

# **A GUIDE TO THE IDENTIFICATION OF THE ANOPHELINE MOSQUITOES (DIPTERA : CULICIDAE) OF SRI LANKA.**

## **II. LARVAE**

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### **ABSTRACT**

The taxonomy of Oriental and SE Asian anopheline mosquitoes has been extensively studied over the past two decades, resulting in greatly improved characterization of species and the definition of taxonomic features that are important in identification. However, published taxonomic keys for Sri Lankan anophelines have not been revised for over 50 years. This paper presents an illustrated key for the identification of larvae of 21 of the 22 currently recognized local anopheline species (the larval stage of the 22nd species is presently unknown), as a guide to workers engaged in malaria surveillance and control in Sri Lanka.

### **INTRODUCTION**

The identification of immature stages of anopheline mosquitoes is an important aspect in malaria surveillance and control strategy throughout the world, taxonomic keys being regularly revised and updated as new information becomes available. However, published keys to identify the species in Sri Lanka have not been revised since the time of Carter (1925), though considerable advances in the taxonomy of the immature anophelines in the Indian and SE Asian regions (including species present in Sri Lanka) have been made during the past 20 years. While the number of anopheline species has not changed substantially from Carter's (1950) checklist, there have been many changes in the identity of the species actually listed, as evidenced by the checklist of Jayasekera and Chelliah (1981). Changes subsequently to this checklist include the invalidation of one species record and description of a new species (Kulasekera *et al.* 1988). This paper presents an updated illustrated key for the identification of larval anophelines occurring on the island. It follows upon a similar key for the identification of adult

females published recently (Amerasinghe, 1990) in an effort to provide a simple and accurate means for the field identification of these medically important insects in Sri Lanka.

## MATERIALS AND METHODS

Primary reference sources consulted during the construction of this key were Christophers (1933), Reid (1968), Harrison and Scanlon (1975), Harrison (1980), Mendis *et al.* (1983), Ramachandra Rao (1984) and Kulasekera *et al.* (1988). Taxonomic characters were checked in relation to Sri Lankan specimens by examination of (i) reference material on deposit at the Department of Zoology, University of Peradeniya, collected and identified under the Biosystematics of the Insects of Ceylon Project of the Smithsonian Institution, USA during 1970-75 and (ii) reference material at the Department of Zoology, University of Peradeniya collected by the author during 1980-88.

Generally, only two of three primary characters are included at each step, with the object of making the key concise and easy to follow. Illustrations are provided to clarify the key characters. Additional characters helpful in identification are provided in the discussion.

Species nomenclature follows Knight and Stone (1977), and abbreviations used in the text follow Reinert (1975). Morphological terminology and chaetotaxy follow Harbach and Knight (1980). The following abbreviations are used: A = Antenna; C = Cephalon; P = Prothorax; M = Mesothorax; T = Metathorax. Abdominal segments are designated by Roman numerals.

Twenty one Sri Lankan species in which the larval stages have been characterized are treated in the key. This includes the newly described *An. peytoni* (Kulasekera *et al.* 1988). *Anopheles insulaeflorum* (listed in the catalogue of Jayasekera and Chelliah, 1981) is excluded as it is no longer regarded as a valid record for Sri Lanka (see Harrison and Scanlon, 1975; Kulasekera *et al.* 1988). *Anopheles ramsayi* is relegated to a junior synonym of *An. pseudojamesi*, which has been shown to have priority (Huda and Harrison, 1985). The larva of *An. reidi* is unknown (Harrison, 1973). Species treated in this work are:

**Subgenus Anopheles** - *aikenii* James 1903, *barbirostris* Van der Wulp 1884, *barbumbrosus* Strickland and Choudhury 1927, *gigas* var. *refutans* Alcock 1913, *interruptus* Puri 1929, *nigerrimus* Giles 1900, *peditaeniatus* (Leicester) 1908, *peytoni* Kulasekera, Harrison and Amerasinghe 1988.

