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A GUIDE TO THE IDENTIFICATION OF THE ANOPHELINE MOSQUITOES OF SRI LANKA. III. PUPAE

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Abstract: An Illustrated key is provided for the identification of the pupal stages of 21 of the 22 species of anopheline mosquitoes occurring in Sri Lanka, for which this life stage has been previously described. The species discussed are: *Anopheles (Anopheles) aitkenii* James, *An. (Ano.) barbirostris* Van der Wulp, *An. (Ano.) barbumbrosus* Strickland & Chowdhury, *An. (Ano.) gigas* Giles var. *refutans* Alcock, *An. (Ano.) interruptus* Puri, *An. (Ano.) nigerrimus* Giles, *An. (Ano.) peditaeniatus* Leicester, *An. (Ano.) peytoni* Kulasekera, Harrison & Amerasinghe, *An. (Cellia) aconitus* Dönitz, *An. (Cel.) annularis* Van der Wulp, *An. (Cel.) culicifacies* Giles, *An. (Cel.) elegans* James, *An. (Cel.) jamesii* Theobald, *An. (Cel.) karwari* (James), *An. (Cel.) maculatus* Theobald, *An. (Cel.) pallidus* Theobald, *An. (Cel.) pseudojamesi* Strickland & Chowdhury, *An. (Cel.) subpictus* Grassi, *An. (Cel.) tessellatus* Theobald, *An. (Cel.) varuna* Iyengar, and *An. (Cel.) vagus* Dönitz. The pupa of one species, *Anopheles (Anopheles) reidi* Harrison, is unknown.

Key words: *Anopheles*, key for identification, pupae, Sri Lanka.

INTRODUCTION

Mosquitoes of the genus *Anopheles* are vectors of human and animal malaria, and are also involved in the transmission cycles of filarial nematodes and viruses.[1-3] Recent research in Sri Lanka has shown that although *An. culicifacies* Giles (species B) is the major vector of malaria, several other species may be involved in seasonal or local transmission in different parts of the country.[4-6] Thus, accurate identification of anopheline species is becoming increasingly important in the context of local malaria entomology. As part of an ongoing study on the taxonomy of Sri Lankan mosquitoes, illustrated keys for the identification of adult and larval stages of local anophelines have been published previously.[7,8]

Anopheline pupal taxonomy, however, has received relatively little attention in the Indian subcontinent. Pupae possess stable taxonomic characters that are readily visible under high power on slide-mounted exuviae. However, these characters are more difficult to visualize on whole pupae under dissecting microscopes, due primarily to pupal shape, colouration, and the generally inconspicuous nature of taxonomically important setae. Pupae often constitute the smaller proportion of field-collected immatures (relative to larvae), and field workers usually go through the laborious process of rearing out adults from collected pupae in order to establish species identity. However, pupae that die during rearing and those directly killed and preserved in the field cannot be identified by such means. The routine identification of field collected pupae is of some importance, as apart from purely taxonomic considerations, quantitative data based on this life stage can provide better estimates of adult production from breeding habitats than those derived from larval stages alone. This is

because mortality within different larval stages, and in the larval-pupal transformation can be extremely high. Following upon the publication of keys for the identification of adults and larvae,[7,8] the present paper provides an illustrated key to the pupae of Sri Lankan anophelines, so as to complete the series on the taxonomy of the local anopheline species.

METHODS AND MATERIALS

The following 21 species are included in the key: *Anopheles (Anopheles) aitkenii* James, *An. (Ano.) barbirostris* Van der Wulp, *An. (Ano.) barbumbrosus* Strickland & Chowdhury, *An. (Ano.) gigas* Giles var. *refutans* Alcock, *An. (Ano.) interruptus* Puri, *An. (Ano.) nigerrimus* Giles, *An. (Ano.) peditaeniatus* Leicester, *An. (Ano.) peytoni* Kulasekera, Harrison & Amerasinghe, *An. (Cellia) aconitus* Dönitz, *An. (Cel.) annularis* Van der Wulp, *An. (Cel.) culicifacies* Giles, *An. (Cel.) elegans* James, *An. (Cel.) jamesii* Theobald, *An. (Cel.) karwari* (James), *An. (Cel.) maculatus* Theobald, *An. (Cel.) pallidus* Theobald, *An. (Cel.) pseudojamesi* Strickland & Chowdhury, *An. (Cel.) subpictus* Grassi, *An. (Cel.) tessellatus* Theobald, *An. (Cel.) varuna* Iyengar, and *An. (Cel.) vagus* Dönitz. The pupa of *An. (Ano.) reidi* Harrison is unknown, and is not included in the key.

