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Phytotelmatomyia, a new Neotropical subgenus of *Culex* (Diptera: Culicidae)

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Abstract

Phytotelmatomyia, a new subgenus in the Neotropical Region, is proposed for four described species, including *Cx. castroi* Casal & García, *Cx. hepperi* Casal & García, *Cx. machadoi* da Silva Mattos, da Silveira Guedes & Hamilton Xavier, and *Cx. renatoi* Lane & Ramalho (type species), and two potentially new species without formal Latin names. Monophyly of the group is supported by cladistic analyses of morphological data. *Phytotelmatomyia* is separated and distinguished from subgenera *Culex* and *Phenacomyia*. Diagnostic and differential characters of the male genitalia, larvae, and pupae of the three subgenera are tabulated and illustrated. Bionomics and distributional data are provided for *Phytotelmatomyia* species.

Key words: Culicidae, mosquitoes, Culicinae, Culicini, *Culex*, *Phytotelmatomyia*, new subgenus

Introduction

While conducting comparative studies of mosquitoes of subgenus *Culex* of genus *Culex* Linnaeus, we noticed that two potentially new species that inhabit the axils of *Eryngium* species (Umbelifera = Apiaceae) share characteristics of the adult, larval, and pupal stages with *Cx*. (*Cux*.) *hepperi* Casal & García and *Cx*. (*Cux*.) *castroi* Casal & García. Furthermore, the same features were observed in two other species that live in Neotropical phytotelmata, *Cx*. (*Cux*.) *renatoi* Lane & Ramalho and *Cx*. (*Cux*.) *machadoi* da Silva Mattos, da Silveira Guedes & Hamilton Xavier, the last one from its published description. The two potentially new species are not described at present due to a paucity of individually reared specimens. Other species whose larvae are found in plants other than *Eryngium* species, including *Culex spinosus* Lutz, *Cx. fernandezi* Casal, García & Cavallieri, and *Cx. dohenyi* Hogue, do not exhibit all of the characteristics observed in the former group, which indicates that it is a heretofore unrecognized group within genus *Culex*. The restricted larval habitat of the species of this group has resulted in adaptations of the immature stages that are sufficiently unique to suggest that it represents a new subgenus. The adaptations are especially evident in the development of the chaetotaxy, as a proliferation of branches that aid movement in a semi-aquatic medium. Based on the results of a cladistic analysis of morphological data that support the monophyly of the group, it is formally recognized as subgenus *Phytotelmatomyia*. Diagnostic and differential characters that distinguish *Phytotelmatomyia* from subgenera *Culex* and *Phenacomyia* Harbach & Peyton are listed and illustrated.

Materials and methods

Morphological structures were examined in the adult, pupal, and fourth-instar larval stages. Diagnostic and differential characters were confirmed in all specimens listed in the **Material examined** section. The morpho-

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logical terminology follows Harbach & Knight (1980, 1982). Life stages are indicated by the symbols M (male), F (female), L (fourth-instar larva), Le (larval exuviae), P (pupa), and Pe (pupal exuviae). Male and female genitalia are denoted by the letter G (genitalia) used in combination with the male and female symbols, respectively.

The phylogenetic relationship of *Phytotelmatomyia* to other generic-level taxa of tribe Culicini was examined by including character data for *Cx. castroi*, *Cx. hepperi*, and *Cx. renatoi* in the unpublished parsimony analysis of St. John (2007; see Harbach, 2007: 621–623). Briefly, 64 characters from fourth-instar larvae, pupae, and adult males and females were coded for 48 species: the three species of *Phytotelmatomyia* (see Appendix for characters and coding), 42 exemplar species representing 26 generic-level taxa of tribe Culicini (*Galindomyia* Stone & Barreto and *Culex* subgenus *Nicaromyia* González Broche & Rodríguez R. were not included), and three outgroup species, *Mansonia africana* (Theobald) (tribe Mansoniini), *Maorigoeldia argyropus* (Walker) (tribe Sabethini), and *Orthopodomyia anopheloides* (Giles) (tribe Orthopodomyiini). The data were analyzed using implied weights, implemented by PIWE version 3.0 (for Windows) (Goloboff, 1997), with the default value of the concavity constant, $K = 3$. The analysis was conducted by heuristic search, using 5000 replications (mult*5000) and holding 25 cladograms per replicate (hold/25). The analysis produced a single most parsimonious cladogram (MPC). To ensure that was the only MPC, a search for successively less fit cladograms was conducted using the commands 'sub *n*' (where *n* is the decrease in fit, in steps of 0.1) and 'find*' (to search for all cladograms of best fit - *n*), up to a maximum of 100,000 cladograms, then applied the 'best' command to this set of 100,000 cladograms to confirm that the included set of MPCs was the same as that with which we started. This procedure confirmed the single MPC was the sole "fittest" cladogram (fit = 286.9). Clade support was assessed using Bremer support (Bremer, 1994).