**Subgenus Cella** - *aconitus* Donitz 1902, *annularis* Van der Wulp 1884, *culicifacies* Giles 1901, *elegans* (James) 1903, *jamesii* Theobald 1901, *karwari* (James) 1902, *maculatus* Theobald 1901, *pallidus* Theobald 1901, *pseudojamesi* Strickland and Chowdhury 1927, *subpictus* Grassi 1899, *tessellatus* Theobald 1901, *vagus* Donitz 1902, *varuna* Iyengar 1924.

## DISCUSSION

The key has been drafted to follow as closely as possible, the current accepted classification of sections, series and groups within the Anophelinae. Generally, members of subgenus Anopheles are characterized by branched seta 1-A and closely situated setae 2-C. However, exceptions such as *aikenii* sens. str., with widely spaced 2-C (Kulasekera *et al.* 1988) and *interruptus*, with simple 1-A and closely spaced 2-C (Harrison and Scanlon 1975, Reid 1968) occur in Sri Lanka, and step 1 in the key has been worded to avoid initial misidentification of larvae of these species into the wrong subgenus. The size of seta 1-A

**KEY TO LARVAE (4th Instar)**

Separated from larvae of other genera by the following combination of characters: No respiratory siphon, the spiracles opening on the body wall of segment VIII: Palmate hairs usually present on segments III - VII, often on segments 3-T, I and II as well.

1. Seta 1-A simple; 2-C inserted at least as far apart as the distance between 2-C and 3-C on one side (Fig. 1a) (Subgenus *Cellia*) 2
- Seta 1-A branched or setae 2-C inserted close together, closer than the distance between 2-C and 3-C on one side, or both (Fig. 1b) (Subgenus *Anopheles*) 14
- 2 (1). Long thoracic pleural setae 9, 10, 12-P, 9, 10-M and 9, 10-T simple (Fig. 2a) (Neomyzomyia series) 3
- Metathorax with at least seta 9 branched, and one or more of long pleural setae may be branched on P and T (Fig. 2b-d) 4
- 3 (2). Seta 1-P weak, 2-5 branched; 1, 2-P arising from separate basal tubercles, only tubercle of 2-P prominent and sclerotized (Fig. 3a) *tessellatus*
- Seta 1-P well developed, with 15-21 branches; 1, 2-P arising from fused prominent basal sclerotized tubercles (Fig. 3b) *elegans*
- 4 (2). Metathorax with only one pleural seta (9-T) branched (Fig. 2b); abdominal segments IV-VII with tergal plate large and enclosing median accessory plate, or smaller with separate median accessory plate and pair of small submedian accessory tergal plates (Fig. 4a,b). (*Myzomyia* series) 5
- Metathorax with both long pleural setae (9, 10-T) branched (Fig. 2c,d); abdominal segments IV-VII with small to moderate sized anterior tergal plate; median accessory tergal plate if present, always separate from tergal plate; submedian accessory tergal plates absent (Fig. 4c) 7
- 5 (4). Tergal plates on abdominal segments III-VII very large, greater than half the width of segment, enclosing median posterior tergal plate (Fig. 4a) (*minimus* gp.) 6
- Anterior tergal plates on III-VII smaller, less than half the width of segment, not enclosing small median accessory tergal plate (Fig. 4b) *culicifacies*
- 6 (5). Seta 2-C with 9-18 short lateral barbs; 4-C with 2-5 branches (rarely simple); 2-P with 10-15 and 8-P with 10-25 branches; 3-T leaflets with short slender tips (Fig. 5a,c) *aconitus*
- Seta 2-C with 1-4 short lateral barbs; 4-C simple (rarely bifid); 2-P with 16-21 and 8-P with 30-40 branches; 3-4-T leaflets with long tapering filamentous tips (Fig. 5b,d) *varuna*
- 7 (4). Prothorax with one long branched pleural seta (9-P) and one short branched pleural seta (11-P); mesothorax with one long branched pleural seta (9-M) (Fig. 2c); setae 2, 3-C with minute barbs or distinct lateral