The primary taxonomic literature references used in constructing the key were the works of Harrison & Scanlon[1] (for subgenus *Anopheles*), Reid[3] and Christophers[9] (for subgenera *Anopheles* and *Cellia*), and Harrison[10] (for the Myzomyia series of subgenus *Cellia*). These works contained taxonomic descriptions of pupae, as well as species-group related keys for the southeast Asian or Oriental regions, that allowed the formulation of key steps for most of the species occurring in Sri Lanka. In addition, Mendis *et al.*[11] and Kulasekera *et al.*[12] were consulted for detailed descriptions of *An. elegans* and *An. peytoni,* respectively.

Pupal stages (slide-mounted exuviae with associated larval and adult stages) of local *Anopheles* collected by the Smithsonian Institution's Medical Entomology Project (MEP), as well as field material collected by the author and deposited at the Department of Zoology, University of Peradeniya, were also examined during the study. These included *An. barbirostris, An. nigerrimus, An. peditaeniatus, An. peytoni, An. aconitus, An. annularis, An. culicifacies, An. elegans, An. jamesii, An. subpictus, An. varuna,* and *An. vagus*. Specimens of *An. tessellatus* were obtained from a colony maintained at the Institute of Fundamental Studies (IFS), Kandy. This material was used to check taxonomic characters used in the key, as well as to formulate the key characters used for the identification of *An. subpictus* and *An. vagus* (pupae of which have been incorrectly separated previously)[3], as well as *An. elegans* and *An. tessellatus* which have not been treated in previous pupal keys.[3]

Pupal chaetotaxy designations used herein follow Harbach & Knight.[13] The illustrations were drawn by the author partly from original material (in the case of species available for direct examination) and partly adapted from previous works.[1,3] They are diagrammatic representations of characters used in the key, meant as a guide to those unfamiliar with the morphology and chaetotaxy of

mosquito immature stages, and follow the standard conventions established for mosquito taxonomic publications.[13] The following points of clarification are made for those unfamiliar with aspects of the modern terminology and conventions that may be found in the key and illustrations: (i) Pupal setal numbers are denoted by arabic numerals and abdominal segment numbers by roman numerals; (ii) The meatal cleft of the trumpet is always located on the inner (mesal) side, and the secondary cleft (if present) located on the outer (lateral) side; (iii) The two borders of the paddle are referred to as lateral (outer) and mesal (inner) borders; (iv) The refractile border of the paddle refers to the region of the lateral border that usually bears marginal serrations ("paddle teeth"), and is refractile to light; (v) the term "mesad" refers to the inner (mesal) side, and "laterad" to the outer (lateral) side of any structure.

KEY TO ANOPHELINE PUPAE

1. Trumpet with longest axis more or less vertical to stem; rim of trumpet simple, without secondary cleft (Fig. 1a) 2

   Trumpet with longest axis transverse to stem; rim of trumpet with or without secondary cleft (Fig. 1b) (Subgenus *Anopheles*; Laticorn section; Myzorhynchus series; barbirostris and hyrcanus gps.) 18

2(1). Trumpet very broad and rounded, transverse axis about as long as vertical; a very large species (Fig. 2a) (Subgenus *Anopheles*; Angusticorn section; Anopheles series; lindesayi gp.) ..... ***gigas***

   Trumpet not so broad, vertical axis longer than transverse; variable size, but much smaller than above (Fig. 2b) 3

3(2). Paddle broad, 1.5 or less times as long as wide; seta 5-V-VII usually no stouter than 1-V-VII; male genital lobes with apical knobs (Fig. 3a) (Subgenus *Cellia*) 4

   Paddle elongate, 1.6 or more times as long as wide; if not elongate then seta 5-V-VII much stouter than 1-V-VII; male genital lobes not ending in knob (Fig. 3b) (Subgenus *Anopheles*; Angusticorn section; Anopheles and Lophoscelomyia series) 16

4(3). Seta 1-Pa short, 0.15 or less length of lateral margin of paddle; 9-V-VII usually less than 0.35 length of lateral margin of their respective segments (Fig. 4a) (Neomyzomyia series) 5

   Seta 1-Pa long, 0.25 or more length of lateral margin of paddle, curved, sinuate or hooked at tip; 9-V-VII usually 0.35 or more length of lateral margin of their respective segments (Fig. 4b) 6

5(4). Seta 1-VI,VII 3-5 branched; seta 9-V-VII, 0.15 or less length of lateral margin of respective segments; genital lobe with clearly defined knobs (Fig. 5a) ..... *tessellatus*

Seta 1-VI,VII usually single; seta 9-V-VII 0.25 or more length of lateral margin of respective segments; genital lobe with indistinctly defined knobs (Fig. 5b) ..... *elegans*

6(4). Seta 9-I simple, rarely branched, long, usually twice or more length of lateral margin of segment I (Fig. 6a) (Pyretophorus series) 7

Seta 9-I simple or branched, shorter to slightly longer than lateral margin of segment I (Fig. 6b) 8

