KEYS FOR THE IDENTIFICATION OF THE IMMATURE STAGES OF GENUS *CULEX* (DIPTERA: CULICIDAE) IN SRI LANKA

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Abstract: This paper provides keys for the identification of the pupae and fourth instar larvae of 34 species of *Culex* mosquitoes (Diptera: Culicidae) occurring in Sri Lanka. These species belong to the subgenera *Culex* (14 species), *Culiciomyia* (6 species), *Eumelanomyia* (3 species), *Lophoceraomyia* (9 species) and *Lutzia* (2 species). The immature stages of 3 species (i.e., 1 species in subgenus *Culiciomyia* and 2 species in subgenus *Eumelanomyia*) that also occur in the island are presently unknown, and are not included in this work.

Keywords: Identification keys, mosquitoes, *Culex*, pupae, larvae.

INTRODUCTION

Thirty seven members of genus *Culex* Linnaeus have been reported to occur in Sri Lanka,¹ and several of these are vectors of arboviral and nematode diseases affecting humans and livestock.^{2,3} Keys for the identification of the adult stages of the species occurring in Sri Lanka have been produced recently.⁴ Studies on the biology and disease relations of mosquitoes involve not only adults but also the immature stages, whose breeding habitats, abundance and seasonal patterns are important disease surveillance parameters. Fundamental to such studies is the capability to accurately identify mosquito immatures at the species level. Taxonomic keys, which provide the means to identify immature stages of the local *Culex* fauna, are presently not available, although regional keys which include many (but not all) local species have been formulated. In addition, there has generally been an emphasis on larval identification, although the pupal stage too offers characters that are useful in species-level recognition. The present paper, therefore, provides keys to both larvae and pupae of Sri Lankan *Culex* to enable workers to reliably identify these species.

METHODS AND MATERIALS

Primary literature sources consulted were detailed species descriptions and keys contained in the works of Belkin,⁵ Bram,⁶ Sirivanakarn^{3,7-11} and Darsie & Pradhan.¹² Once formulated, key characters were checked (and modified when necessary) against Sri Lankan reference material present in the collections at the Department of Zoology, University of Peradeniya. Illustrations were drawn by the author, from original material. All scale bars included with the illustrations are in mm. Reference to general illustrations appropriate for each key is provided immediately below each heading, and specific illustrations are cited as required at specific key steps. The terminology used follows Harbach & Knight.¹³ The keys have been prepared for use with specimens in reasonably good condition. Damaged immatures with missing setae will, in most cases, be difficult or impossible to identify.

The following species of genus *Culex* are treated in this work:

(a) Subgenus *Culex* Linnaeus: *bitaeniorhynchus* Giles, *fuscocephala* Theobald, *gelidus* Theobald, *hutchinsoni* Barraud, *infula* Theobald, *jacksoni* Edwards, *mimulus* Edwards, *pseudovishnui* Colless, *quinquefasciatus* Say, *sinensis* Theobald, *sitiens* Wiedemann, *tritaeniorhynchus* Giles, *vishnui* Theobald, *whitmorei* (Giles).

(b) Subgenus *Culiciomyia* Theobald: *bailyi* Barraud, *fragilis* Ludlow, *nigropunctatus* Edwards, *pallidothorax* Theobald, *scanloni* Bram, *spathifurca* (Edwards).

(c) Subgenus *Eumelanomyia* Theobald: *brevipalpis* (Giles), *malayi* (Leicester), *pluvialis* Barraud).

(d) Subgenus *Lophoceraomyia* Theobald: *bicornutus* (Theobald), *infantulus* Edwards, *lasiopalpis* Sirivanakarn, *mammilifer* (Leicester), *minutissimus* (Theobald), *quadripalpis* (Edwards), *rubithoracis* (Leicester), *uniformis* (Theobald), *wardi* Sirivanakarn.

(e) Subgenus *Lutzia* Theobald: *fuscus* Wiedemann, *halifaxii* Theobald.