Culex subgenus *Phytotelmatomyia* Rossi & Harbach, new subgenus

Type species. *Culex renatoi* Lane & Ramalho, 1960. Species included: *Culex castroi* Casal & García, 1967a, *Culex hepperi* Casal & García, 1967b, *Culex machadoi* da Silva Mattos, da Silveira Guedes & Hamilton Xavier, 1978, and two undescribed species (sp. 1 and sp. 2) from Argentina.

Morphological study revealed a suite of characters that distinguishes this group of species from subgenera *Culex* and *Phenacomyia*. The distinctive characters that diagnose this group are listed and compared with homologous characters of subgenera *Culex* and *Phenacomyia* in Table 1, and the numbered characters in Table 1 correspond to the numbered arrows on the larval and pupal stages illustrated in Figs. 3–8. Whereas the larvae and pupae of the group are easily distinguished from the larvae and pupae of *Culex* and *Phenacomyia*, the adults are scarcely distinguishable. Despite this, the results of the cladistic analysis (see discussion below) reveal that *Phytotelmatomyia* is a distinct monophyletic lineage.

Diagnosis. *Adults*: The scutum has a pattern of pale scales, principally on the margins, which is inconspicuous in *Cx. castroi* and *Cx. machadoi*. Females have a patch of pale scales on the maxillary palpus, except in *Cx. castroi* and *Cx. machadoi*, which lack pale scales, and a pale patch or band is more or less evident on the proboscis. The tarsi have broad bands across the joints (except in *Cx. castroi* and *Cx. machadoi*). The postgenital lobe of the female genitalia is trapezoidal, at least in *Cx. castroi*, *Cx. hepperi*, and the two undescribed species. The male genitalia (Fig. 2) differ from those of *Phenacomyia*, which have a small hair-like seta *f* and a nearly straight, short seta *h*, and are similar to the male genitalia of various *Cx.* (*Cux.*) species in having a relatively large, curved seta *f* and a strong, hooked seta *h* on the subapical lobe of the gonocoxite. The male genitalia otherwise do not exhibit distinctive characteristics except for the number of teeth on the lateral plate of the phallosome and the number of setae on the ninth tergal lobes.

The principal distinctions of *Phytotelmatomyia* are found in the immature stages. *Larvae*: Setae 2–4-A forked or bifid; antenna very short, <0.33 length of head, nearly cylindrical, seta 1-A small with few branches;

TABLE 1. Comparison of diagnostic and differential characters of subgenera *Phytotelmatomyia*, *Culex*, and *Phenacomyia*. The numbers in the second column correspond to the numbered arrows in Figs. 3–8.

		Character	*Phytotelmatomyia*	*Culex*	*Phenacomyia*
Adults		Scutum	With pale areas	Variable	With pale areas
		Last palpomere ♀	With pale scales (except *castroi*)	Usually unicolorous	Usually unicolorous
		Postgenital lobe ♀	Trapezoidal	Variable	Variable
		IX tergal lobe ♂	5–22 setae in 1–2 rows	2–17 setae in 1–3 rows	12–29 in 2–5 rows
Larvae	1	Antenna	Short, 0.33 length of head, nearly cylindrical	Long, 0.75 length of head, narrower beyond seta 1-A	Short, 0.35 length of head, nearly cylindrical
	2	Seta 1-A	Poorly developed, with few short branches	Strongly developed, with numerous branches	Poorly developed, with few short branches
	3	Setae 2–4-A	Bifid or forked, 4-A sometimes pinnate	Single, simple, rigid	Single, simple, rigid
	4	Seta 1-C	Short, stout, branched or forked	Variable but usually fine, simple	Relatively long, fine, simple
	5	Seta 4-C	Long, similar to 5,6-C, 4–10 branches	Variable, usually short, single	Usually 2–3branches
	6	Seta 9-C	Anterior to 8-C, branched, same length as 8,10-C	Normally anterior to 8-C, branched, shorter than 10-C	Posterior to 8-C, branched, smaller than 8,10-C
	7	Seta 14-C	Posterior to 13-C	In line or anterior to 13-C	Posterior to 13-C
		Maxillary brush	Shorter than maxillary body	Longer than maxillary body	Shorter than maxillary body
		Seta 1-Mx	Near 0.60 from base	Near 0.50 from base	Near 0.67 from base
		Maxillary spiculose area	With spicules	With or without spicules	Without spicules
		Seta 0-P	Large, posterior to 5-P	Small, usually posterior to 4-P	Small, posterior to 4-P
	8	Seta 3-P	Shorter than 1,2-P, multiple branched	Long, about length of 1,2-P, usually single	Shorter than 1,2-P, usually branched
	9	Seta 4-P	Short, about 0.35 length of 1-P, multiple branched	Long, about 0.65 length of 1-P, single or double	Short, about 0.35 length of 1-P, single
	10	Seta 14-P	With 4–17 branches (except *renatoi*)	Usually single (1,2)	Usually single (1,2)