7(6). Seta 9-V-VII with distinctly blunt tips (Fig. 7a) ..... *subpictus*

Seta 9-V-VII with distinctly sharp pointed tips (Fig. 7b) ..... *vagus*

8(6). Seta 9-IV usually 0.67 or more length of 9-V, with same tapering sharp pointed shape as 9-V; 1-II with 8 or more branches (Fig. 8a) (Myzomyia series) 9

Seta 9-IV 0.15 to 0.67 length of 9-V, broader with more rounded apex than 9-V; 1-II with 2-10 branches, usually less than 8 (Fig. 8b) (Neocellia series) 11

9(8). Seta 1-V-VII simple; seta 7-VI,VII same length or slightly shorter than 9-VI,VII, approximately 0.35 to 0.70 length of segment VI, VII lateral margins; paddle fringe not extending mesad of seta 1-Pa (Fig. 9a) ..... *culicifacies*

Seta 1-V-VII usually 2-5 branched; seta 7-VI,VII much longer than 9-VI,VII, equal to or slightly longer than segment VI, VII lateral margins; paddle fringe extending mesad of seta 1-Pa (Fig. 9b) 10

10(9). Paddle fringe spicules mesad of seta 1-Pa short, widely spaced, approximately 0.5 length of spicules just laterad of 1-Pa, mesal spicules not extendingto mesal angle of paddle; trumpet pinna distally rounded, venter convex at apex; sum of branches of both setae 1-III, 14- 32; sum of branches of both setae 5-III, 9-22 (Fig. 10a) ..... *aconitus*

Paddle fringe spicules mesad of seta 1-Pa long, closely spaced, equal in length to spicules just laterad of 1-Pa, mesal spicules extending to mesal angle of paddle; trumpet pinna distally flattened, venter concave at apex; sum of branches on both setae 1-III, 31-39; sum of branches on both setae 5-III, 22-37 (Fig. 10b) ..... *varuna*

11(8). Seta 9-IV usually less than 0.1 length of lateral margin of segment, less than 0.25 length of 9-V (Fig. 11a) ..... *karwari*

Seta 9-IV 0.2 or more length of lateral margin of segment, 0.25 or more length of 9-V (Fig. 11b) 12

12(11). Seta 1-Pa, unstraightened, about 0.33 or more length of paddle; fringe spicules extend mesad of seta 1-Pa (Fig. 12a) ..... *maculatus*

Seta 1-Pa, unstraightened, less than 0.33 length of paddle; fringe spicules do not extend mesad of seta 1-Pa (Fig. 12b) 13

13(12). Seta 2-VII usually simple; trumpet meatus about 0.33 trumpet length (Fig. 13a) ..... *pseudojamesi*

Seta 2-VII usually 2-4 branched; trumpet meatus about 0.25 trumpet length (Fig. 13b) 14

14(13). Seta 9-I usually triple; lower refractile border of paddle with non-filamentous spicules (Fig. 14a) ..... *annularis*

Seta 9-I simple or bifid; lower refractile border of paddle with filamentous spicules (Fig. 14b) 15

15(14). Seta 1-Pa strongly coiled (Fig. 15a) ..... *jamesii*

Seta 1-Pa hooked, but not coiled (Fig. 15b) ..... *pallidus*

16(3). Trumpet without meatal cleft; seta 1-Pa with 2-5 branches from midpoint; phytotelmic habitats (Fig. 16a) (Lophoscelomyia series; asiaticus gp.) ..... *interruptus*

Trumpet with deep meatal cleft; seta 1-Pa simple; ground water habitats (Fig. 16b) (Anopheles series, aitkenii gp.) 17

17(16). Seta 1-IV with 2-5 branches; seta 9-IV with blunt, rounded tip; paddle refractile margin long (0.6) (Fig. 17a) ..... *aitkenii*

Seta 1-IV with 5-9 branches; seta 9-IV with sharp tip; paddle refractile margin short (0.4-0.5) (Fig. 17b) ..... *peytoni*

18(1). Opening of trumpet narrow, very transverse when viewed from above; seta 1-VII a strong tuft of 15-50 branches (Fig. 18a) (barbirostris gp.) 19

Opening of trumpet more expanded when viewed from above; seta 1-VII a weaker tuft of 1-7 branches (Fig. 18b) (hyrcanus gp.) 20

19(18). Trumpet without secondary cleft, but with thickened seam; abdominal seta 9 dark brown to black; seta 9-VII 6-8 times as long as thick (Fig. 19a) ..... *barbumbrosus*

Trumpet with secondary cleft, without seam; abdominal seta 9 yellow to light brown; seta 9-VII 4-6 times as long as thick (Fig. 19b) ..... *barbirostris*

20(18). Trumpet with thickened saw-toothed areas on rim; seta 9-VIII branching reduced or absent (Fig. 20a) ..... *peditaeniatus*

Trumpet without thickened saw-toothed areas, but with dark border area delineating thin, uniform rim; seta 9-VIII with well developed branches (Fig. 20b) ..... *nigerrimus*

(The pupa of *An. (Ano.) reidi* of the barbirostris group is unknown. However, based on group characteristics it should key out to the barbirostris group at step 18).