The larval and pupal stages of *Cx. (Culiciomyia) bahri* (Edwards), *Cx. (Eumelanomyia) campilunati* Carter & Wijesundara, and *Cx. (Eum) castrensis* Edwards which also occur in Sri Lanka are unknown, and thus these species are not included in this work. At present, they can be identified only in the adult stage.⁴

1. KEY TO SUBGENERA OF CULEX IN SRI LANKA - PUPAE

(Refer Figs. 1A-D)

1. Setae 2, 3-CT very close together; paddle clearly emarginate at tip (Fig. 1D) *Lutzia*
(*fuscus* & *halifaxii*, inseparable)
- Setae 2, 3-CT usually widely separated; paddle evenly rounded or only slightly emarginate at tip (Fig. 1C) 2
- 2(1). Seta 9-VIII at or near caudolateral angle of segment VIII *Eumelanomyia*
Seta 9-VIII well cephalad from caudolateral angle of segment VIII (Fig. 1C) 3
- 3(2). Seta 10-CT multiple, rarely 3-4 branched *Culex*
Culiciomyia
- Seta 10-CT single or double, rarely 3-branched *Lophoceraomyia*

NOTE: Pupae of the subgenera *Culex* and *Culiciomyia* cannot be separated with certainty, and other associated life stages will be required for the separation to be made.

2. KEY TO SPECIES OF SUBGENUS *CULEX* - PUPAE

(Refer Figs. 1A-G, 2A-C)

1. Seta *1-II* multiple, strongly dendritic or with more than 10 branches; trumpet yellowish; paddle entirely pale or transparent 2
 Seta *1-II* single, forked or with less than 10 branches, if more, then trumpet dark brown or paddle with conspicuous darkened areas 4
- 2(1). Setae *8-CT* and *6-III-V* double or triple 3
 Setae *8-CT* and *6-III-V* 4-6 branched *fuscocephala*
- 3(2). Seta *1-CT* 3-4 branched; seta *6-VI* 4-5 branched; pinna of trumpet strongly oblique (Fig. 2B) *quinquefasciatus*
 Seta *1-CT* double; seta *6-VI* double or triple; pinna of trumpet weakly oblique (Fig. 2C) *hutchinsoni*
- 4(1). Trumpet asymmetrically funnel shaped; pinna strongly widened or flared toward apex (Fig. 2A) 5
 Trumpet cylindrical; pinna same diameter as meatus or slightly widened toward apex (Fig. 2B-C) 7
- 5(4). Trumpet whitish or lightly darkened on meatus; cephalothorax and abdomen very pale or whitish *sinensis*
 Trumpet, cephalothorax and abdomen yellowish to dark brown; abdominal segments *III-VIII* strongly yellow or dark brown, with or without pattern of dark alveolar spots; paddle with darkened or infuscated areas 6
- 6(5). Setae *10-CT* and *3-I-III* usually single *bitaeniorhynchus*
 Setae *10-CT* and *3-I-III* double *infula*
- 7(4). Seta *8-CT* double or triple 8
 Seta *8-CT* 4-6 branched 12
- 8(7). Seta *1-II* 15-20 branched *gelidus*
 Seta *1-II* 1-10 branched 9
- 9(8). Seta *6-V, VI* 1-2 branched 10
 Seta *6-V, VI* 3-4 branched 11
- 10(9). Trumpet short, index about 5, pinna strongly oblique; seta *1-II* forked into 4-8 branches *sitiens*
 Trumpet longer, index about 7, pinna weakly oblique; seta *1-II* single or double *jacksoni*

- 11(9). Trumpet index 8-13; Seta 1-VI triple, 1-VII 2-3 branched
(Fig. 1A,C) (in part) *mimulus*
Trumpet index 5-9; Seta 1-VI 4-5 branched, 1-VII 3-4
branched *vishnui*
- 12(7). Cephalothorax and abdomen with dark areas on leg and
wing cases, metanotum and abdominal segments I-IV;
setae 5-V, VI 6-8 branched, their length much shorter
than segments following *whitmorei*
Cephalothorax and abdomen without dark areas or with
dark areas not forming definite pattern; setae 5-V,
VI double, their length as long as or longer than segments
following 13
- 13(12). Setae 6-IV-VI 2-4 branched; trumpet index 8-13
(Fig. 1A,C) (in part) *mimulus*
Setae 6-IV-VI 4-7 branched; trumpet index
6-8 14
- 14(13). Trumpet dark brown; seta 1-II with more than
10 branches *tritaeniorhynchus*
Trumpet pale yellowish; seta 1-II with less than
10 branches *pseudovishnui*