- branches (Fig. 6a,b); 1,2-P with well developed sclerotized bases (Fig. 6d) (Neocellia series) 8
- Pro- and mesothorax with long pleural setae simple or with one pleural seta on each segment with 2-3 distal branches (Fig. 2d); setae 2, 3-C simple (Fig. 6c); 2-P with very weakly sclerotized bases (Fig. 6e) (Pyretophorus sereis) 13
- 8 (7). Seta 3-C simple or with only short side branches not more than 1/4th the length of the seta, not brush-like in appearance (Fig. 6a) 9
- Seta 3-C with long lateral branches, brush-like (Fig. 6b) 11
- 9 (8). Seta 1-P small, usually with 7-14 branches; shortest prothoracic pleural seta (11-P) stout and bluntly barbed along its length (Fig. 7a) *pseudojamesii*
- Seta 1-P usually with 16-24 branches; shortest prothoracic pleural seta (11-P) not stout and barbed, but with few medium to long branches (Fig. 7b) 10
- 10 (9). Abdominal setae 6-V, VI with 6-16 branches; filament of abdominal palmate setae blunt-ended (Fig. 8a) *karwari*
- Abdominal setae 6-V, VI with 3-6 branches; filament of abdominal palmate setae sharply pointed (Fig. 8b) *maculatus*
- 11 (8). Seta 8-C simple, sometimes bifid distally (Fig. 9a) 12
- Seta 8-C split near base into 2-10 branches (Fig. 9b) *pallidus*
- 12 (11). Palmate seta 1-I well developed, with obvious leaflets (Fig. 10b) *annularis*
- Palmate seta 1-I undeveloped, without obvious leaflets, the branches more filamentous (Fig. 10a) *jamesii*
- 13 (7). Seta 3-C half or more as long as 2-C; 4-C nearly as long as 3-C, and placed far back and wide apart (Fig. 11a) *subpictus*
- Seta 3-C short, 1/3rd as long as 2-C; 4-C short, placed far forward and close together (Fig. 11b) *vagus*
- 14 (1). Branches of seta 1-A not reaching much beyond middle of antenna shaft (Fig. 12a) (Angusticorn section) 15
- Branches of seta 1-A extend close to or beyond end of antennal shaft, whole seta usually greater than half the length of shaft (Fig. 12b) (Laticorn section, Myzorhynchus series) 17
- 15 (14). Setae 5,6 7-C well developed and feathered; 4-C branched from base (Fig. 13a) (Anopheles series; aitkenii and lindesayi gps.) 16
- Setae 5,6 7-C reduced, some or all short and few-branched or simple; seta 4-C simple or branched only on distal half (Fig. 13b) (Lophoscelomyia series; asiaticus gp.) *interruptus*
- 16 (15). Palmate setae 3-T, 1-II with obvious leaflets but undifferentiated filaments (Fig. 14a) (aitkenii gp.) 17
- Palmate setae 3-T, 1-II without leaflets but with filiform branches

- (Fig. 14b). (Largest anopheline larva in the Indian region, 7-10 mm. length) *gigas*
- 17 (16) Setae 2-C bifid or trifid, separated by a distance approximately equal to distance between 2-C and 3-C on one side; 6-III with 20 or more branches (Fig. 15a,c) *aitkenii*
- Setae 2-C simple, very close together, separated by less than distance between 2-C and 3-C on one side; 6-III with 5-12 branches (Fig. 16b,d) *peytoni*
- 18 (14) Seta 1-P with long branches from near base (Fig. 16a) (*barbirostris* gp.) 19
- Seta 1-P simple or with 2-5 short branches near tip (Fig. 16b) (*hyrcanus* gp.) 20
- 19 (18). Seta 3-C with 12-36 thin attenuated branches, usually lax and spread out (Fig. 17b); palmate seta 1-II usually not pigmented *barbumbrosus*
- Seta 3-C with 19-95 thick branches, usually stiff and broom-like (Fig. 17a); palmate seta 1-II usually darkly pigmented. *barbirostris*
- 20 (18). Seta 4-M with sinuate spreading branches arising close together at base; 8-C with 4-9 branches; 9-C with 3-7 branches (Fig. 18a,c) *peditaeniatus*
- Seta 4-M with stiff, erect branches arising along the stem; 8-C with 12-24 branches; 9-C with 8-14 branches (Fig. 18b,d) *nigerrimus*
- (NOTE: The larva of *reidi* in the *barbirostris* gp. is unknown, but should key out as step 19 on the basis of the major character of the group).

