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Revised morphological identification key to the larval anopheline (Diptera: Culicidae) of Sri Lanka

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PEER REVIEW

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Comments

This study is very important because the identification of mosquito species is always the first step in all surveillance and control of malaria strategies. It describes an updated and easy way to use illustrated key that will be useful for Sri Lankan malaria control personnel and researchers interested in local anopheline fauna.

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ABSTRACT

Objective: To revise morphological identification keys to the anophelines in Sri Lanka.

Method: Samples were collected from selected entomological sites in different districts in the country. Stage III and IV larvae were identified under a light microscope with an objective (× 10) using standard larval keys developed for Sri Lankan anophelines. Key larval characters were recorded for each species based on original observations and previous usage in literature.

Results: This manuscript describes an illustrated key for the identification of 22 of 23 mosquitoes which are currently recognized as local anopheline species in Sri Lanka, as a guide to workers engaged in malaria surveillance and control in the country.

Conclusions: Revised morphological keys to the larval of these species may be helpful in easy and accurate identification at the field level.

KEYWORDS

Anopheline, Immature, Mosquitoes, Control, Keys

1. Introduction

The identification keys to the immature stages of *Anopheles* mosquitoes have long been a necessity for entomologists dealing with malaria vectors. Many of the illustrated keys to the *Anopheles* of Sri Lanka are of limited value[1,2], as these were published more than 20 years ago and significant advances in our knowledge of the *Anopheles* mosquitoes have occurred in the intervening years. The number of anopheline species has not changed substantially from Carter's checklist; there have been many changes in the identity of the species actually listed, as evidenced

by the checklist of Jayasekera and Chelliah[2]. Changes subsequently to this checklist include the invalidation of one species record and description of *Anopheles jeyporiensis* (*An. jeyporiensis*). This paper presents an updated illustrated key for the identification of larval anophelines occurring in the country.

The purpose of the keys presented here is to assist field surveillance teams to identify the larval stages of *Anopheles* mosquitoes. The keys can be used initially to identify specimens to species group and then to species. Discriminating characteristics are highlighted in drawings wherever possible.

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2. Materials and methods

2.1. Collecting samples

Samples were collected from 17 entomological sites in Ampara, Batticaloa, Mannar, Trincomalee and Killinochchi districts as part of the malaria elimination program of Tropical and Environmental Diseases and Health Associates (TEDHA) Pvt. Ltd. Further, wet zone specimens were obtained by the entomological teams attached to the Anti Malaria Campaign.

2.2. Identification of field samples

Stage III and IV larvae were placed individually in microscopic slides and identified under a light microscope with an objective ($\times 10$) using standard larval keys developed for Sri Lankan anophelines[2]. Key larval characters were recorded for each species. Further, larval species identification was reconfirmed through adult identification[3].

2.3. Revising larval identification key for Sri Lankan *Anopheles*

The morphological characters used here are based on original observations and previous use in the literature. The following were referred to during the construction of this key: Amarasinghe, 1992[2], Christophers, 1933[4], Colless, 1957[5], Reid, 1968[6], Harrison, 1980[7], Harrison and Scanlon, 1975[8], Linton *et al.*, 2001[9], Junkum *et al.*, 2005[10], Linton *et al.*, 2005[11], Rattanarithikul *et al.*, 2005[12], Sallum *et al.*, 2005[13] and Rattanarithikul *et al.*, 2006[14]. Taxonomic characteristics were cross checked in relation to Sri Lankan specimens by examining the reference materials archived at the Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Sri Lanka.

