marshallii* s.str. *A. transvaalensis* is a synonym of *marshallii*. While *pseudocostalis* and *pitchfordi* do not seem to belong to any of the four known groups, they should nevertheless remain synonyms of *marshallii* until more information is available. Likewise, because the sample size of species E is so small, it should await a formal description until more material has been collected and studied in detail.

No available names appear to exist for species A and C and so they are here formally described as new species and *marshallii* is redescribed.

**Species descriptions and notes**

*Anopheles marshallii* (Theobald)

*Pyretophorus marshallii* Theobald, 1903. Holotype ♀, ZIMBABWE: 'Salisbury, Mashunaland' (BMNH) [examined].

*Pyretophorus pitchfordi* Giles, 1904. Syntype ♀, SOUTH AFRICA: 80 miles north of 'Eshowe, Zululand' (BMNH) [examined].

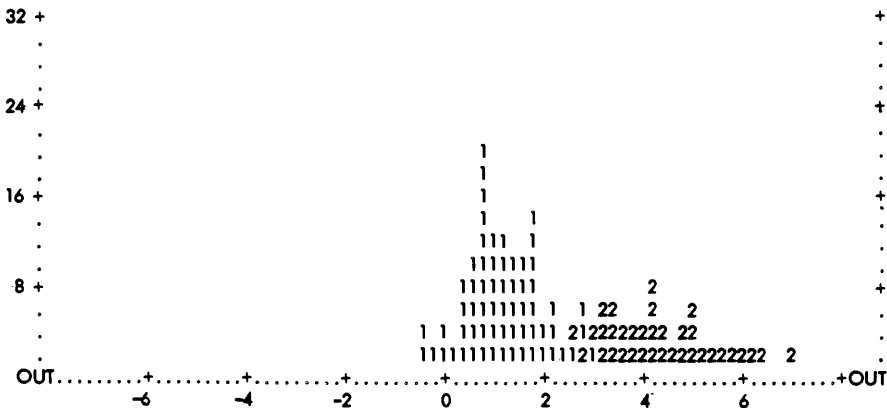


FIG. 4. An example of the discrimination between two species of the *Anopheles marshallii* complex using the SPSS stepwise discriminant analysis program (Nie *et al.*, 1975).

*Pyretophorus pseudocostalis* Theobald, 1910.

Syntypes, 2♀♀, ANGOLA: Bihe (BMNH) [examined].

*Pyretophorus transvaalensis* Carter, 1910.

Syntype ♀, SOUTH AFRICA: Transvaal, Leydsdorp (BMNH) [examined].

#### Adult

Palps smooth with three pale bands, apical dark band about equal in width to either of adjacent pale bands. Most tarsomere segments very narrowly pale apically. Wings, cibarial armature, mesonotum and male genitalia as described by Gillies & de Meillon (1968).

#### Pupa

*Cephalothorax*. Seta 1, 4–7 branches; 2, 5–7 branches; 3, 6–10 branches; 4, 5–10 branches; 5, 9–18 branches; 6, 4–8 branches; 7 long, bifid; 8 simple or with up to 3 branches; 9, 6–13 branches; 10, 1–3 branches; 11, 3–7 branches; 12 long, 6–10 branches. *Abdominal segment I*. Seta 2, 5–11 branches; 3 as long as 2, simple or rarely bifid; 4, 6–13 branches; 5, long simple or rarely branched; 6, 3–6 branches; 7, 7–14 branches; 9, 1–3 branches. *Segment II*. Seta 0 small, simple; 1 large, 5–11 branches; 2, 6–15 branches; 3, 2–5