		Character	*Phytotelmatomyia*	*Culex*	*Phenacomyia*
	11	Seta 1-M	Large, multiple branched	Usually small, single	Small, single
	12	Seta 1-T	Usually with 3–14 branches	Usually with 1–5 branches	Usually single
	13	Seta 1-I,III	Usually with 3–11 branches	Usually with <3 branches	Usually with <3 branches
	14	Thorax and abdomen	Spiculate	Usually without spicules	Spiculate
	15	Seta 13-III,V	Usually with 4–8 branches	Usually with 1–6 branches	Usually with 2(1–3) branches
	16	Saddle	Largely spiculate, long or strong spicules on posterior border	With or without spicules on posterior border	With spicules on posterior border
	17	Seta 2-X	Usually with 2–7 branches	Usually with 2(1–3) branches	Usually single
	18	Ventral brush	Comprised of 4 pairs of setae	Normally comprised of 5 or more pairs of setae	Usually comprised of 6–8 pairs of setae
		Siphon Index (width at base)	<4	Usually >4	<4
		Pecten Row Index	0.34–0.58	0.31–0.35	0.37–0.61
		Larval movement	Sinuous fast	Irregular sustained	Sinuous fast
		Larval habitat	Apiaceae or Umbeliferae ground phytotelmata	Principally ground water habitats	Various (bamboo, tree holes, ground pools)
Pupae	19	Seta 1-CT	0.7 longer than 2-CT	0.3 longer than 2-CT	0.7 longer than 2-CT
	20	Seta 7-I,II	Similarly developed	Variable	Similarly developed
	21	Seta 6-III–VI	Usually single	Usually single to triple	Usually single
	22	Seta 5-IV	Longer than segment	Shorter than segment	Longer than segment
	23	Seta 9-VII,VIII	Large, at least 0.5 length of paddle	Small, distinctly <0.5 length of paddle	Small, distinctly <0.5 length of paddle
	24	Paddle, shape	Usually emarginate	Usually oval	Usually oval
	25	Paddle, spiculation	Anterior 0.4 of surface minutely spiculate; marginal serrations posteriorly	With or without fine spicules on 0.5 of outer border	Without spicules

seta 1-C short, stout, branched or forked; seta 4-C long, reaching anterior margin of head, fan-like, developed similar to 5,7-C; maxilla short, stout, seta 1-Mx inserted 0.60 from base; maxillary brush relatively short, slightly shorter than maxillary body, spiculose area lateral to seta 5-Mx poorly developed; seta 0-P large, inserted posterior to 5-P; seta 3-P multiple branched, short, about 0.33 length of 1-P; seta 4-P similar to 3-P; setae 14-P, 1-M,T and 1-I,II usually with multiple branches; seta 1-I,III multiple branched; seta 7-II long, similar to 7-I; saddle largely covered with spicules that grade into long spicules posteriorly, especially dorsally; ventral brush comprised of 4 pairs of setae. *Pupae*: Seta 1-CT significantly longer than 2,3-CT; seta 7-II as long as 6-II; seta 5-IV as long as 5-V,VI, about 1.5 length of following tergum; paddle spiculate on anterior 0.4 of surface, with distinct serration posteriorly on margins of inner and outer parts.

Etymology. *Phytotelmatomyia* is derived from three Greek words: *Phyton* (n. plant, combining form *phyto-*); *telma*, *-tos* (n. standing water, combining form *telmato-*), and *myia* (f. fly). The name is feminine in gender. The three-letter abbreviation *Phy.* is recommended for the subgeneric name.

Distribution. The species of *Phytotelmatomyia* are distributed in the Neotropical Region, along the Río Paraná Basin from Brazil to Argentina, including Paraguay and Uruguay. The collection sites of the species are indicated in Fig. 1. This distribution corresponds to the Tupi area of dispersion of Lane (1953). The occurrence of *Cx. hepperi* in Cosquín, Cordoba Province reported by Brewer *et al*. (1991) is doubtful.