Figures 1-20 are diagrammatic representations of key characters, numbered in accordance with the key steps. Abbreviations are as follows: GL = genital lobe, MC = meatal cleft, MP = metanotal plate, Pa = paddle, S = seam, SC = secondary cleft, Tr = trumpet.

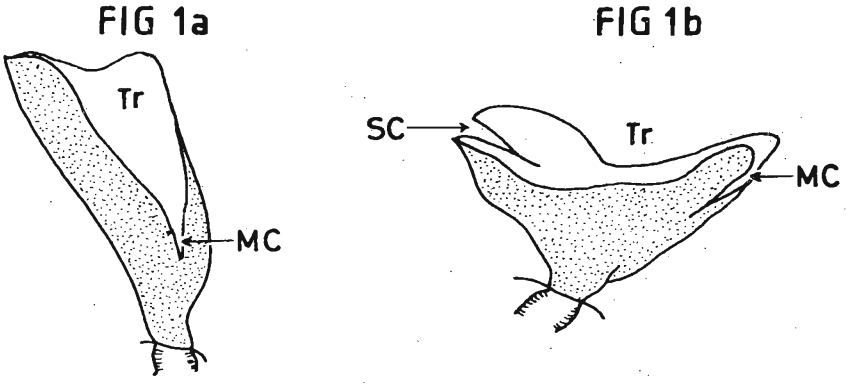


Figure 1a,b: Lateral view of angusticorn and laticorn trumpets.

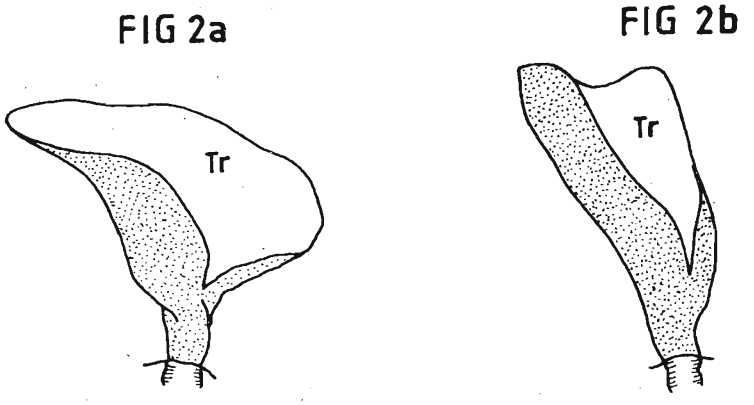


Figure 2a,b: Lateral view of two angusticorn trumpets.

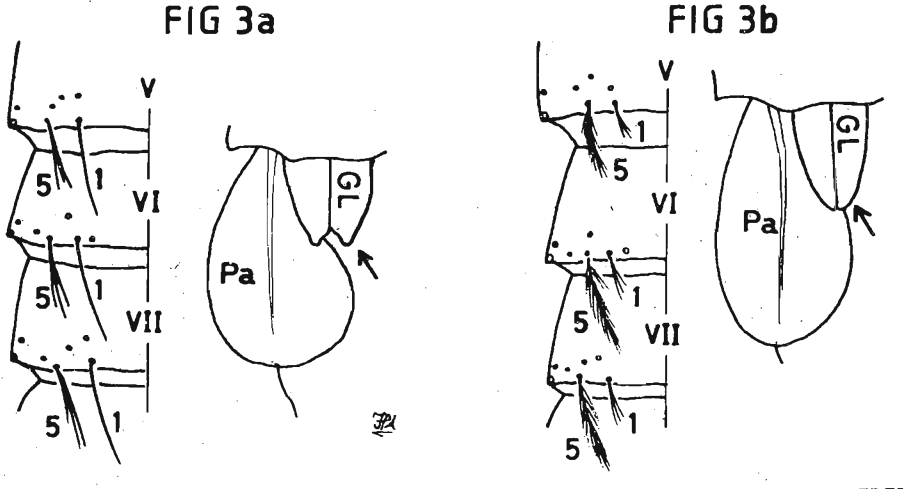


Figure 3a,b: Dorsal view of abdominal segments V-VII to show seta 1,5-V-VII, and ventral view of genital lobe.