branches; 4, 6–12 branches, 5, 4–10 branches; 6, large, 3–7 branches; 7, 8–15 branches; 8, 4–8 branches; 9 very small stout, rounded; 10 simple or bifid, always present. *Segment III*. Setae 0, 1 as on II; 2, 7–17 branches; 3, 3–7 branches; 4, 5–12 branches; 5, 6–13 branches; 6, 6–13 branches; 7, 3–8 branches; 8, 2–7 branches; 9 small, stout, longer than II; 10, 4–7 branches; 11 small, simple; 14 small, simple. *Segment IV*. Setae 0, 1, 11, 14 as on III; 2, 5–10 branches; 3, 6–14 branches; 4, 3–6 branches; 5, 7–15 branches; 6, 4–8 branches; 7, 4–8 branches; 8, 2–5 branches; 9 small, spine-like; 10, 2–4 branches. *Segment V*. Setae 0, 10, 14 as on IV; 1 long, simple or with up to 4 branches; 2, 4–8 branches; 3, 4–8 branches; 4, 5–10 branches; 5, 4–12 branches; 6, 4–7 branches; 7, 5–9 branches; 8 simple or with up to 4 branches; 9 simple, 0.3 length of segment; 11 simple or rarely bifid. *Segment VI*. Setae 0, 1, 11, 14 as on V; 2, 3–7 branches; 3, 2–7 branches; 4, 3–8 branches; 5, 5–10 branches; 6, 3–7 branches; 7 long, 2–6 branches; 8, 2–4 branches; 9 simple, 0.3–0.5 length of segment; 10, 3–8 branches. *Segment VII*. Setae 0, 1, 9, 14 as on VI; 2, 3–8 branches; 3 and 4, 3–7 branches; 5, 3–8 branches; 6, 2–4 branches; 7 long, simple or with up to 5 branches; 8, 4–8 branches; 10, 3–7 branches; 11 simple, or

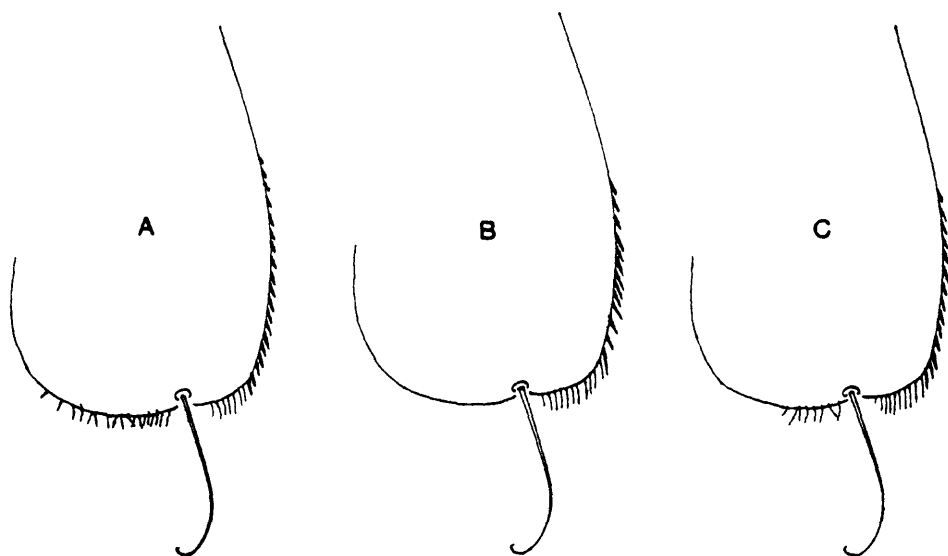


FIG. 5. Differences between the pupal paddles of species of the *Anopheles marshallii* complex: A, *letabensis*; B, *marshallii*; C, *hughi*.

with up to 5 branches. *Segment VIII*. Setae 0, 14 as on VII; 1 small, indistinct; 4, 3–7 branches; 9, 7–15 branches. *Paddle*. Seta 1 simple 0.4 length of paddle; 2, 4–8 branches; fringe consists of spines changing rather abruptly to hairs near the apex, not continued beyond 1-P (Fig. 5).