NOTE: Sirivanakarn³ uses the presence of a dark spot at the apex of the paddle midrib to characterize *Cx. gelidus* (key step 8). This character was absent in the local material examined during this study, and is not included as a key character here.

3. KEY TO SPECIES OF SUBGENUS *CULICIOMYIA* - PUPAE

(The pupa of *Cx. bahri* is unknown)

(Refer Figs: 1A-C)

1. Seta 5-IV-VI short, not reaching posterior margin of following
segment *nigropunctatus*
At least seta 5-IV,V long, reaching or
exceeding posterior margin of following
segment (Fig. 1C) 2
- 2(1). Seta 10-CT with 3 branches 3
Seta 10-CT with 5-6 branches 4
- 3(2). Seta 12-CT double; setae 5-II-III 4-branched; seta 1-VI more than
half the length of seta 5-VI *scanloni*
Seta 12-CT 4-branched; setae 5-II-III double; seta 1-VI less than
half the length of seta 5-VI *bailyi*

- 4(2). Seta 1-IV,V less than half the length of the corresponding
seta 5-IV,V *pallidothorax*
Seta 1-IV,V equal to or greater than half the
length of the corresponding seta 5-IV,V 5
- 5(4). Paddle 1.5 or less times as long as broad *spathifurca*
Paddle 2.0 times as long as broad *fragilis*

4. KEY TO SPECIES OF SUBGENUS *EUMELANOMYIA* - PUPAE

(The pupae of *Cx. campilunati* and *Cx. castrensis* are unknown)

(Refer Figs. 1C, 1E-G, 2D)

1. Trumpet pinna with narrow slit extending to meatus
(Fig. 2D) *brevipalpis*
Trumpet pinna without narrow slit extending to
meatus 2
- 2(1). Seta 5-IV-VI and 9-VII,VIII with smooth branches
(Fig. 1E) *pluvialis*
Seta 5-IV-VI with lightly aciculate branches;
seta 9-VII,VIII with strongly aciculate branches,
often dendritic (Figs. 1F,G) *malayi*

5. KEY TO SPECIES OF SUBGENUS *LOPHOCERAOMYIA* - PUPAE

(Refer Figs 1A-C)

1. Pinna of trumpet without slit extending to meatus 2
Pinna of trumpet with slit extending to meatus
(Fig. 2D) 3
- 2(1). Seta 5-CT 5-6 branched; trumpet index 8 *uniformis*
Seta 5-CT 2-4 branched; trumpet index
at least 10 *bicornutus*
- 3(1). Seta 5-V long, exceeding posterior margin of following segment
(Fig. 1C) 4
Seta 5-V short, not reaching posterior margin of following
segment 5
- 4(3). Seta 8-CT 3-4 branched; seta 5-IV double or triple, long,
extending well beyond posterior margin of following
segment *lasiopalpis*
Seta 8-CT usually double; seta 5-IV with 5-6 branches, shorter,
reaching but not extending much beyond posterior margin
of following segment (Fig. 1C) *wardi*
mammilifer

- 5(3). Setae 6-III-IV usually 2-4 branched6
 Setae 6-III-IV usually 5-6 branched*minutissimus*
infantulus
- 6(5). Seta 5-V usually 5-6 branched; seta 6-IV-VI usually 4-6
 branched*quadripalpis*
 Seta 5-V usually 2-4 branched; seta 6-IV-VI usually 2-4
 branched*rubithoracis*

NOTE: Separations at key steps 5 and 6 are tenuous, since setal ranges often overlap.