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is also of some use, since it is generally small to minute in subgenus *Cellia* and large in many members of subgenus *Anopheles*. However, 1-A is relatively small in the *Angusticorn* section of the latter subgenus (eg. *An. interruptus*, *An. gigas*, *An. aitkenii*, *An. peytoni*) and is not used as a key character for Sri Lankan anophelines. Steps 1, 2, 4, 7 and 14 of the key are critical since species level identification is based on larvae being properly assigned to their respective sections and series.

In general, Sri Lankan anopheline larvae can be easily identified on the basis of the key characters. Difficulties may, however, be encountered in some cases, especially when routinely processing whole larvae in malaria entomological surveys. In the *Myzomyia* Series of Subgenus *Cellia*, for instance, separating *An. aconitus* from *An. varuna* may sometimes be problematic when the barbed nature of setae 2-C, 3-C and branching of 4-C in *aconitus* are obscured by dirt or the larval mouth brushes. It may also be difficult at times to decide on the nature of the 3-T leaflets. Additional characters (branching on setae 2-P and 8-P), based on Harrison's (1980) revision of the species group in Thailand, are given in the key as these setae are easily seen on the larvae. Another character, the presence of distinct shoulders on the leaflets of palmate seta 1-I in *varuna* (shoulders poorly defined or absent in *aconitus*) appears to be variable in both species in Sri Lanka, and thus of limited value in identification. The closely related *An. minimus* Theobald is

## LEGENDS FOR FIGURES

A = Antenna; C = Cephalon; P = Prothorax; M = Mesothorax; T = Metathorax; TP = Tergal Plate; MP = Median Accessory Tergal Plate; SP = Submedian Accessory Tergal Plate. Setae are designated by arabic numerals and abdominal segments by Roman numerals.

- Fig. 1 a, b: Dorsal view of head.
- Fig. 2 a - d: Ventral view of thorax.
- Fig. 3 a, b: Dorsal view of prothorax.
- Fig. 4 a - c: Dorsal view of abdominal segment IV.
- Fig. 5 a - d: Dorsal view of head (5 a, b) and thorax (5 c, d).
- Fig. 6 a - e: Dorsal view of head (6 a, b, c) and prothorax (6 d, e)
- Fig. 7 a, b: Dorsal and ventral views of prothorax.
- Fig. 8 a, b: Dorsal view of abdominal segments V and VI.
- Fig. 9 a, b: Dorsal view of head.
- Fig. 10 a, b: Dorsal view of abdominal segment I.
- Fig. 11 a, b: Dorsal view of head.
- Fig. 12 a, b: Dorsal view of antenna.
- Fig. 13 a, b: Dorsal view of head.
- Fig. 14 a, b: Dorsal view of metathorax and abdominal segments I and II.
- Fig. 15 a - d: Dorsal view of head (15 a, b) and abdominal segments III (15 c, d).
- Fig. 16 a, b: Dorsal view of prothorax.
- Fig. 17 a, b: Dorsal view of head.
- Fig. 18 a - d: Dorso-lateral view of mesothorax (18 a, b) and dorsal view of head (18 c, d).

