J. Natn. Sci. Coun. Sri Lanka 1996 24(1): 37-50

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

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(Received: 25 August 1995; accepted: 03 November 1995)

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 paraméters. 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 Departmentof 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: fuscanus 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 (fuscanus & 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
3(2).	Seta 10-CT multiple, rarely 3-4 branchedCulex
	Seta 10-CT single or double, rarely 3-branchedLophoceraomyia

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
	Seta <i>1-II</i> single, forked or with less than 10 branches, if more, then trumpet dark brown or paddle with conspicuous darkened areas
	Setae 8-CT and 6-III-V double or triple
	Seta 1-CT 3-4 branched; seta 6-VI 4-5 branched; pinna of trumpet strongly oblique (Fig. 2B)
	Trumpet asymmetrically funnel shaped; pinna strongly widened or flared toward apex (Fig. 2A)
	Trumpet whitish or lightly darkened on meatus; cephalothorax and abdomen very pale or whitishsinensis 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
	Setae 10-CT and 3-I-III usually singlebitaeniorhynchus Setae 10-CT and 3-I-III doubleinfula
7(4).	Seta 8-CT double or triple
8(7).	Seta 1-II 15-20 branchedgelidus Seta 1-II 1-10 branched9
9(8).	Seta 6-V, VI 1-2 branched
10(9).	Trumpet short, index about 5, pinna strongly oblique; seta 1-II forked into 4-8 branchessitiens Trumpet longer, index about 7, pinna weakly oblique; seta 1-II single or doublejacksoni

11(9)	* · · · · · · · · · · · · · · · · · · ·
	(Fig. 1A,C)(in part) mimulus
	Trumpet index 5-9; Seta 1-VI 4-5 branched, 1-VII 3-4
	branchedvishnui
12(7)	wing cases, metanotum and abdominal segments I-IV; setae 5-V, VI 6-8 branched, their length much shorter than segments following
	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
	following13
13(12	(Fig. 1A,C)(in part) mimulus Setae 6-IV-VI 4-7 branched; trumpet index
	6-814
14(13). Trumpet dark brown; seta 1-II with more than 10 branchestritaeniorhynchus
	Trumpet pale yellowish; seta 1-II with less than
	10 branches
to cha	E: Sirivanakarn³ uses the presence of a dark spot at the apex of the paddle midrib racterize Cx . $gelidus$ (key step 8). This character was absent in the local material ned during this study, and is not included as a key character here.
	B. 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
	segmentnigropunctatus
	At least seta 5-IV,V long, reaching or
	exceeding posterior margin of following segment (Fig. 1C)
2(1).	Seta 10-CT with 3 branches
	Seta 10-CT with 5-6 branches
3(2).	Seta 12-CT double; setae 5-II-III 4-branched; seta 1-VI more than half the length of seta 5-VI
	nan the length of seta 5-71
	nan the length of seta 5-v1bunyt

	Seta 1-IV,V less than half the length of the corresponding seta 5-IV,Vpal	lidathanau
	Seta 1-IV,V equal to or greater than half the length of the corresponding seta 5-IV,V	
5(4).	Paddle 1.5 or less times as long as broadsi Paddle 2.0 times as long as broad	
4.	. KEY TO SPECIES OF SUBGENUS <i>EUMELANOMYIA</i> - F (The pupae of <i>Cx. campilunati</i> and <i>Cx. castrensis</i> are unknow (Refer Figs. 1C, 1E-G, 2D)	
1.	Trumpet pinna with narrow slit extending to meatus (Fig. 2D)	revipalpis
٠.	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 Pinna of trumpet with slit extending to meatus (Fig. 2D)	
2(1).	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8 Seta 5-CT 2-4 branched; trumpet index	uniformis
2(1).	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8	uniformis
	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8 Seta 5-CT 2-4 branched; trumpet index at least 10 Seta 5-V long, exceeding posterior margin of following segment (Fig. 1C)	uniformis picornutus
	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8 Seta 5-CT 2-4 branched; trumpet index at least 10 Seta 5-V long, exceeding posterior margin of following segments	3 uniformis picornutus t
3(1).	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8 Seta 5-CT 2-4 branched; trumpet index at least 10 Seta 5-V long, exceeding posterior margin of following segmen (Fig. 1C) Seta 5-V short, not reaching posterior margin of following segment Seta 8-CT 3-4 branched; seta 5-IV double or triple, long, extending well beyond posterior margin of following	3 uniformis oicornutus t4
3(1).	Pinna of trumpet with slit extending to meatus (Fig. 2D) Seta 5-CT 5-6 branched; trumpet index 8 Seta 5-CT 2-4 branched; trumpet index at least 10 Seta 5-V long, exceeding posterior margin of following segmen (Fig. 1C) Seta 5-V short, not reaching posterior margin of following segment Seta 8-CT 3-4 branched; seta 5-IV double or triple, long,	3 uniformis picornutus t45