### Larva

*Head*. Inner clypeal setae (2-C) widely separated, long, simple; outer clypeals (3-C) 0.5 lengths of 2-C, simple or bifid; posterior clypeals (4-C) short, reaching to just beyond base of 2-C, mostly with 3–4 branches but rarely simple or bifid; 5-C, 13–20 branches; 6-C, 12–18 branches; 7-C, 13–22 branches; 8-C, 1–3 branches; 9-C, 2–6 branches; 10-C, 2–6 branches; 11-C, large, plumose; 12-C, 4–8 branches; 13-C, 4–10 branches; 14-C, very short, much branched; 15-C, 5–9 branches; 6-MP, 10–16 branches; antennae clothed in spicules of about equal size; 1-A small, simple; 4-A, 5–9 branches. *Prothorax*. Seta 0 small, simple or bifid; 1, 14–20 branches; 2, 11–18 branches; 3, simple rarely bifid; 4, 13–20 branches; 5 large, plumose; 6 simple or with up to 4 branches; 7 large, plumose, branches on one side of stem markedly shorter than the other; 8 large, plumose; 9 long, simple; 10 long, simple or with up to 3 branches; 11, 4–9 branches; 12 long, 12–22 branches; 13, 4–7 branches; 14, 5–9 branches. *Mesothorax*. Seta 1 much branched; 2, 2–5 branches; 3 quite long, simple; 4, 5–8 branches; 5 longer than 3, simple or with up to 3 branches; 6, 4–8 branches; 7, 4–7 branches; 8 large, plumose; 9 and 10 long, simple, small basal spine; 11 small, simple; 12, 3–6 branches; 13, 6–12 branches; 14, 11–22 branches. *Metathorax*. Seta 1, 3–5 branches; 2 simple or bifid; 3, 14–20 lanceolate leaflets; 4, 4–7 branches; 5, 7 and 8 large, plumose; 6, 4–9 branches; 9 long, 11–20 branches; 10 long, simple or bifid; 11 tiny, spine-like; 12, 5–10 branches; 13, 4–7 branches. *Abdominal segment I*. Seta 1, 10–15 leaflets, fully developed but smaller than on the rest of abdomen; 2 small, 2–6 branches; 3 long, simple or bifid; 4, 5–14 branches; 5, 4–8 branches; 6 and 7 large, plumose; 9, 5–10 branches; 10, 3–6 branches; 11 large, 4–6 branches; 12, 5–9 branches;

13, 7–12 branches. *Segment II*. Seta 0, 1–3 branches, very small; 1, 11–17 fully developed leaflets; 2, 7–13 branches; 3 long, simple or with up to 3 branches; 4, 7–13 branches; 5, 7–10 branches; 6 and 7 large, plumose; 8, 2–6 branches; 9, 7–14 branches; 10 small, 2–6 branches; 11, 4–7 branches; 12, 4–7 branches; 13, 5–9 branches; 14 very small, 1–3 branches. *Segment III*. Setae 0, 1, 6, 13, 14 as on II; 2, 4–9 branches; 3 long, simple or bifid; 4, 3–6 branches; 5, 6–11 branches; 7, 8–13 branches; 8, 3–6 branches; 9, 7–12 branches; 10, 5–8 branches; 11 small, 2–6 branches; 12, 2–6 branches. *Segment IV*. Setae 0, 1, 13, 14 as on III; 2 simple or with up to 3 branches; 3, 2–5 branches; 4, 2–4 branches; 5, 6–11 branches; 6 long, 4–8 branches; 7, 7–14 branches; 8, 2–6 branches; 9, 7–13 branches; 10, 4–8 branches; 11 small, 2–5 branches; 12, 2–5 branches. *Segment V*. Setae 0, 1, 6, 9, 11, 12, 14 as on IV; 2 simple or bifid; 3 simple or with up to 4 branches; 4, 2–7 branches; 5, 7–12 branches; 7, 8–12 branches; 8, 2–5 branches; 10, 4–6 branches; 13, 5–7 branches. *Segment VI*. Setae 0, 1, 3, 14 as on V; 2 simple or with up to 4 branches; 4 simple or with up to 3 branches; 5, 8–13 branches; 6 long, 5–9 branches; 7, 7–11 branches; 8, 2–6 branches; 9, 8–12 branches; 10, 5–8 branches; 11 small, 2–6 branches; 12, 3–6 branches; 13, 8–14 branches. *Segment VII*. Setae 0, 1, 7, 14 as on VI; 2, 4–8 branches; 3, 2–5 branches; 4 simple or with up to 4 branches; 5, 9–14 branches; 6 small, 4–7 branches; 8, 4–8 branches; 9, 6–12 branches; 10, 6–11 branches; 11 small, 2–4 branches; 12, 2–5 branches; 13 large, 4–6 branches. *Segment VIII*. Setae 0 and 14 as on all other segments; 1, 3–6 branches; 2, 8–12 branches; 3 large, 7–15 branches; 4, 4–7 branches; 5, 5–9 branches; 6, 2–6 branches; 8, 5–8 branches; 9, 3–7 branches. *Pecten*. Consists of 13 teeth, 5 long ones, all teeth with basal serrations; seta 1 large, 6–10 branches; 2, 6–12 branches. *Saddle hair*. 5–10 branches (Fig. 6).