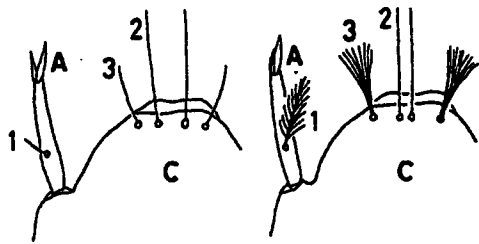


FIG 1a

FIG 1b

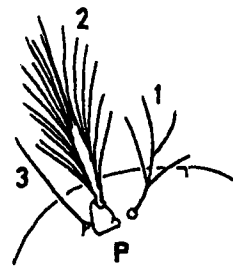


FIG 3a

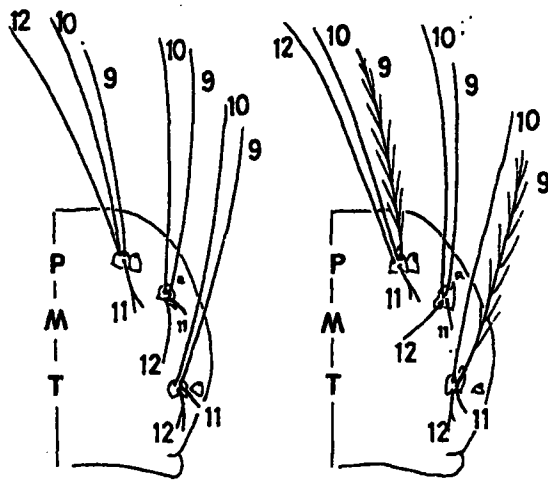


FIG 2a

FIG 2b

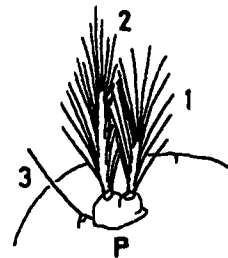


FIG 3b

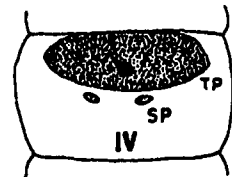


FIG 4a

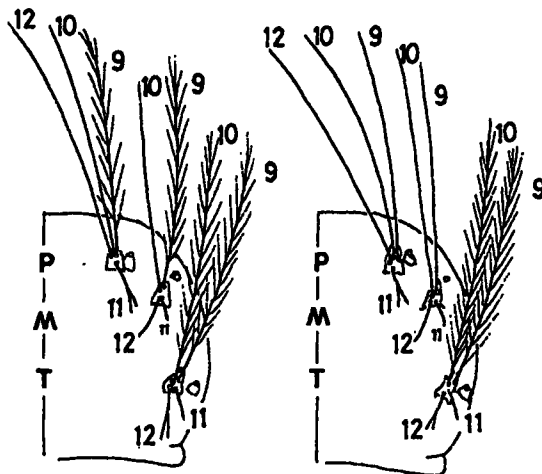


FIG 2c

FIG 2d

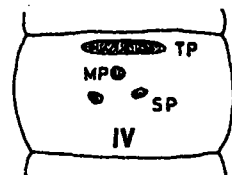


FIG 4b

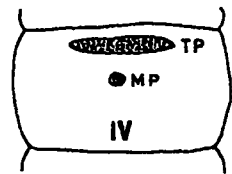


FIG 4c

77A

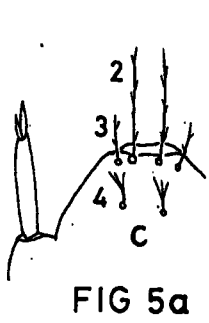