Species nomenclature follows that proposed by Knight and Stone[15], and abbreviations used in the text follow that used by Reinert[16,17]. Morphological terminology and chaetotaxy follow that used by Harbach and Knight[18]. Abdominal segments are numbered by roman numerals. Twenty two Sri Lankan anopheline species have been included into this key. The species considered include:

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

Subgenus *Cellia*: *Anopheles aconitus* Donitz 1902 (*An. aconitus*), *Anopheles annularis* Vander Wulp 1884, *Anopheles culicifacies* Giles 1901 (*An. culicifacies*), *Anopheles elegans* (James) 1903 (*An. elegans*), *Anopheles jamesii* Theobald 1901,

An. jeyporiensis (James) 1902, *Anopheles karwari* (James) 1902, *Anopheles maculatus* Theobald 1901, *Anopheles pallidus* Theobald 1901, *Anopheles pseudojamesi* Strickland and Chowdhury 1927, *Anopheles ramsayi* Covell 1927, *Anopheles subpictus* Grassi 1899 (*An. subpictus*), *Anopheles tessellatus* Theobald 1901 (*An. tessellatus*), *Anopheles vagus* Donitz 1902 (*An. vagus*), *Anopheles varuna* Iyengar 1924 (Figure 1).

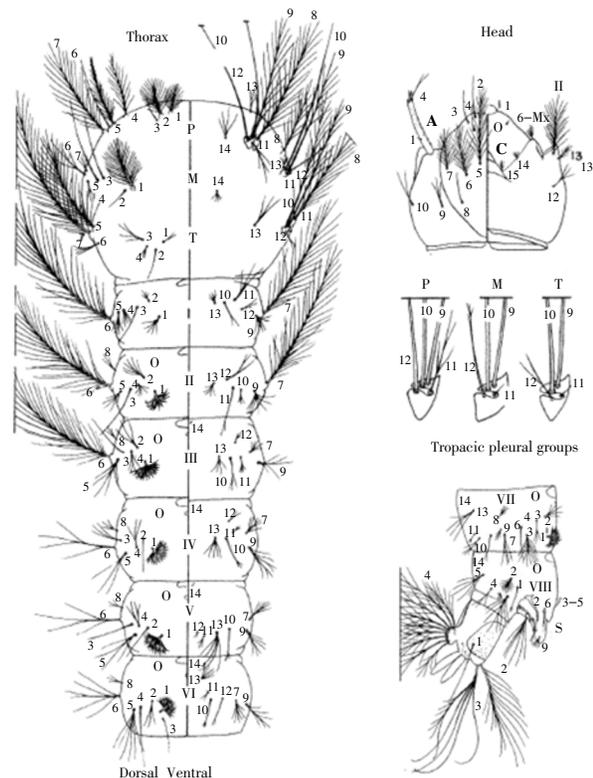


Figure 1. Larval morphology.

Head: A=Antenna, C=Cranium head capsule; Thorax: P=Pro-thorax, M=Meso-thorax, T=Meta-thorax, 1–14=setae on designated areas (seta 1M, setae 1–T); Abdomen: I–VII, X=Abdominal segments, S=Spiracular apparatus, 1–14=Setae on designated areas (seta 1–I, seta 5–IV).

3. Results

The revised larval morphological key is shown below. This key presents an illustrated key for the identification of larvae of 22 of 23 currently recognized local anopheline species in Sri Lanka. Morphological features noted in different regions of the body were recorded: head (antennal hairs, inner clypeal hairs, outer clypeal hairs, frontal hairs and sutural hairs), thorax (thoracic palmate hairs, shoulder hairs, pro, meso and meta thoracic hairs), abdomen (abdominal tergal plates, palmate hairs in abdominal segments, lateral hairs in the abdominal segments).

The inclusion of *An. jeyporiensis* is based on the reported collection during the TEDHA Malaria Elimination Program in 2011. This species was included in this key as local workers may encounter the species and may difficulty in its identification.

Generally, members of subgenus *Anopheles* are characterized by branched seta 1–A and closely situated setae 2–C. However, exceptions such as *An. aitkenii* sens. str., with widely spaced 2–C[2] and *An. interruptus*, with

simple 1–A and closely spaced 2–C[2,6,8] have been reported in Sri Lanka. Figure 2 in the key has been worded to avoid initial misidentification of larvae.

4. Discussion

The key to the Sri Lankan anopheline larvae has been revised according to the currently accepted classification of sections, series and groups in the Anophelinae.