### Egg

Exochorion normally continuous between floats but leaving two decks exposed at either end of the dorsal surface, surrounded by a

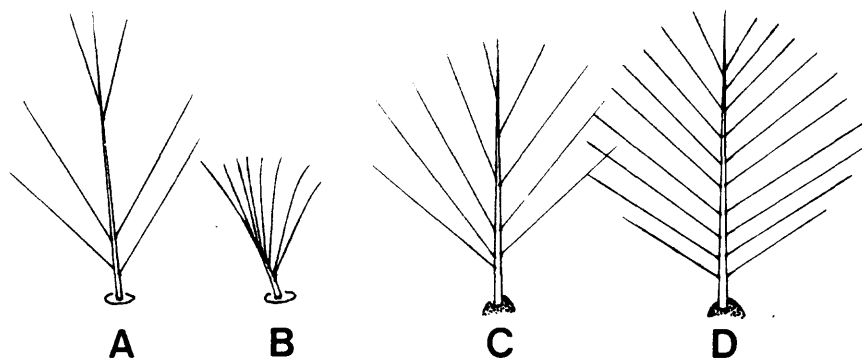


FIG. 6. Differences between larval setae of species of the *Anopheles marshallii* complex: A, Seta 13-II of *letabensis* and *marshallii*; B, Seta 13-II of *hughi*; C, Seta 1-P of *letabensis*; D, Seta 1-P of *marshallii*.

narrow frill. Floats very close, sometimes touching, in mid-dorsal line. Stippling on exochorion very fine (Fig. 7).

#### Larval habitat

Usually fresh, flowing streams with large areas of vegetation (Gillies & de Meillon, 1968; Lambert, 1979, 1980, 1981).

#### Adult biology

Both exophilic and zoophilic. Found commonly biting cattle in South Africa and Zimbabwe. Only two specimens have been caught biting man (Lambert, 1979, 1980, 1981).

#### Distribution

A widespread, mainly high altitude mosquito. Recorded from Salisbury, Zimbabwe; Makonde, Johannesburg, Pretoria and Rustenburg, South Africa (Lambert, 1979, 1980, 1981).

#### *Anopheles letabensis* sp.n. (Figs. 5–7)

[Lambert's species A of the *marshallii* complex (Lambert, 1979, 1980, 1981).]

#### Adult

As *marshallii* but with apical dark band on palps much reduced and pale bands on tarsomeres more prominent.

#### Pupa

As *marshallii* but with the following differences: Seta 3-II, simple or bifid; paddle fringe extends for 20–35 hairs beyond 1-P (Fig. 5).

#### Larva

As *marshallii* but with the following differences: Seta 4-C, simple; 15-C, 3–5 branches; 1-P, 9–14 branches; 1-T, 1–3 branches (Fig. 6).

#### Egg

Similar to *marshallii* but with floats well separated and polar deck openings slightly more rounded (Fig. 7).

#### Larval habitat

As *marshallii* (Lambert, 1979, 1980, 1981).

#### Type material

*Holotype* ♀, SOUTH AFRICA (TRANSVAAL PROVINCE): Grey Stones (30° 15' E, 23° 44' S), 16.iv.1980 (no. GRS 1.19).

*Paratypes*. SOUTH AFRICA (TRANSVAAL PROVINCE): Grey Stones (30° 15' E, 23° 44' S), 16.iv.1980 4 ♀♀ nos. GRS 1.2, GRS 1.4, GRS 1.7 and GRS 1.21. 4 ♂♂ nos. GRS 1.5, GRS 1.6, GRS 1.8, and GRS 1.10.