6. KEY TO SUBGENERA OF *CULEX* IN SRI LANKA -

4TH INSTAR LARVAE

(Refer Figs. 2E, H, 3A, B)

1. Pecten extending nearly to apex of siphon; lateral palatal brushes composed of laminated plates (Fig. 2H); seta 1-A short, single, simple, inserted on proximal 0.5 of antennal shaft; large species *Lutzia*
(*fuscanus* & *halifaxii*, inseparable)

Pecten restricted to basal 0.5 of siphon; lateral palatal brushes composed of filaments; seta 1-A generally multiple, inserted on distal 0.5 of antennal shaft; small to moderate sized species.....2

- 2(1).. Seta 4-X with 4 pairs of fan-like setae inserted on grid; seta 1-C
fine, filamentous; seta 3-P more slender than 1-P and about
0.5 its length *Culiciomyia*
Seta 4-X with 5 or more pairs of fan-like setae on grid; seta 1-C
robust, or if seta 1-C filamentous, then 1, 3-P single, subequal
in size 3

- 3(2). Seta 3-*P* always single, subequal to 1-*P* *Culex*
 Seta 3-*P* single or branched, more slender than 1-*P* and ≤ 0.5
 its length 4

- 4(3). Seta 5, 6-C short, weak, 5-C about 0.5 length of 6-C; pecten teeth fringed with many parallel, closely placed denticles distally and a few coarse, widely placed denticles proximally *Eumelanomyia*
- Seta 5, 6-C strong, long, subequal in length; pecten teeth without 2 distinct types of denticles as described above, although proximal 2-3 denticles may be enlarged *Lophoceraomyia*

7. KEY TO SPECIES OF SUBGENUS *CULEX* - 4TH INSTAR LARVAE

(Refer Figs. 2E-G, 2I-J, 3A-E)

1. Median labral plate of head capsule present as a distinct transverse bar separated from dorsal apotome (Fig. 2E,G) 2
- Median labral plate of head capsule not seen as distinct bar separated from dorsal apotome (Fig. 2F) 12
- 2(1). Seta *1-C* pale, slender; distally tapered or filamentous 3
- Seta *1-C* dark, stout, spiniform or foliform, abruptly pointed or blunt apically 4
- 3(2). Setae *5, 6-C* double; mental plate with 6-7 lateral teeth on each side of median tooth; siphon slender, cylindrical, tapered *fuscocephala*
- Setae *5, 6-C* 4-6 branched; mental plate with 10-12 lateral teeth on each side of median tooth; siphon short, basal 0.5 distinctly swollen *quinquefasciatus*
- 4(2). Siphon short, fusiform, swollen at middle, with a strong subapical spine on dorsal surface (Fig. 3C); setae *1-M, 1-T* very strongly developed *hutchinsoni*
- Siphon length variable, without subapical spine on dorsal surface; setae *1-M, 1-T* short and weak 5
- 5(4). Seta *7-I* double *tritaeniorhynchus*
- Seta *7-I* single 6
- 6(5). Siphon short, fusiform, swollen at middle, index 3-4 *gelidus*
- Siphon longer, subcylindrical, index at least 5 7
- 7(6). Seta *4-P* double or multiple 8
- Seta *4-P* single 11
- 8(7). Seta *1-C* slender or spiniform, with pointed apex (Fig. 2E); anal papillae as long or longer than saddle length (Fig. 3B) 9
- Seta *1-C* flattened or foliform, with blunt apex (Fig. 2G); saddle complete; anal papillae short, rounded, less than 0.5 length of saddle (Fig. 3D) *sitiens*