Brief notes are provided at each step in the identification process along with illustrative diagrams. It is emphasized that taxonomic keys are only a rapid and convenient guide

to identification based on the examination of a few important characters. In case of doubt, it is essential to consult published literature with detailed descriptions of species characters.

4.1. Subgenus Anopheles

4.1.1. Myzorhynchus series

The Myzorhynchus series can be separated from other Sri Lankan representatives of subgenus *Anopheles* by the outer clypeal hair (3–C) being strongly branched. Two species groups are present in Sri Lanka: the Hyrcanus and

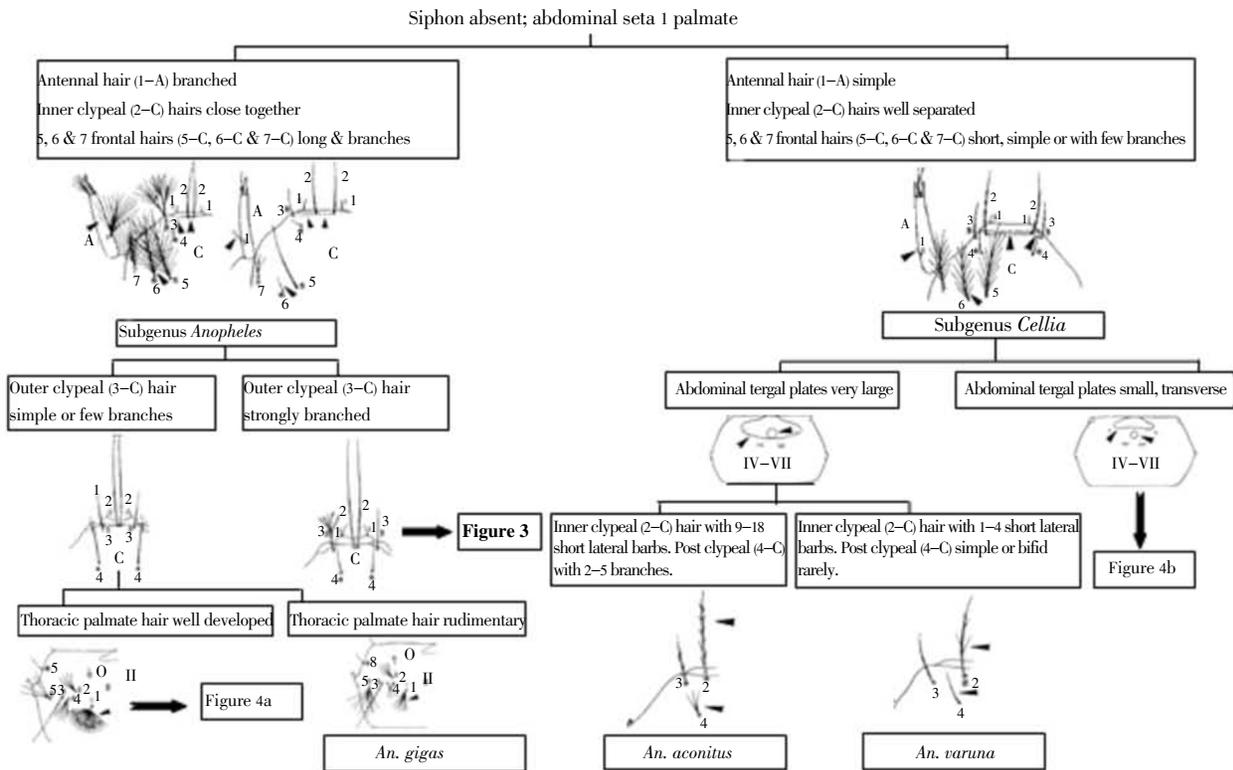


Figure 2. Sri Lankan anopheline mosquito – key to the fourth instar larvae.