FIG 5a

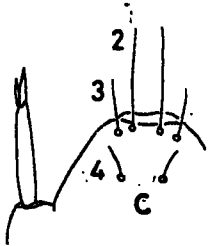


FIG 5b

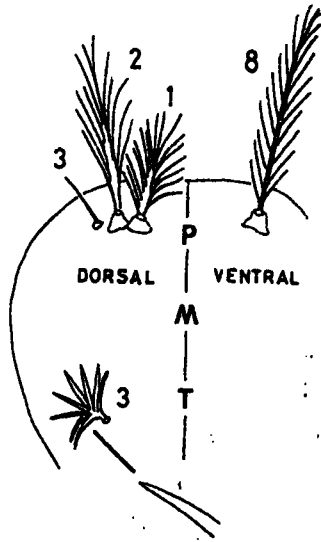


FIG 5c

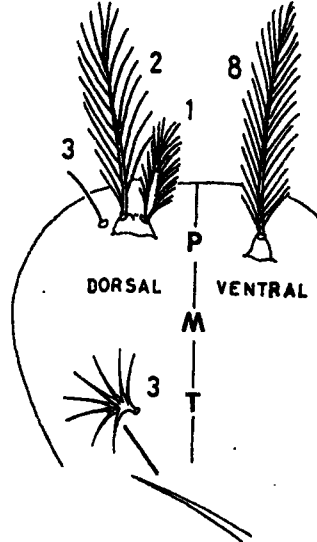


FIG 5d

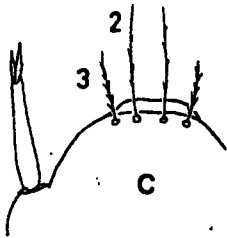


FIG 6a

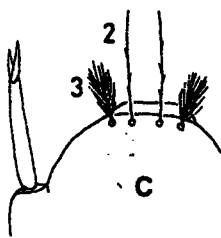


FIG 6b

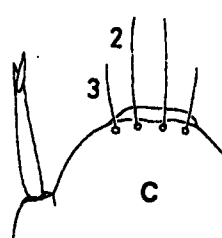


FIG 6c

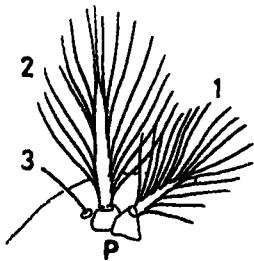


FIG 6d

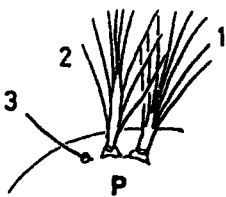


FIG 6e

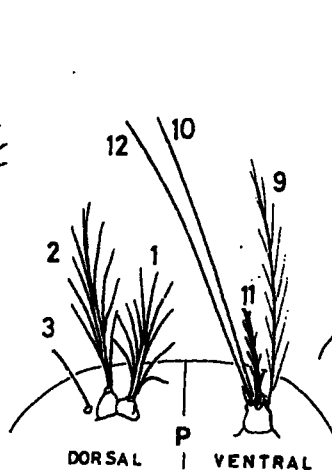