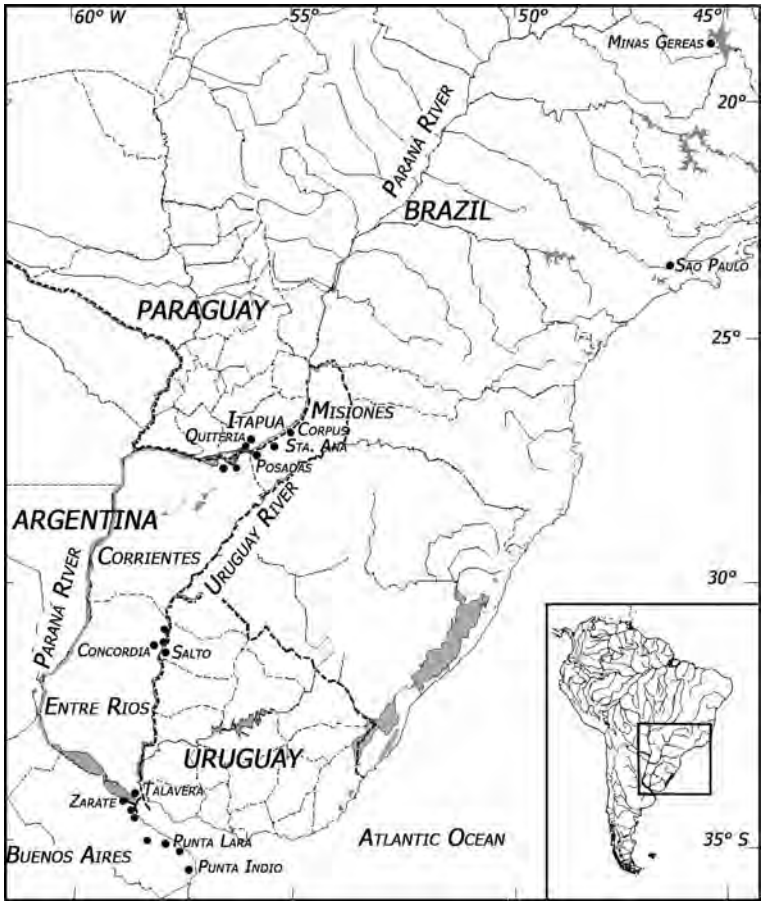

FIGURE 1. Map of the Paraná Basin. Dots (●) indicate collection sites.

Bionomics. The daily cycle of activity of *Phytotelmatomyia* species is unknown. Adults are attracted to humans (see **Material examined**), but have never been collected inside or outside houses or in CDC light traps (during more than 8,000 hours of trapping in the vicinity of *Eryngium* plants in Buenos Aires, Entre Ríos, Corrientes, and Misiones Provinces in Argentina, Salto in Uruguay, and Itapua in Paraguay). Only the capture of immature stages is reported previously in published literature. Campos and Lounibos (1999) found

Cx. hepperi, *Cx. castroi*, and *Cx. renatoi* in two species of *Eryngium* in Punta Lara, Buenos Aires Province, Argentina, which is concordant with collections made for the present study. Eggs are deposited in small rafts of 4–8 eggs or as single eggs placed in different axils. Eggs are small but generally resemble the eggs of *Cx.* (*Cux.*) species.