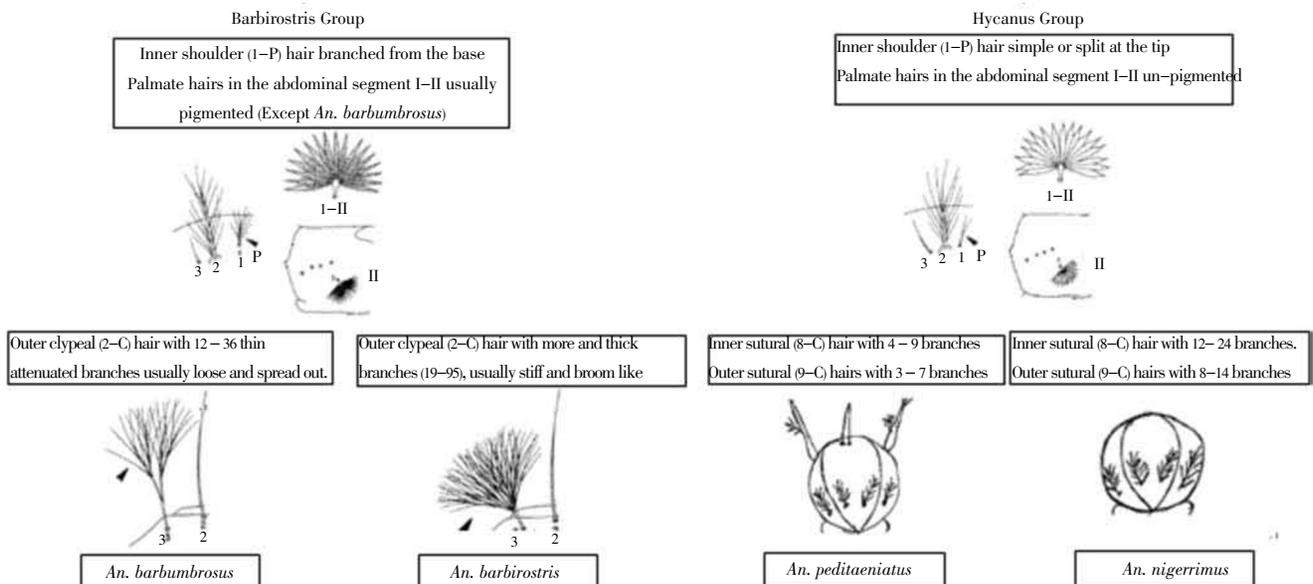


Figure 3. Outer clypeal hair strongly branched.

Barbirostris group (Figure 2).

4.1.1.1. Hyrcanus group

The Hyrcanus group can be separated from the Barbirostris group by the morphological characters given in the key. This group bears a superficial resemblance to members of the Hyrcanus group, all of which have pale or un-pigmented I-II; the separation of *An. peditaeniatus* and *An. nigerrimus* needs care. The definitive identifying feature is the basally branched, sinuous seta 4-M of *An. peditaeniatus* which is small and often difficult to observe. This may be confused

with 7-M resulting in misidentification as *An. nigerrimus*. In this instance, branching of setae 8-C and 9-C are useful in confirmation. The correct identification of *An. nigerrimus* is important as it is a suspected vector of malaria in Sri Lanka while *An. peditaeniatus* is considered a non-vector (Figure 3).

4.1.1.2. Barbirostris group

Within the Barbirostris group of subgenus *Anopheles*, the orientation of the inner shoulder hair (1-P) can be used as the primary character: 1P seta with 4 or more branches