5(3).	Setae 6-III-IV usually 2-4 branched
	Setae 6-III-IV usually 5-6 branchedminutissimus infantulus
6(5).	Seta 5-V usually 5-6 branched; seta 6-IV-VI usually 4-6
	branchedquadripalpis
	Seta 5-V usually 2-4 branched; seta 6-IV-VI usually 2-4
	branchedrubithoracis
NOTI	E: Separations at key steps 5 and 6 are tenuous, since setal ranges often overlap.
	6. KEY TO SUBGENERA OF <i>CULEX</i> IN SRI LANKA - 4 TH 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
	(fuscanus & halifaxii, inseparable)
	Pecten restricted to basal 0.5 of siphon; lateral palatal brushes
	composed of filaments; seta <i>1-A</i> generally multiple, inserted on distal 0.5 of antennal shaft; small to moderate
	sized species
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
	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(2).	Seta 3-P always single, subequal to 1-P
	Seta 3-P single or branched, more slender than 1 -P and ≤ 0.5 its length
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
	proximallyEumelanomyia
	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 enlargedLophoceraomyia

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)
• .	Median labral plate of head capsule not seen as distinct bar separated from dorsal apotome (Fig. 2F)12
2(1).	Seta <i>1-C</i> pale, slender, distally tapered or filamentous
3(2).	Setae 5, 6-C double; mental plate with 6-7 lateral teeth on each side of median tooth; siphon slender, cylindrical, tapered
	teeth on each side of median tooth; siphon short, basal 0.5 distinctly swollenquinquefasciatus
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
5(4).	Seta 7-I double tritaeniorhynchus Seta 7-I single
6(5).	Siphon short, fusiform, swollen at middle, index 3-4gelidus Siphon longer, subcylindrical, index at least 57
7(6).	Seta 4-P double or multiple
8(7).	Seta 1-C slender or spiniform, with pointed apex (Fig. 2E); anal papillae as long or longer than saddle length (Fig. 3B)
	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
	seta 4-P always double; pleural areas of thorax
	spiculatevishnui
	spiculate
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 doublewhitmorei
	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 multiplepseudovishnui
	designations, social 12 double of management and provide the control of the contr
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-Ssinensis
	Setae 2, 3-A located almost apically (Fig. 2J); seta 4-P
	strong and distinct; siphon with 3-4 pairs of seta 1-S
	Strong and distinct, signal with a report of restriction
13(12).	Siphon usually with 3 pairs (total 6) of seta 1-Sinfula
	Siphon usually with 4 pairs (total 8) of seta 1-S bitaeniorhynchus
5 setae (At Key step 13, some specimens have an unpaired seta 1 - S , resulting in a total of (2.5 pairs) in Cx . infula and (3.5 pairs) in $(3.5 p$
	8. KEY TO SPECIES OF SUBGENUS CULICIOMYIA - 4 TH INSTAR LARVAE
	(The larva of Cx. bahri is unknown)
	(Refer Figs. 2E, 3A-B, 3F)
•	Setae 1 2 3-P single 2
1.	Setae 1, 2, 3-P single
	single or with 2 or more branches
	Single of with 2 of more stationes.
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).	tooth robust, markedly larger than other lateral barbs
	Siphon index 6 or less; basal barb of each pecten tooth equal to or smaller than other lateral barbs
4(3).	Setae 5, 6-C 3-4 branched; individual siphon tufts single
	Setae 5, 6-C 5-7 branched; individual siphon tufts 2-3 branched
5(1).	Siphon narrow, cylindrical, seta 1-S tufts single or double; pecten with more than 10 teethbailyi Siphon swollen at middle, seta 1-S tufts 3-4 branched; pecten with less than 10 teethpallidothorax
	9. KEY TO SPECIES OF SUBGENUS <i>EUMELANOMYIA</i> - 4 TH 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 tuftbrevipalpis Setae 2, 3-A placed subapically; siphon moderately long, index 7-8, with strong seta 1-S tufts
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
10.	KEY TO SPECIES OF SUBGENUS LOPHOCERAOMYIA - 4 TH 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