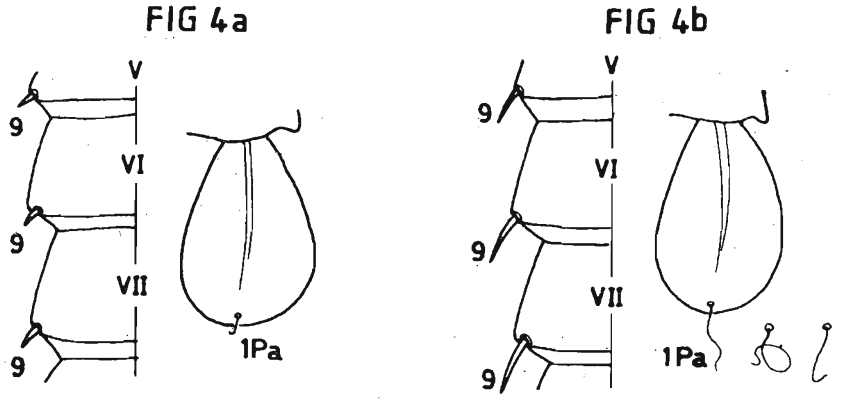


Figure 4a,b: Dorsal view of abdominal segments V-VII to show seta 9-V-VII, and of paddle to show seta 1-Pa.

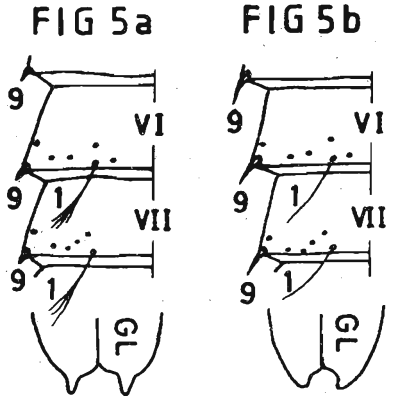


Figure. 5a,b: Dorsal view of segments VI-VIII to show seta 1-VI-VII, and ventral view of male genital lobe.

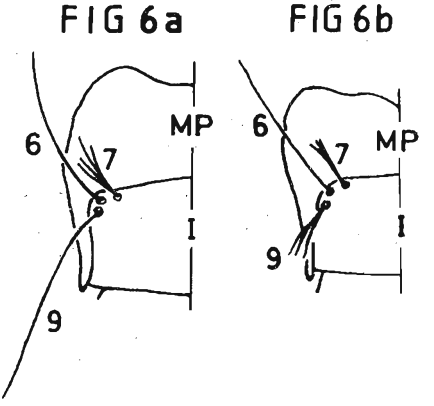


Figure. 6a,b: Dorsal view of abdominal segment I, to show seta 9-1.

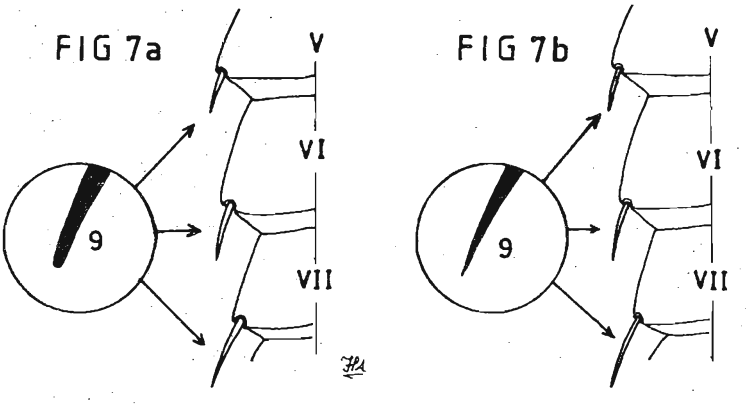


Figure. 7a,b: Dorsal view of abdominal segments V-VII, to show seta 9-V-VII.

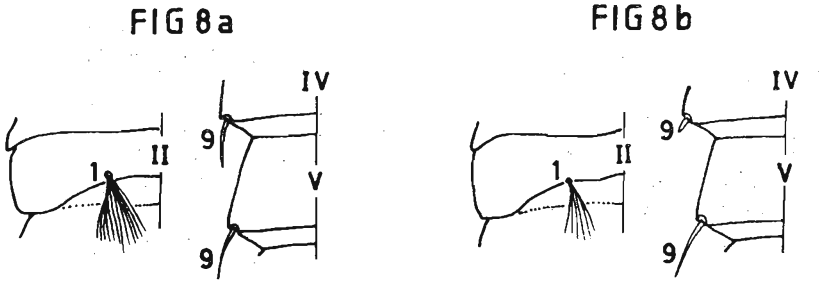


Figure. 8a,b: Dorsal view of abdominal segments II and IV-V, to show seta 1-II and 9-IV-V, respectively.