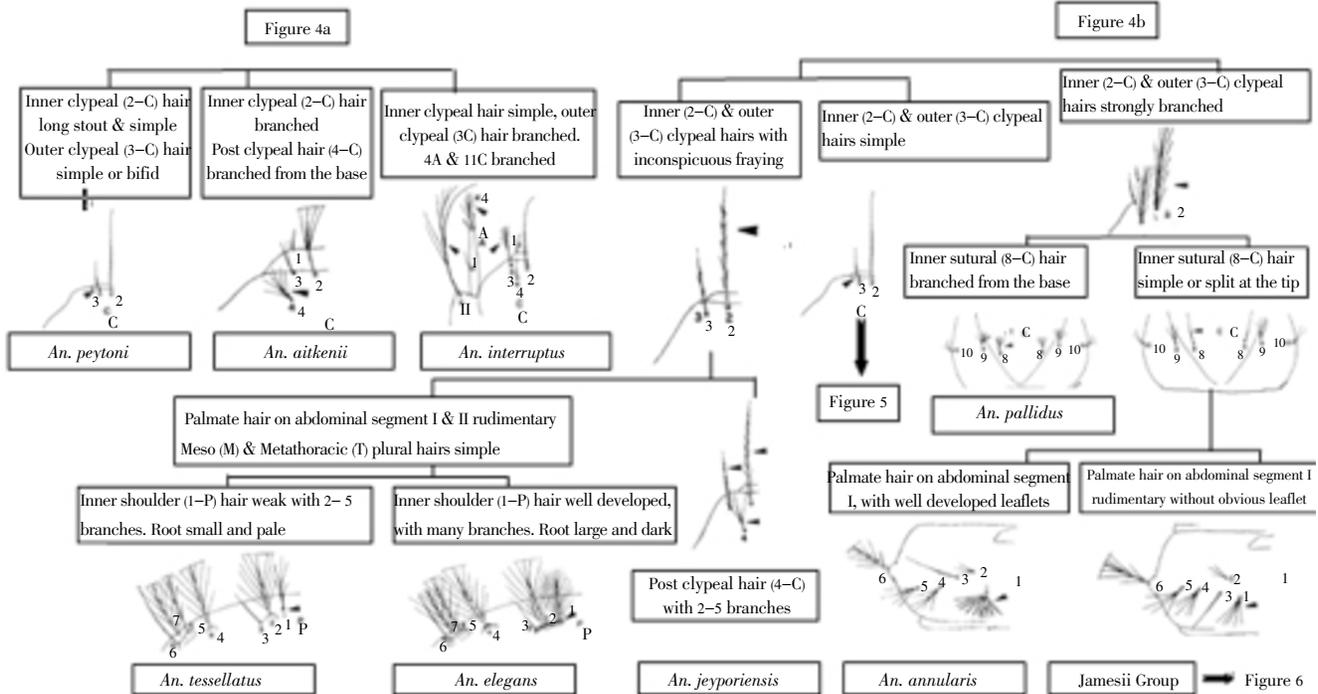


Figure 4. Thoracic palmate hair and abdominal tergal plates.

a: Thoracic palmate hair well developed; b: Abdominal tergal plates small, transverse.