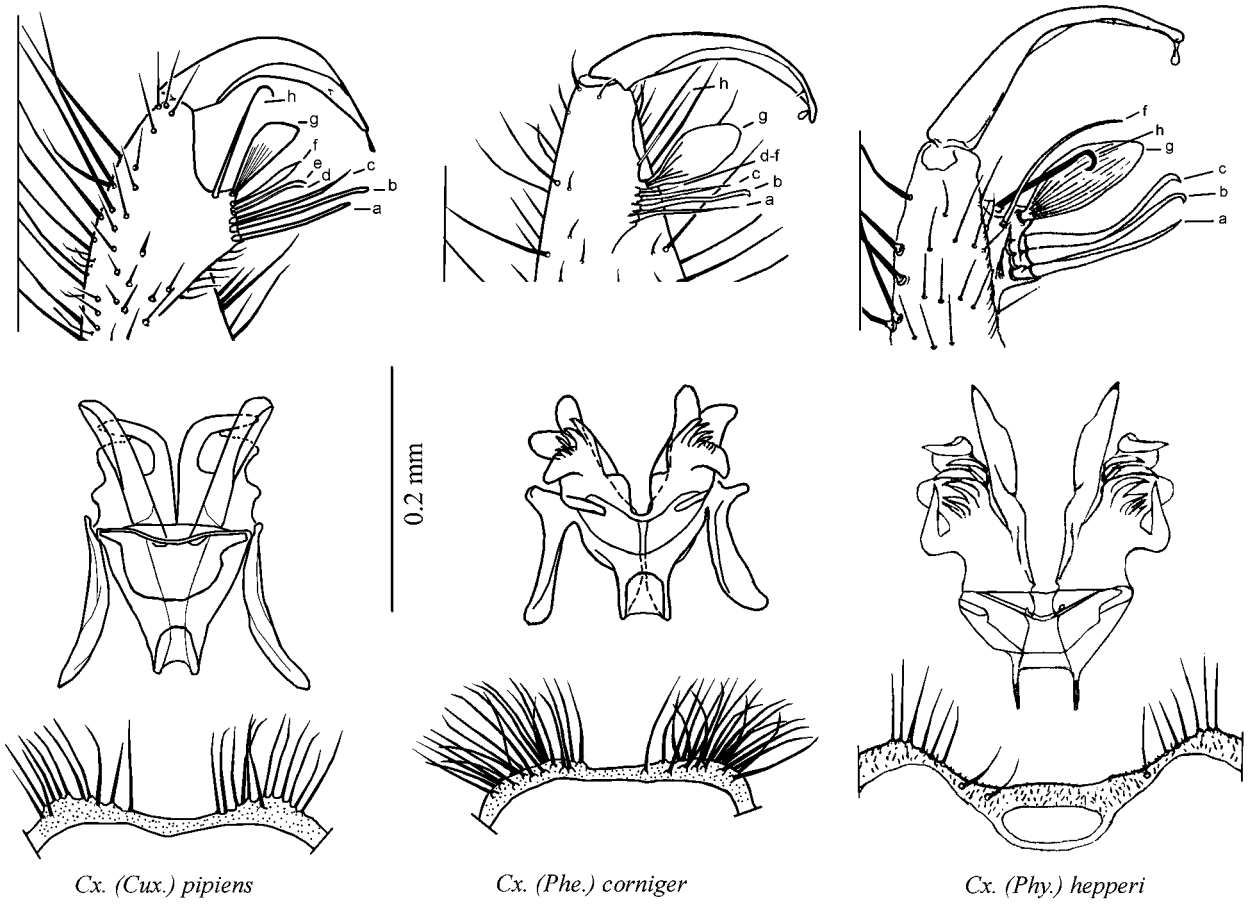

FIGURE 2. Male genitalia of *Cx.* (*Cux.*) *pipiens*, *Cx.* (*Phe.*) *corniger*, and *Cx.* (*Phy.*) *hepperi*.

Material examined. Specimens included pinned adults reared from fourth-instar larvae collected from the axils of *Eryngium* species, adult females captured landing on the legs of collectors, dissected male and female genitalia, fourth-instar larvae, and the larval and/or pupal exuviae (of reared adults) mounted on microscope slides in Canada balsam. A total of 605 specimens were examined: 85 M, 89 GM, 65 F, 5 GF, 215 Pe, 122 Le, 2 P, 22 L.

Culex hepperi. 33 M, 30 GM, 13 F, 4 GF, 88 Pe, 44 Le, including holotype, allotype, and paratypes as follow: **Argentina**, Buenos Aires Province, Zárate, Canal 6 y Paraná de las Palmas River, 1 M (holotype), 8 GM, 1 F, 4 GF, 57 Pe, 30 Le, holotype and paratypes, Casal & García leg, Administración Nacional de Laboratorios de Salud (ANLIS) "Dr. Carlos G. Malbrán" (formerly Instituto Nacional de Microbiología); Zárate, Talavera Island near Paraná de las Palmas River, 10-03-2004, 1 M, 1 MG, 3 F, 4 Pe, D. Carpintero leg; same locality, 10-18-2004, 2 M, 2 GM, 5 F, 7 Pe, 4 Le, Rossi leg; Magdalena, Punta Lara, 2 M, 2 GM, 1 Pe, 1 Le, 10-13-1987, Rossi leg; idem, 08-06-1988, 3 M, 3 GM, 1 F, 4 Pe, 2 Le, Rossi & García legs; same locality, 07-14-1988, 1 GM, Rossi leg; idem, 11-02-1988, 4 M, 1 GM, 3 Pe, 1 Le; same locality, 08-14-1989, 8 M, 8 GM, 1 F, 2 Pe, Campos & Maciá legs; same locality, 08-28-1989 1 M, 1 GM, Rossi leg; same locality, 10-23-1989, 1 M, 1 GM, Campos & Maciá legs; same locality, 08-31-1995, 1 M, 1 GM, Campos leg; Berisso, Los Talas, 11-30-1988, 1 M, 1 GM, Rossi leg; Punta Indio, Balneario Sarandí, 7 M, 1 F, 8 Pe, 4 Le, 04-06-2006, Rossi leg; **Paraguay**, Itapua, Encarnación, Quiteria, 10-03-2000, 1 M, 1 GM, 1 F, 2 Pe, 2 Le, López & Ortiz leg.