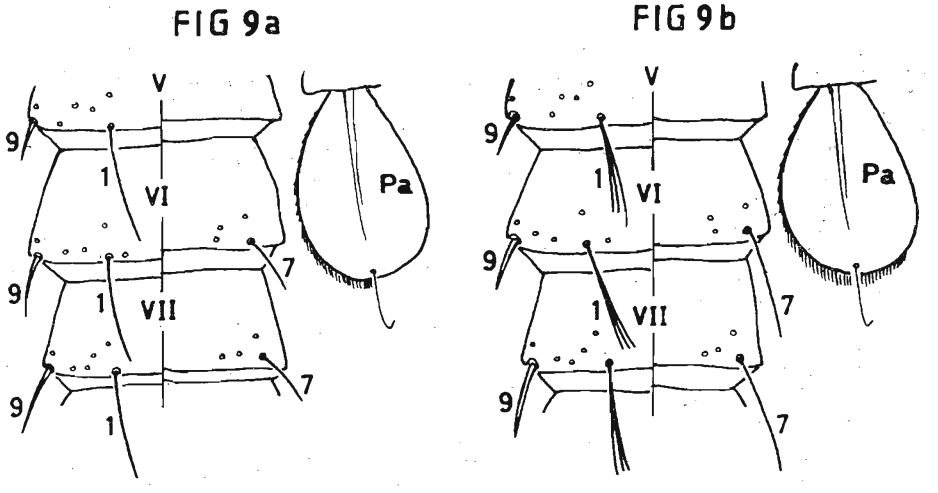


Figure. 9a,b: Dorsal (left) and ventral (right) view of abdominal segments V-VII to show seta 1,7,9-V-VII, and dorsal view of paddle to show distribution of fringe spicules.

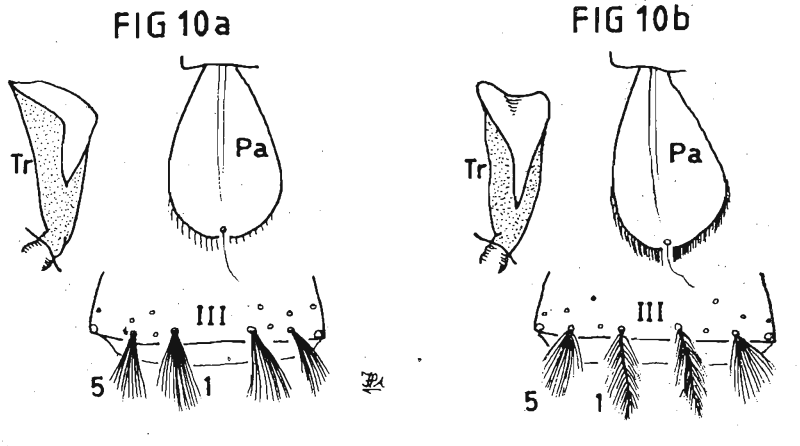
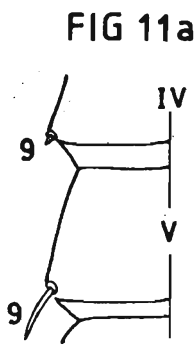


Figure. 10a,b: Lateral view of trumpet to show shape of pinna, dorsal view of paddle to show distribution of fringe spicules, and dorsal view of abdominal segment III to show seta 1,5-III.



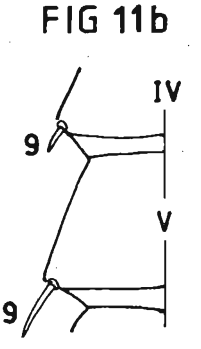
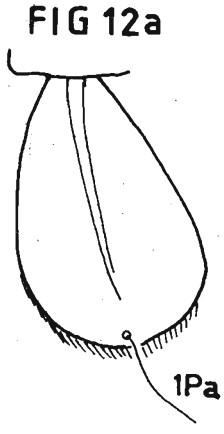


Figure 11a,b: Dorsal view of abdominal segments IV,V to show seta 9-IV,V.



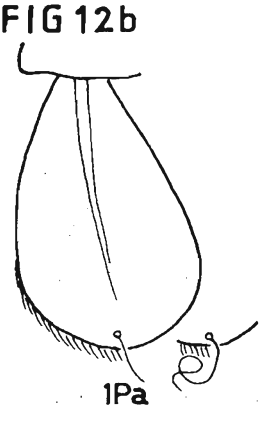
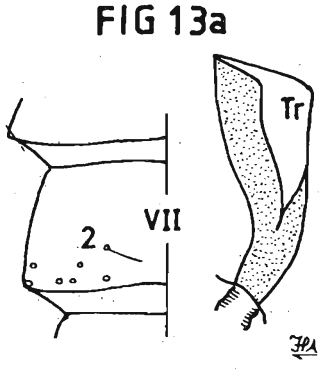


Figure 12a,b: Dorsal view of paddle to show seta 1-Pa and distribution of fringe spicules.



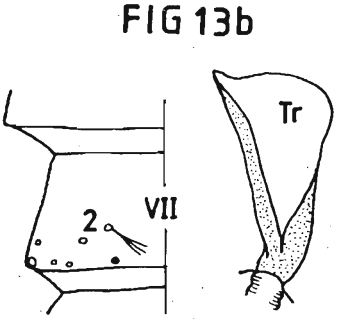


Figure 13a,b: Dorsal view of abdominal segment VII to show seta 2-VII, and lateral view of trumpet to show relative meatal length.