FIG 7a

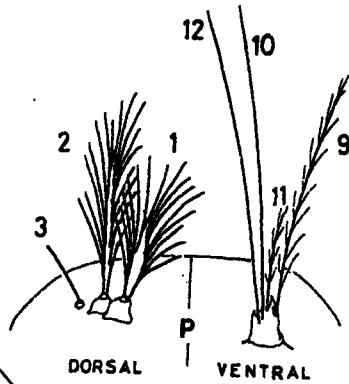
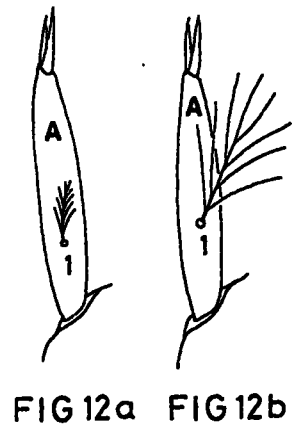
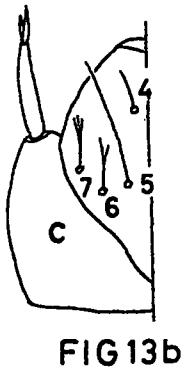
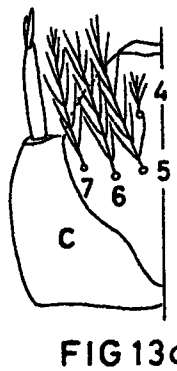
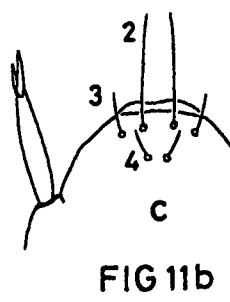
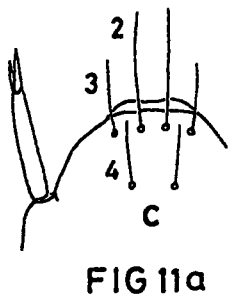
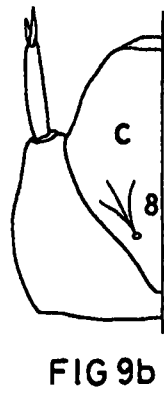
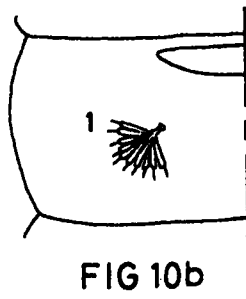
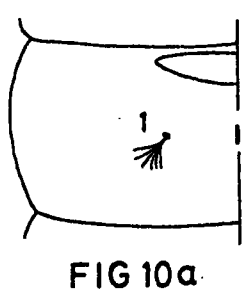
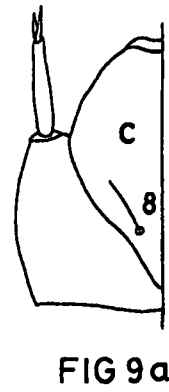
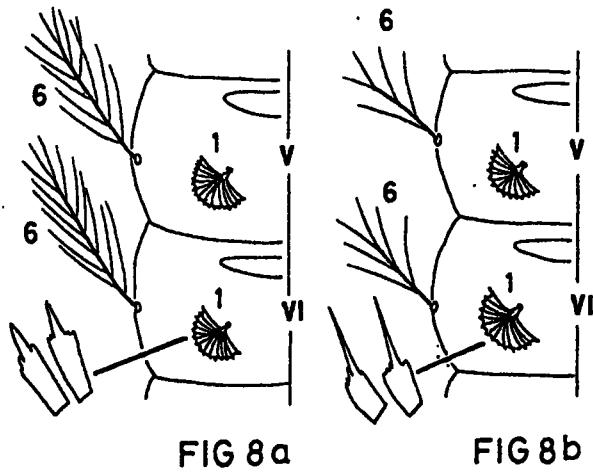