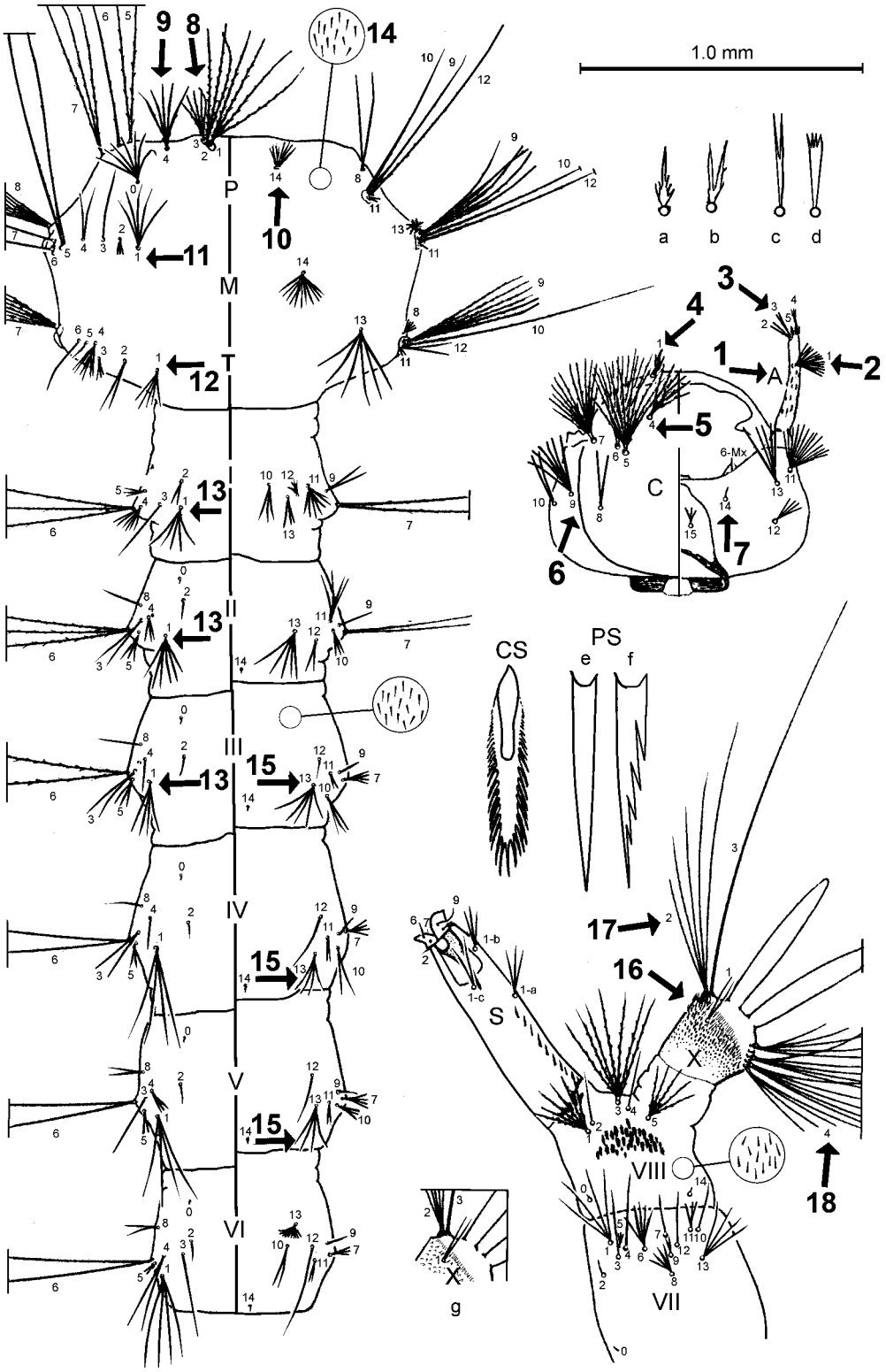

FIGURE 3. Larva of *Cx*. (*Phy.*) *hepperi*. a–d: Distinct forms of seta 1-C. e,f: Pecten spines of *Cx*. (*Phy.*) *hepperi* and *Cx. (Phy.) castroi*. *g*: Spicules on saddle of *Cx*. (*Phy.*) *renatoi*.

Culex castroi. 32 M, 41 GM, 31 F, 1 GF, 97 Pe, 56 Le, 2 P, 9 L including holotype and paratypes as follow: **Argentina**, Buenos Aires Province, Zárate, Canal 6 y Paraná de las Palmas River, 1 M (holotype), 13 GM, 1F, 33 Pe, 16 Le, Casal & García legs, Administracion Nacional de Laboratorios de Salud (ANLIS) "Dr.