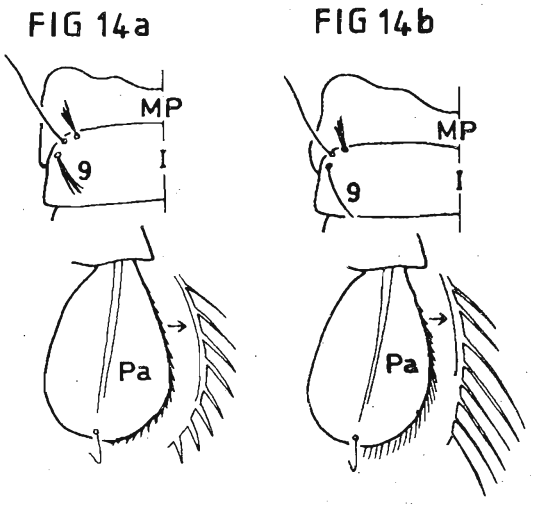


Figure 14a,b: Dorsal view of abdominal segment I to show seta 9-I, and of paddle to show fringe spicules.

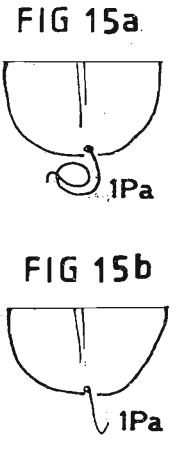


Figure 15a,b: Dorsal view of paddle to show seta 1-Pa.

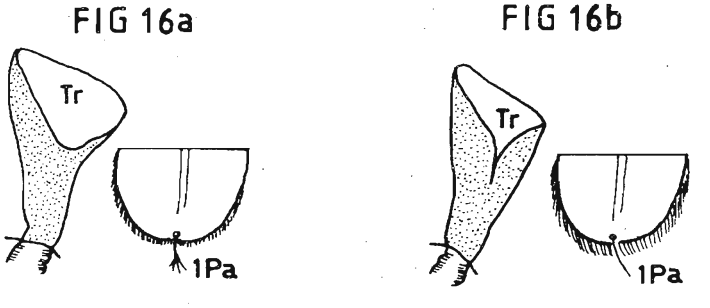


Figure 16a,b: Lateral view of trumpet to show meatal cleft, and dorsal view of paddle to show seta 1-Pa.

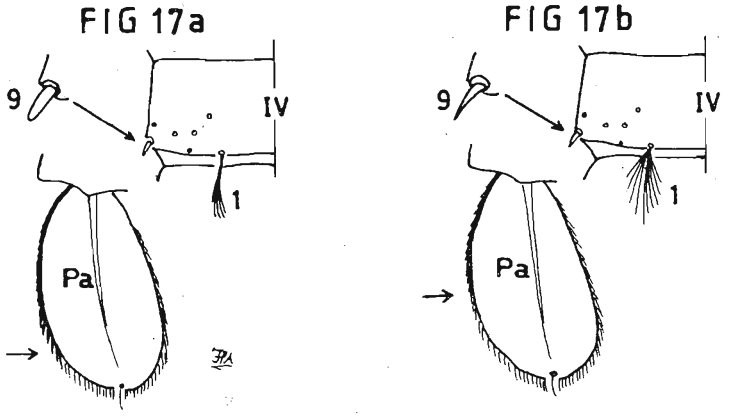
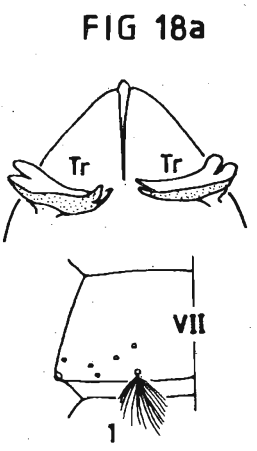


Figure 17a,b: Dorsal view of abdominal segment IV to show seta 9-IV, and of paddle to show refractile border.



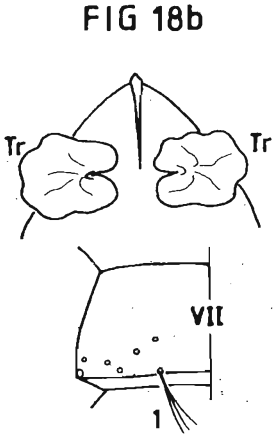
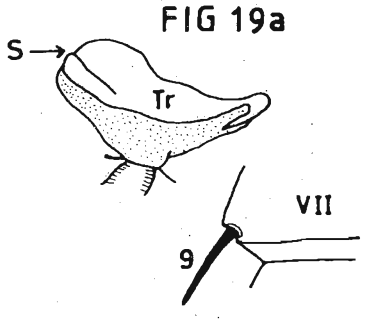


Figure 18a,b: Dorsal view of trumpets to show transverse and expanded nature, and of abdominal segment VII to show seta 1-VII.