- 9(8). Comb with single row of 4-8 large spiniform scales;
 seta 4-P double or multiple; pleural areas of thorax
 not spiculate 10
 Comb with 16-20 or more small scales in 3-4 rows;
 seta 4-P always double; pleural areas of thorax
 spiculate *vishnui*
- 10(9). Siphon strongly tapered, distally upcurved; siphonal tufts
 very strong, 2-3 branched each, 4-5 times as long as
 siphonal width at point of attachment; seta 4-P always
 double *whitmorei*
 Siphon moderately tapered, straight or very slightly distally
 curved; siphonal tufts weak, 4-6 branched each, as long
 or only slightly longer than siphonal width at point of
 attachment; seta 4-P double or multiple *pseudovishnui*
- 11(7). Siphon with some prominent ventral spines on the apical
 half (Fig. 3E) *jacksoni*
 Siphon without spines as above (Fig. 3B) *mimulus*
- 12(1). Setae 2, 3-A located half way between apex and base of seta 1-A
 on antennal shaft (Fig. 2I); 4-P short and minute,
 indistinct; siphon with 5 pairs of seta 1-S *sinensis*
 Setae 2, 3-A located almost apically (Fig. 2J); seta 4-P
 strong and distinct; siphon with 3-4 pairs of seta 1-S 13
- 13(12). Siphon usually with 3 pairs (total 6) of seta 1-S *infula*
 Siphon usually with 4 pairs (total 8) of seta 1-S *bitaeniorhynchus*

NOTE: At Key step 13, some specimens have an unpaired seta 1-S, resulting in a total of 5 setae (2.5 pairs) in *Cx. infula* and 7 setae (3.5 pairs) in *Cx. bitaeniorhynchus*. The setae 1-S are also referred to as subventral tufts.

8. KEY TO SPECIES OF SUBGENUS *CULICIOMYIA* - 4TH INSTAR LARVAE

(The larva of *Cx. bahri* is unknown)

(Refer Figs. 2E, 3A-B, 3F)

1. Setae 1, 2, 3-P single 2
 Setae 1-P double, 2-P single and 3-P either
 single or with 2 or more branches 5
- 2(1). Siphon with a false joint - an irregular ring distal to middle
 due to lack of sclerotization (Fig. 3F) *nigropunctatus*
 Siphon without a false joint (Fig. 3B) 3

- 3(2). Siphon index 8 or greater; basal barb of each pecten tooth robust, markedly larger than other lateral barbs *scanloni*
Siphon index 6 or less; basal barb of each pecten tooth equal to or smaller than other lateral barbs 4
- 4(3). Setae 5, 6-C 3-4 branched; individual siphon tufts single *spathifurca*
Setae 5, 6-C 5-7 branched; individual siphon tufts 2-3 branched *fragilis*
- 5(1). Siphon narrow, cylindrical, seta 1-S tufts single or double; pecten with more than 10 teeth *bailyi*
Siphon swollen at middle, seta 1-S tufts 3-4 branched; pecten with less than 10 teeth *pallidothorax*

9. KEY TO SPECIES OF SUBGENUS *EUMELANOMYIA* - 4TH INSTAR LARVAE

(The larvae of *Cx. campilunati* and *Cx. castrensis* are unknown)
(Refer Figs. 2E, 2J, 3A-B)

1. Setae 2, 3-A placed apically (Fig. 2J); siphon very long,
 index 12-15, with weak seta 1-S tuft *brevipalpis*
 Setae 2, 3-A placed subapically; siphon moderately long,
 index 7-8, with strong seta 1-S tufts 2
- 2(1). Seta 5-C half the length of 6-C; segment VIII with 15-25
 comb scales usually in a single row; distal pecten teeth
 4-5 times as long as basal ones *malayi*
 Seta 5-C about one fourth the length of 6-C; segment VIII
 with 45-50 comb scales in at least two rows; distal pecten
 teeth 2-3 times as long as basal ones *pluvialis*

10. KEY TO SPECIES OF SUBGENUS LOPHOCERAOMYIA - 4TH INSTAR LARVAE

(Refer Figs. 2E, 3A-B)

1.	Seta 1-M less than half length of 3-M.....	2
	Seta 1-M as long as or longer than 3-M.....	4
2(1).	Seta 14-P usually single; 2-VIII double	3
	Seta 14-P double; 2-VIII single	<i>minutissimus</i>
<i>infantulus</i>

Figure 1: (A) Lateral view of whole pupa to show cephalothoracic structures and setal positions (*Cx. mimulus*); (B) Cephalothorax of pupal exuvium to show setal positions (*Cx. mimulus*); (C) Metanotum and abdomen of pupal exuvium to show dorsal (left) and ventral (right) pupal structures and setal positions (*Cx. mimulus*); (D) Dorsal view of paddle with emarginate apex (*Cx. halifaxii*); (E) Seta with smooth branches (seta 6-VII of *Cx. pluvialis*); (F) Seta with aciculate branches (seta 5-IV of *Cx. brevipalpis*); (G) Dendritic seta (seta 6-VII of *Cx. brevipalpis*). All scale bars are in mm.