All adults have associated larval and pupal pelts. The type series is the progeny of a single wild-caught female which was chromosomally identified. The holotype and paratypes

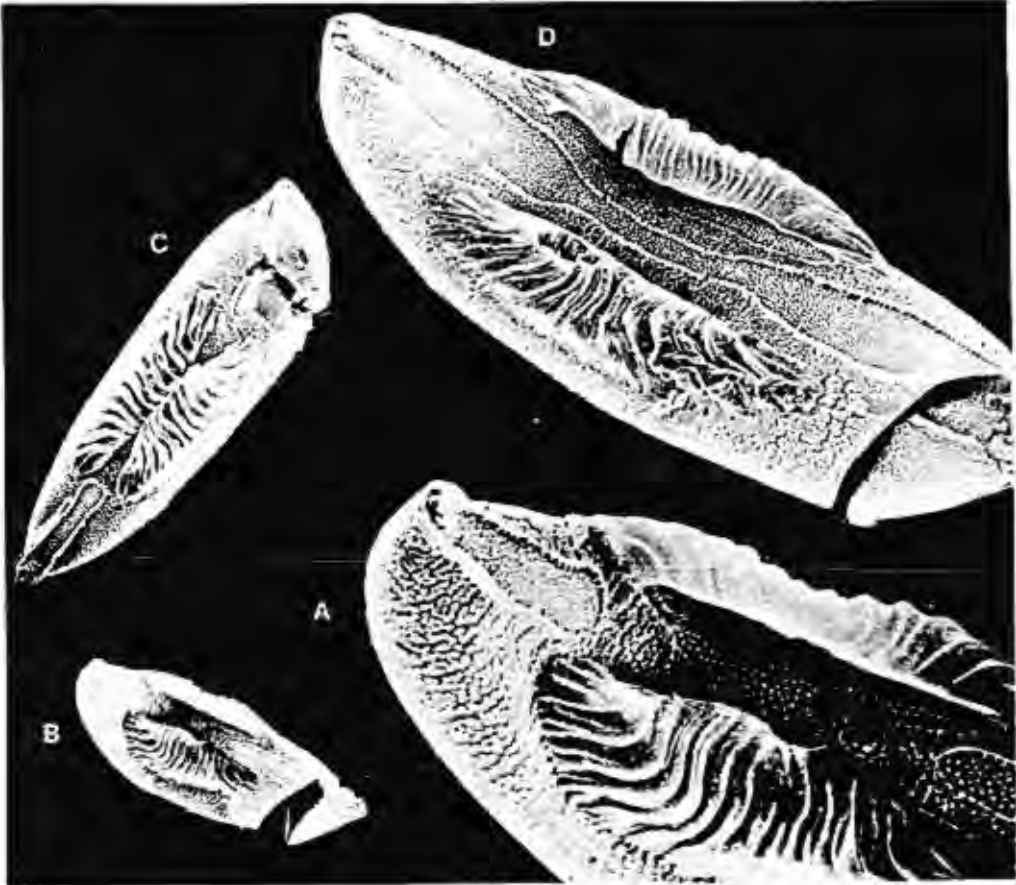


FIG. 7. Scanning electron micrographs of eggs of species of the *Anopheles marshallii* complex: A, B, *letabensis*; C, *marshallii*; D, *hughi*.

nos. GRS 1.2, GRS 1.4, GRS 1.5 and GRS 1.6 are deposited in the Smithsonian Institute, Washington. Paratypes nos. GRS 1.7, GRS 1.21, GRS 1.8 and GRS 1.10 are deposited in the Department of Entomology, South African Institute for Medical Research.

#### Adult biology

One of us (M.C.) caught a total of five females resting inside houses in Tzaneen, Transvaal, over a 4-month period in 1978. Most specimens, however, were caught biting man outdoors. The precipitin tests positive for man (Bruce-Chwatt & Gockel, 1960) might well be confined to this species. Nothing is known of its capacity to transmit malaria but this should be investigated.

#### Distribution

A widespread species found commonly in the low-lying areas of the eastern Transvaal. Occasionally found at higher altitudes, e.g. Salisbury, Zimbabwe.

#### *Anopheles hughi* sp.n. (Figs. 5–7)

[Lambert's species C of the *marshallii* complex (Lambert, 1979, 1980, 1981).]

#### Adult

As *marshallii* but with pale bandings on hind tarsomeres more prominent than either *marshallii* or *letabensis*.