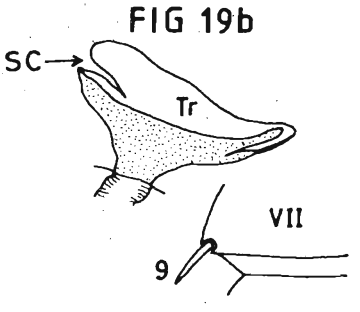
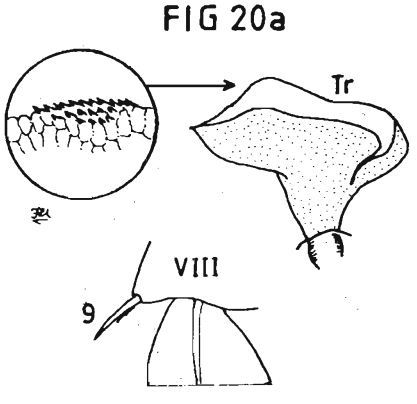


Figure 19a,b: Lateral view of trumpet to show nature of secondary cleft, and dorsal view of abdominal segment VII to show seta 9-VII.



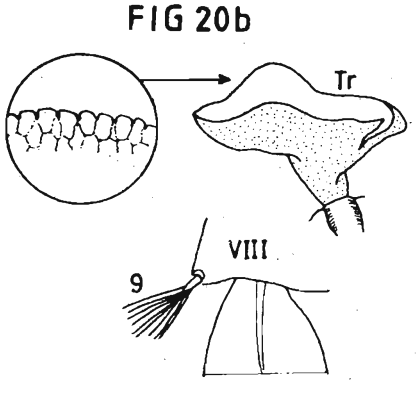


Figure 20a,b: Lateral view of trumpet to show nature of rim, and dorsal view of abdominal segment VIII to show seta 9-VIII.

NOTES ON THE KEY

1. Much of the emphasis in the present key is on characters of the pupal trumpet, paddle, and the more prominent abdominal setae which can be visualized without too much difficulty even on whole pupae. In most instances, however, the magnification provided by dissecting microscopes will be insufficient, and it will be necessary to make a temporary mount of the pupae on microscopical slides and view at magnifications upto 100x under a transmitted-light microscope for a definite identification to be made.

2. The key does not include all possible characters useful in identifications, as this would make it unwieldy and time consuming to use. The characters selected for use in the key provide the most rapid and unambiguous means of separating the different species. Additional characters that can be used in the separation of some species pairs are listed below.

3. The pupae of *An. elegans* and *An. tessellatus* have not been separated previously. The characters used in the key (key step 5) are based on the examination of Sri Lankan material of both species. Whilst *An. elegans* reportedly occurs only in South India outside of Sri Lanka, *An. tessellatus* is widespread throughout the Oriental region and the variability in its pupal characters on a regional scale is unknown. Thus, the characters used in the key should be regarded as provisional, and applicable only to the local populations of these two species.

4. The separation of *An. subpictus* and *An. vagus* pupae (key step 7) should be regarded as provisional as it is based on a single character seen in Sri Lankan material. Reid[3] used the length and branching of seta 6-IV (short and double in *An. subpictus*, long and single in *An. vagus*) and the nature of the lower paddle spicules (tips hooked in *An. subpictus,* not hooked in *An. vagus*) to separate the two species.

However, Reid's keys were based on descriptions and/or the examination of very few specimens of these species from the Southeast Asian region. I have found the appearance of the paddle spicule tips and the length/branching of seta 6-IV to be unreliable for the separation of Sri Lankan *An. subpictus* and *An. vagus*. Indeed, contrary to Reid, most local *An. subpictus* have 6-IV single, and *An. vagus* have seta 6-IV double. In the present work, seta 9-V-VII (blunt tip in *An. subpictus*, sharply pointed tip in *An. vagus*) has been used to separate the two species, based on an examination of local material.

5. *An. interruptus* can be separated from the aitkenii group (key step 16) on the basis of the following additional characters: seta 9-V-VII hooked at tip, seta 5-V-VII with long, strong axis and short branches along its length, and seta 1-V-VII very small and weak in *An. interruptus*, contrasted with seta 9-V-VII not hooked at tip, seta 5-V-VII with weaker axis and long branches along its length, and seta 1-V-VII well developed in the aitkenii group.[1]

6. *An. barbumbrosus* and *An. barbirostris* can be separated on the basis of an additional character at key step 19. The sum of the branches of both setae 5-III less than 30 in *An barbumbrosus*, whilst this sum is grea ter than 30 in *An. barbirostris*.[1]

7. *An. peditaeniatus* and *An. nigerrimus* can be separated by an additional character at key step 20. *An. peditaeniatus* has seta 1-V with 1-6 branches whilst *An. nigerrimus* has seta 1-V with 8 or more branches.[1]

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