FIG 7b



PA

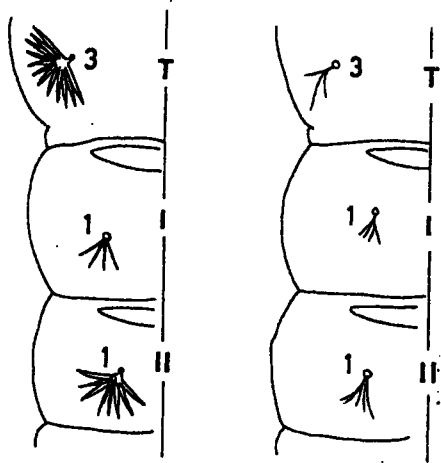


FIG 14 a

FIG 14 b

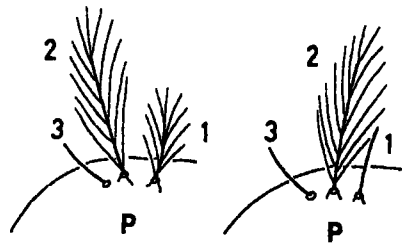


FIG 16 a

FIG 16 b

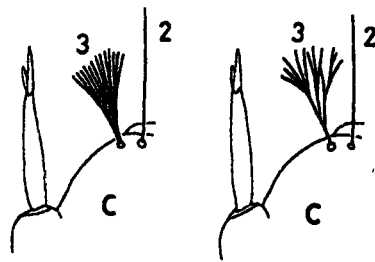


FIG 17 a

FIG 17 b

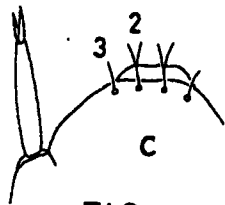


FIG 15 a

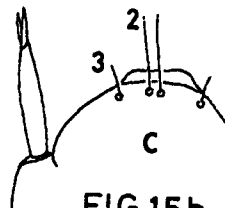


FIG 15 b

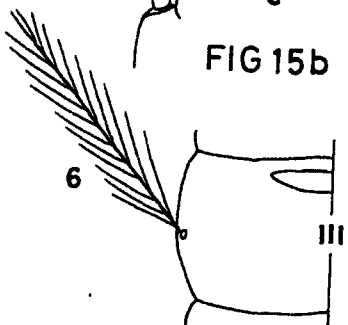


FIG 15 c

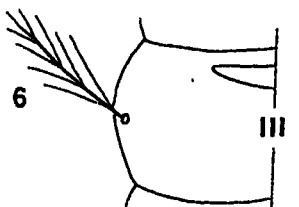


FIG 15 d

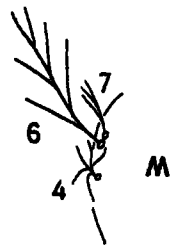


FIG 18 a

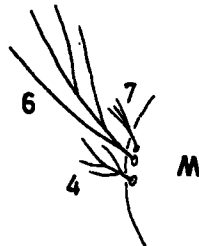


FIG 18 b

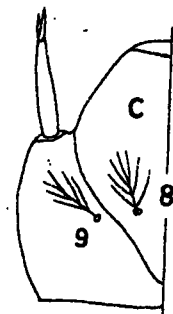


FIG 18 c



FIG 18 d

no longer considered to occur in Sri Lanka (Harrison 1980). However, clinal variation sometimes results in adults of the species being virtually indistinguishable from the above 2 species (Harrison, 1980) and it is worth outlining diagnostic larval characteristics for purpose of checking *Minimus* group collected in Sri Lanka. They are; (i) *minimus* (seta 2, 3-C simple, unbarbed; 4-C simple) vs *aconitus* (2, 3-C barbed; 4-C branched) and (ii) *minimus* (seta 3-T leaflets blunt; 6-C with 12-15, 2-I with 3-6, 9-I with 5-7 and 13-III with 6-11 branches) vs *varuna* (3-T leaflets sharp pointed; 6-C with 15-18, 2-I with 2-3, 9-I with 4-5 and 13-III with 3-5 branches). *Anopheles culicifacies*, currently the only known Sri Lankan member of the *Myzomyia* series with small abdominal tergal plates, is easily separable from *aconitus* and *varuna* which have large tergal plates.

The 6 species of Sri Lankan *Neocellia* are equally divided into those with bushy setae 3-C (*jamesii*, *annularis*, *pallidus*) and those with simple or barbed 3-C (*maculatus*, *kawari*, *pseudojamesi*). A further character that separates the two groups is the length of the filaments in abdominal palmate setae IV-VII, these being equal to or greater than 1/2 the length of the blades in the former and 1/3rd or less in the latter. The characters given in the key appear to be the only reliable ones to distinguish between the different species of *Neocellia*. The same is true for the 2 local species in the *Pyretophorus* series, *An. subpictus* and *An. vagus*. Reid (1968) records the branching range for seta 1-M as 21-34 in *subpictus*, the corresponding range being 11-20 in *vagus* var. *vagus* (Gater 1934, in Reid 1968) the form reported to occur in Sri Lanka (see Jayasekera and Chelliah, 1981) and 9-17 in *vagus* var. *limosus* (King 1932, in Reid 1968). However, in local *vagus* examined by this author, seta 1-M consistently had more than 21 branches and thus the character cannot be used in separation. This also throws into question the definition of the Sri Lankan form as *vagus* ssp. *vagus* given in Jayasekera and Chelliah (1981).

Within the *Barbirostris* group of subgenus *Anopheles*, the pigmentation of seta 1-II is a useful secondary character in separating *barbirostris* (dark seta) from *barbumbrosus* (pale or colourless seta; see step 19 in key). The latter species thus bears a superficial resemblance to members of the *Hyrchanus* group all of which have pale or unpigmented 1-II, and it is essential that the branching of seta 1-P (see step 18 in key) be carefully checked during identification. The separation of *Hyrchanus* group members *peditaeniatus* and *nigerrimus* too, needs care. The definitive identifying feature, the basally branched, sinuous seta 4-M of *peditaeniatus* is small and often difficult to see; it may be confused with 7-M (see Fig. 18a,b) resulting in misidentification as "*nigerrimus*". In this case the branching of setae 8-C and 9-C are useful additional checks (see step 20 in key). This separation is particularly important because *nigerrimus* is a suspected field vector of malaria in Sri Lanka (AMC 1987) while *peditaeniatus* is regarded as a non-vector.

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