A = antenna, CE = compound eye, CT = cephalothorax, Lat = lateral, Lg = legs, Mxp = maxillary palp, MW = mesothoracic wing, P = proboscis, PA = paddle, Tr = trumpet, I-IX = abdominal segments,

Figure 2: (A) Flared pupal trumpet (*Cx. bitaeniorhynchus*); (B) Pupal trumpet with strongly oblique pinna (*Cx. quinquefasciatus*); (C) Pupal trumpet with weakly oblique pinna (*Cx. tritaeniorhynchus*); (D) Pupal trumpet pinna with slit extending to meatus (*Cx. malayi*); (E) Larval head, showing dorsal (left) and ventral (right) structures and setal positions (*Cx. mimulus*); (F) Anterior dorsal view of larval head showing median labral plate not distinct from dorsal apotome (*Cx. bitaeniorhynchus*); (G) Anterior dorsal view of larval head showing median labral plate as a bar distinct from dorsal apotome, and flattened, blunt-ended seta 1-C (*Cx. sitiens*); (H) Ventral view of laminated plate-like larval lateral palatal brushes (*Cx. halifaxii*); (I) Larval antenna showing setae 2,3-A situated midway between base of seta 1-A and antennal apex (*Cx. sinensis*); (J) Larval antenna showing setae 2,3-A situated apically (*Cx. brevipalpis*). All scale bars are in mm.

A = antenna, DA = dorsal apotome, LPB = lateral palatal brush, MLP = median labral plate, MP = mental plate

Figure 3: (A) Dorsal (left) and ventral (right) setal positions on larval thorax and abdominal segment I (*Cx. mimulus*); (B) Lateral view of structures and setal positions on posterior abdomen (*Cx. mimulus*); (C) Lateral view of siphon with dorsal spine (*Cx. hutchinsoni*); (D) Lateral view of segment X with short, stubby anal papillae (*Cx. sitiens*); (E) Lateral view of siphon with distal ventro-lateral spines (*Cx. jacksoni*); (F) Lateral view of mid-region of siphon showing false joint (*Cx. nigropunctatus*). All scale bars are in mm.

AP = anal papillae, CS = comb scales, FJ = false joint, M = mesothorax, P = prothorax, PT = pecten teeth, S = siphon, SP = spine, T = metathorax, I-X = abdominal segments.

- 3(2). Seta 3-P 1-2 branched; 7-I double; thoracic integument non-spiculate; proximal pecten teeth with strong basal denticles *quadripalpis*
- Seta 3-P 4-10 branched; 7-I single; thoracic integument spiculate; proximal pecten teeth without strongly differentiated basal denticles *rubithoracis*
- 4(1). Thorax and abdomen heavily spiculate, spicules large, hair-like, very distinct under low magnification; siphon with 4 pairs of strong seta 1-S tufts, 2-3 times as long as siphon width at point of insertion, proximal pair placed among pecten *uniformis*
- Thorax and abdomen non-spiculate or very lightly spiculate, spicules minute, weak, not distinct as above; siphon with 3 pairs of weak seta 1-S tufts, as long as or slightly longer than siphon width at point of insertion, proximal pair placed above pecten 5
- 5(4). Seta 8-P single; head capsule, siphon, and saddle dark yellow to brown; thorax and abdomen lightly spiculate *bicornutus*
- Seta 8-P double; head capsule, siphon, and saddle pale yellowish to white; thorax and abdomen non-spiculate *mammilifer*
lasiopalpis
wardi

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