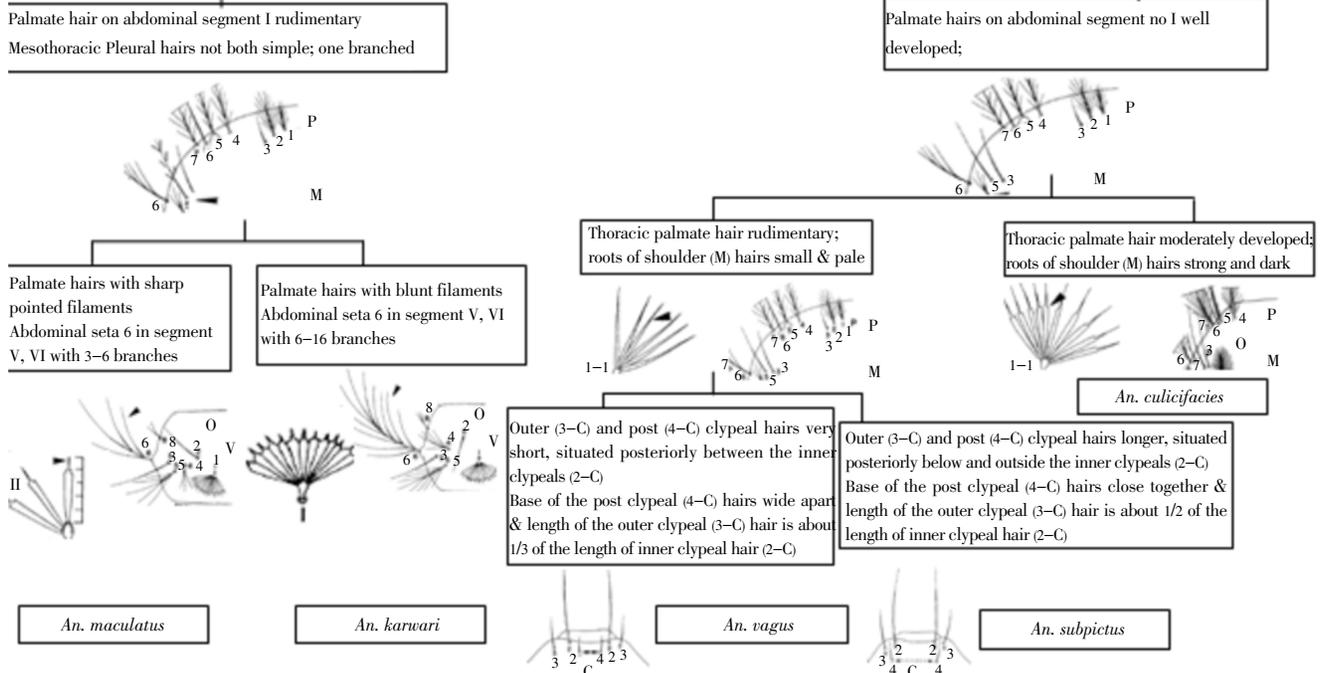


Figure 5. Palmate hairs on abdominal segments.

On abdominal segment II always well developed; hairs on segment I sometimes rudimentary; Meso and Metathoracic Pleural hairs not all simple; one or more branched.

is characteristic of this group. This character should be checked carefully during identification. The pigmentation of seta I–II is an useful secondary character in separating *barbirostris* from *barbumbrosus* (pale or colorless seta). In addition, seta orientation of the outer clypeal hair (3–C) can be used to separate these two species as the optional character in order to confirm the identification. It can be described as follows; 3–C of *An. barbumbrosus* has 12–36 thin attenuated branches usually loose and separated out, whereas 3–C of *An. barbirostris* has more branches which are thick (19–95), usually stiff and broom like (Figure 3). The larval morphology of *An. reidi* has still not been reported.

4.1.2. *Lophoscelomyia* series

An. interruptus is the only species in this series reported in the country. It can be distinguished by the simple inner clypeal hair (2–C), and branched outer clypeal hair (3–C), 4–A and 11–C. In the previous key for Sri Lankan anopheline larvae, the identification was confirmed through the presence of reduced setae 5, 6 and 7–C, some or all of which were short and a few-branched or simple; seta 4–C simple or branched only in distal half [2]. These characters are rather difficult in identification and controversial. Therefore, the new character which has been included in this key is useful to minimize misidentification of this species (Figure 4).

4.1.3. *Anopheles* series

There are three species in the country, namely: *Anopheles gigas*, *An. aitkenii* and *Anopheles peytoni* (*An. peytoni*). *An. peytoni* is an endemic species in Sri Lanka and had been confused with *An. insulaeflorum*; the larvae of *An. peytoni* has bases of setae 2–C nearly as wide apart as 2–C and 3–C on one side which is different from the larval morphology of *An. insulaeflorum*. In this key the following characters have been included to confirm identification of *An. peytoni*: 2–C long and simple, 3–C simple or bifid. These features facilitate the separation of *An. aitkenii* which has branched 2–C and 4–C (Figure 4).

4.2. Subgenus *Cellia*

4.2.1. *Myzomyia* series

Separating *Anopheles varuna* and *An. aconitus* in the *Myzomyia* series of subgenus *Cellia* is sometimes difficult when the barbed nature of setae 2–C, 3–C and branching of 4–C in *An. aconitus* are observed by drift or the larval mouth brushes. It is more difficult to distinguish *An. jeyporiensis* as it separates only by having a small tergal plate and 4–5 branches in 4–C (Figure 2).