### Pupa

As *marshallii* and *letabensis* but with the following differences: Seta 7-C usually with 3 branches; 12-C, 3–6 branches; 9-I mainly simple; 2-II, 3–6 branches; 3-II as in *letabensis*; 8-II, 1–3 branches; 10-II absent; 2-III, 3–7 branches; 3-III, 1–3 branches; 2-IV, 3–5 branches; 5-IV, 3–7 branches; 1-V, 3–7 branches; 6-V, 2–4 branches; paddle fringe extends for 8–20 hairs beyond 1-P (Fig. 4).

### Larva

As *marshallii* but with the following differences: Seta 4-C simple as in *letabensis*; 2-P, 8–13 branches; 13-II, 8–13 branches but half the size of *marshallii* and *letabensis* (Fig. 6).

### Egg

Differs from the other two species in that it has a continuous open deck between the floats (Fig. 7).

### Larval habitat

As *marshallii* (Lambert, 1979, 1980, 1981).

### Type material

*Holotype* ♀, SOUTH AFRICA (TRANSVAAL PROVINCE): Makonde (30° 31' E, 22° 46' S), 20. ix. 1980 (no. MAK 27.18).

*Paratypes*. SOUTH AFRICA (TRANSVAAL PROVINCE): Makonde (30° 31' E, 22° 46' S), 20. ix. 1980. 4 ♀♀ (nos. MAK 27.4, MAK 27.7, MAK 27.10 and MAK 27.25. 4 ♂♂ nos. MAK 27.2, MAK 27.15, MAK 27.20 and MAK 27.21).

All adults have associated larval and pupal pelts. The type series is the progeny of a single wild-caught female which was chromosomally identified. The holotype and paratypes nos. MAK 27.4, MAK 27.7, MAK 27.2 and MAK 27.15 are deposited in the Smithsonian Institution, Washington. Paratypes nos. MAK 27.10, MAK 27.25, MAK 27.20 and MAK 27.21 are deposited in the Department of Entomology, South African Institute for Medical Research.

### Adult biology

Mainly exophilic and zoophilic (Lambert, 1979, 1980, 1981).

### Distribution

Known only from Salisbury, Zimbabwe and Makonde, Transvaal (Lambert, 1979, 1980, 1981).

### Discussion

The results presented here concerning '*Anopheles marshallii*' are another example of the lack of reliability of gross morphological characters as useful indicators of species in *Anopheles* mosquitoes. Just as '*Anopheles gambiae*' was shown to be a complex of species (Paterson, 1962, 1963, 1964), a series of further studies have revealed similar cases. The common Australian species, '*Anopheles annulipes*', for example, is composed of a number of morphologically similar species (Green, 1972). Similarly '*Anopheles farauti*' is known to be a complex of species (Bryan, 1970; Mahon *et al.*, 1981). Indeed, it seems almost a characteristic of *Anopheles* that cryptic species are common. (The term cryptic species is used here in preference to sibling species. The latter term implies a close genetic relationship. This may or may not be so. The term 'cryptic' only implies that they are difficult to tell apart. It is argued here that this should be the limit of our decisions regarding such species).

Often characteristics such as the ability to transmit malaria and man-biting behaviour appear to be species-specific in *Anopheles*. The fact that '*Anopheles marshallii*' is not a single species but a group of genetically distinct but morphologically extremely similar species requires an alternative interpretation for the data collected by Bruce-Chwatt & Gockel (1960) for example. These authors report that overall 29.5% of '*A. marshallii*' females were positive for human blood. In the light of this study it may be that the great bulk of those individuals were *A. letabensis* since this species appears to bite humans commonly while the other species do not (Lambert, 1979, 1980). The large

morphological variation within *A.marshallii* sensu lato (Gillies & de Meillon, 1968) can now be seen in its proper perspective. Whole groups of species seem certain to have been included within the taxon 'marshallii' and hence the variation previously described as intraspecific variation is actually interspecific.

As previously suggested (Lambert, 1979), *Anopheles letabensis*, because of its man-biting characteristics, should be examined in order to establish its status as a malaria vector. This study has provided chromosomal and morphometric as well as taxonomic methods which allow *letabensis* to be separated from the other species of the *A.marshallii* complex.

We feel that the work presented here adequately emphasizes the advantages of taxonomists and geneticists collaborating in the field of mosquito systematics.

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