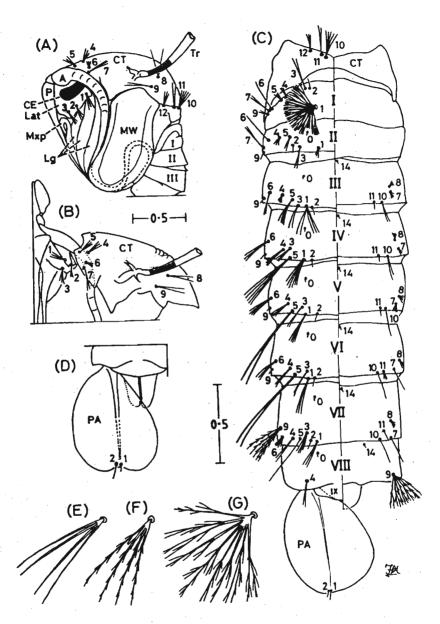


Figure 1: (A) Lateral view of whole pupa to show cephalothoracic structures and setal positions (Cx. mimulus); (B) Cephalothorax of pupal exuvium to show setal positions (Cx. mimulus); (C) Metanotum and abdomen of pupal exuvium to show dorsal (left) and ventral (right) pupal structures and setal positions (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 = lateralia, Lg = legs, Mxp = maxillary palp, MW = mesothoracic wing, P = proboscis, PA = paddle, Tr = trumpet, I-IX = abdominal segments,

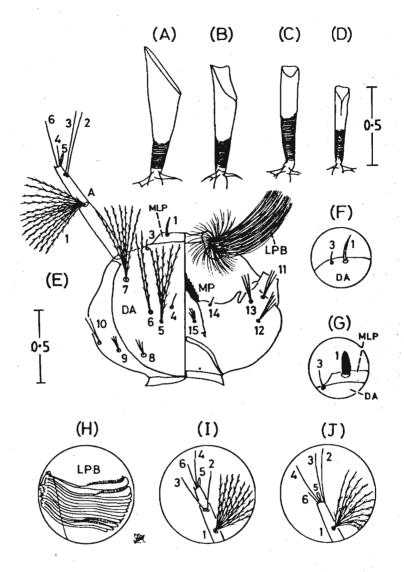


Figure 2: (A) Flared pupal trumpet (Cx. bitaeniorhynchus); (B) Pupal trumpet with strongly oblique pinna (Cx. quinquefasciatus); (C) Pupal trumpet with weakly oblique pinna (Cx. tritaeniorhynchus); (D) Pupal trumpet pinna with slit extending to meatus (Cx. malayi); (E) Larval head, showing dorsal (left) 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

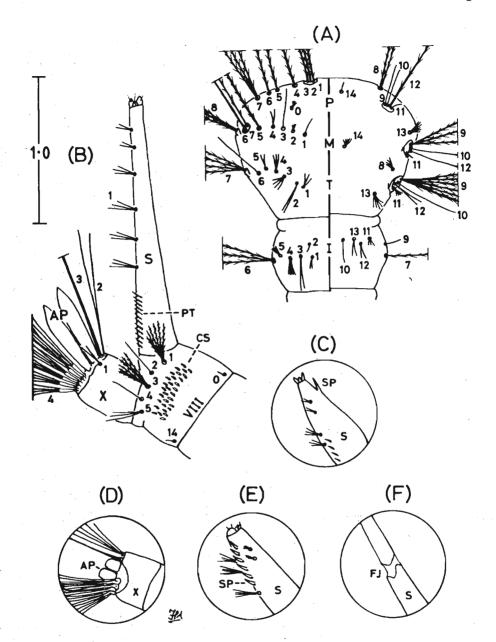


Figure 3: (A) Dorsal (left) and ventral (right) setal positions on larval thorax and abdominal segment I (Cx. mimulus); (B) Lateral view of structures and setal positions on posterior abdomen (Cx. mimulus); (C) Lateral view of siphon with dorsal spine (Cx. hutchinsoni); (D) Lateral view of segment X 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	rubith oracis
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 tim	es
	as long as siphon width at point of insertion,	
	proximal pair placed among pecten	uniformic
	Thorax and abdomen non-spiculate or very lightly spicu	lata
	spicules minute, weak, not distinct as above; siphor	
	with 3 pairs of weak seta 1-S tufts, as long as or	1
	slightly longer than siphon width at point of inserti	
	proximal pair placed above pecten	5
5(4).	Seta 8-P single; head capsule, siphon, and saddle dark y	allow
0(1).	to brown; thorax and abdomen lightly spiculate	
	Seta 8-P double; head capsule, siphon, and saddle pale	oicornutus
		•
	yellowish to white; thorax and abdomen	
	non-spiculate	mammilifer
		lasiopalpis
		wardi

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