An. culicifacies, the main vector of malaria in Sri Lanka can be separated very easily among the members in *Myzomyia* series of subgenus *Cellia* by the presence of small tergal plates, unbranched 2–C and 3–C and moderately developed thoracic palmate hairs (Figure 5).

4.2.2. *Pyrethophorus* series

An. subpictus and *An. vagus* are the only two recorded species in this series in Sri Lanka. These two species can be easily separated from *An. culicifacies* by the moderately developed thoracic palmate hair and strong and dark roots of shoulder hairs. *An. vagus* has very short 3–C and 4–C, 4–C is situated between 2–C with the base of 4–C being wide apart, and the length of 3–C being about 1/3 of the length of 2–C; *An. subpictus* has longer 3–C and 4–C, and 4–C seta is situated below and outside 2–C; the base of the 4–C seta and length of the 3–C is about 1/2 of the length of 2–C (Figure 5).

4.2.3. *Neocellia* series

Six species of Sri Lankan *Neocellia* can be divided into those with bushy setae 3–C (*Anopheles jamesii*, *Anopheles annularis*, *Anopheles pallidus*) and those with simple or barbed 3–C (*Anopheles maculatus*, *Anopheles kawari*, *Anopheles 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 (Figure 6).

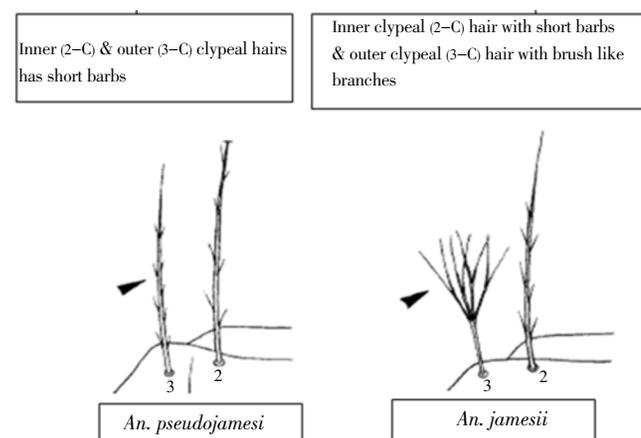


Figure 6. Palmate hair on abdominal segment I rudimentary without obvious leaflet.

4.2.4. *Neomyzomyia* series

Two species, *An. tessellatus* and *An. elegans* (the latter belonging to the *leucosphyrus* group) have been reported in Sri Lanka. *An. elegans* is an endemic species in the country. Identification of *An. tessellatus* is important in malaria vector surveillance programmes in the Sri Lanka as it has been incriminated as a secondary vector of malaria transmission in the country. Identification of this species can be confirmed by the presence of 2–5 branches in the inner shoulder hair (1–P) with small and pale roots. Individuals with opposite morphological features can be regarded as *An. elegans* (Figure 4).

Sri Lankan anopheline larvae can be differentiated easily by observing basic characters. However, this may become a difficult task when processing whole larvae in routine malaria entomological surveys.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Morphological taxonomic keys are indispensable tools in malaria vectors surveillance and control. They help field operators to easily and accurately identify anopheline species in the field. In Sri Lanka, the published keys are outdated and need to be revised as knowledge on anophelines have advanced.

Research frontiers

This paper is carried out to revise a larval morphological identification key of the anopheline species of Sri Lanka.

Related reports

This key has reported one more species than the guide to the identification of anopheline mosquitoes of Sri Lanka published by Amarasinghe in 1992.

Innovations and breakthroughs

This paper contains the first illustrated key that allows the identification of the immature of 22 anopheline species currently known in Sri Lanka.

Applications

This key will assist field workers involved in malaria surveillance and control in Sri Lanka to firmly identify the anopheline species currently known in this island.

Peer review

This study is very important because the identification of mosquito species is always the first step in all surveillance and control of malaria strategies. It describes an updated and easy way to use illustrated key that will be useful for Sri Lankan malaria control personnel and researchers interested in local anopheline fauna.

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