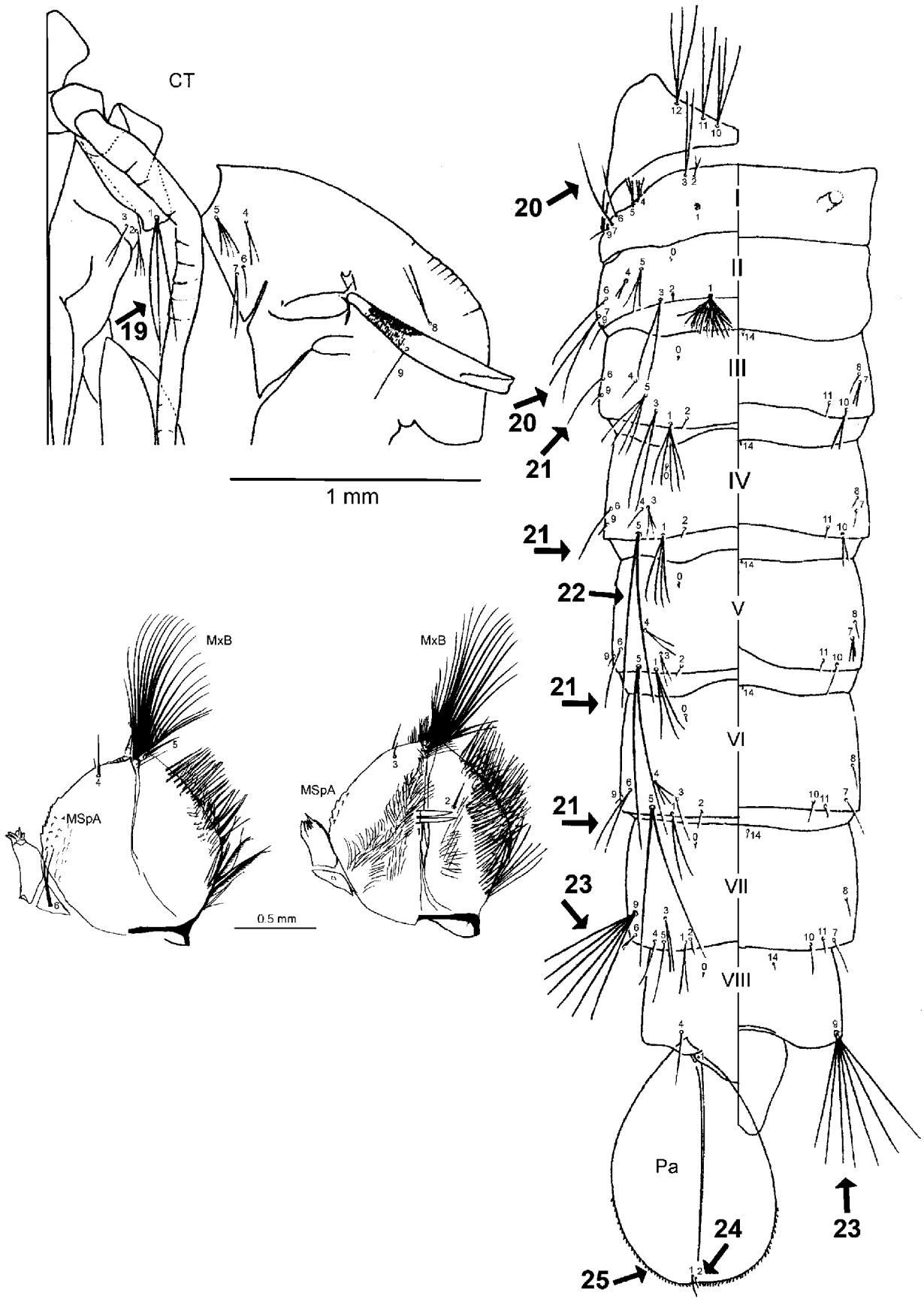

FIGURE 4. Pupa and larval maxilla of *Cx.* (*Phy.*) *hepperi.*

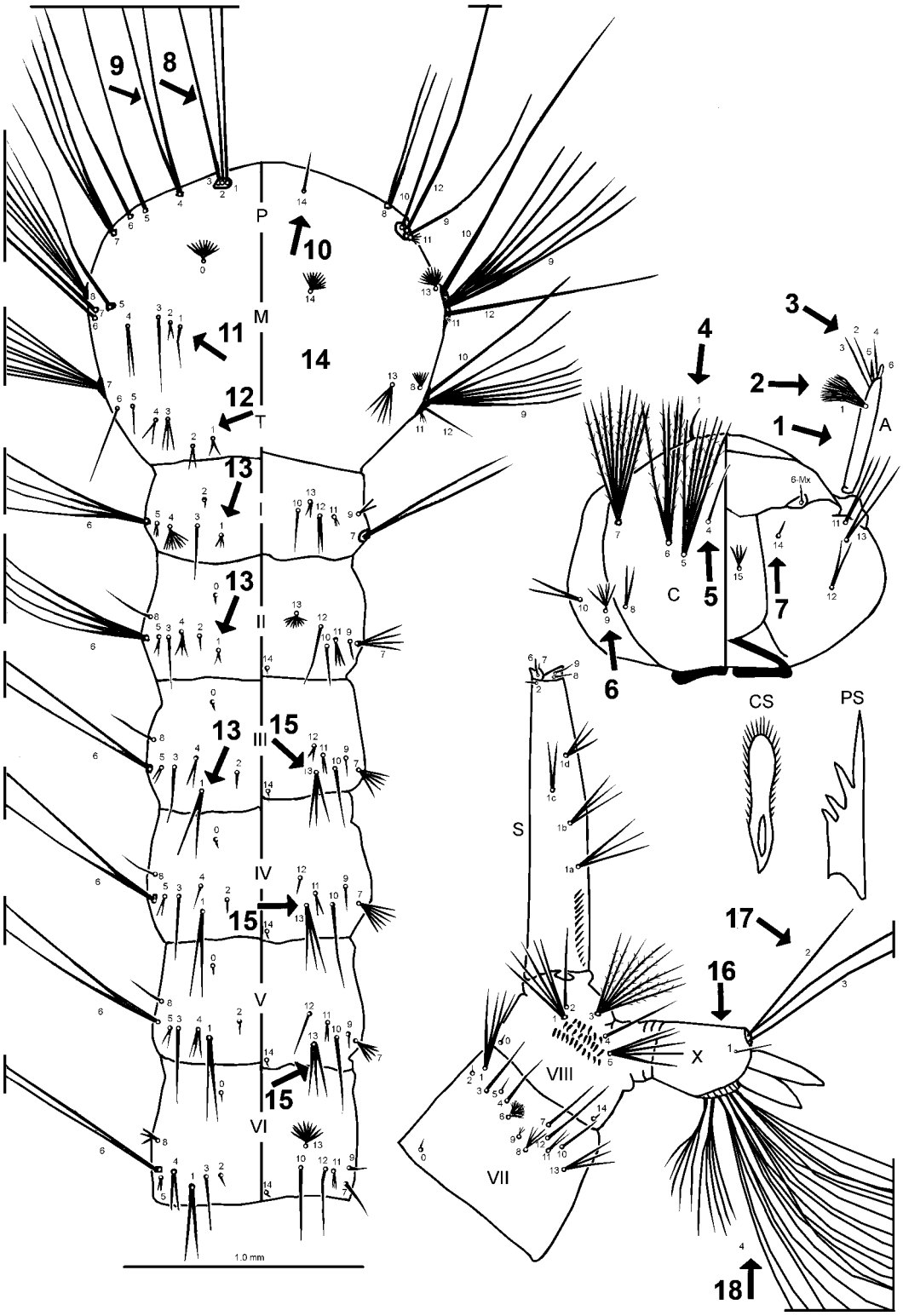

FIGURE 5. Larva of *Cx*. (*Cux.*) *pipiens.*

Carlos G Malbrán" (formerly Instituto Nacional de Microbiología); Zárate, Talavera Island, near Paraná de las Palmas River, 03-10-2004, 2 M, 2 GM, 7 F, 1 GF, 10 Pe, 8 Le, D. Carpintero leg; idem, 18-10-2004, 6 M, 6 GM, 4 F, 9 Pe, 1 P, Rossi leg; Cañada Honda at Route 9, 03-03-2004, 2 M, 2 GM, 1 F, 3 Pe, 2 Le, Rossi leg; La Cruz stream at Route 9, 2 M, 2 Pe, 1 Le; Areco River at Route 9, 6 M, 6 GM, 2 F, 7 Pe, 4 Le, all same date and

collector; Los Talas, Berisso, 3 M, 3 GM, 1 F, 2 Pe, 10-30-1988, Rossi leg; Punta Indio, Balneario Sarandí, 1 M, 1 Pe, 04-06-2006, Rossi leg; Corrientes Province, Garapé, 09-23-1999, 3 M, 3 GM, 6 F, 8 Pe, 1 P, 9 Le, 2 L, Rossi leg; Misiones Province, Maní Port, Corpus, 09-17-1999, 1 F, 1 Pe, 1 Le, Pascual leg; **Paraguay**, Itapua, Quiteria River, 10-15-1998, 3 L, Spinelli leg; idem, 10-03-2000, 5 M, 9 F, 15 Pe, 1 P, 13 Le, López & Ortiz legs; Encarnación, Mboy Cae, 10-27-1999, 4 F, 4 Pe, 2 Le, 4 L, Rossi leg; **Uruguay**, Salto, 01-03-2001, 1 M, 1 GM, Rossi leg.

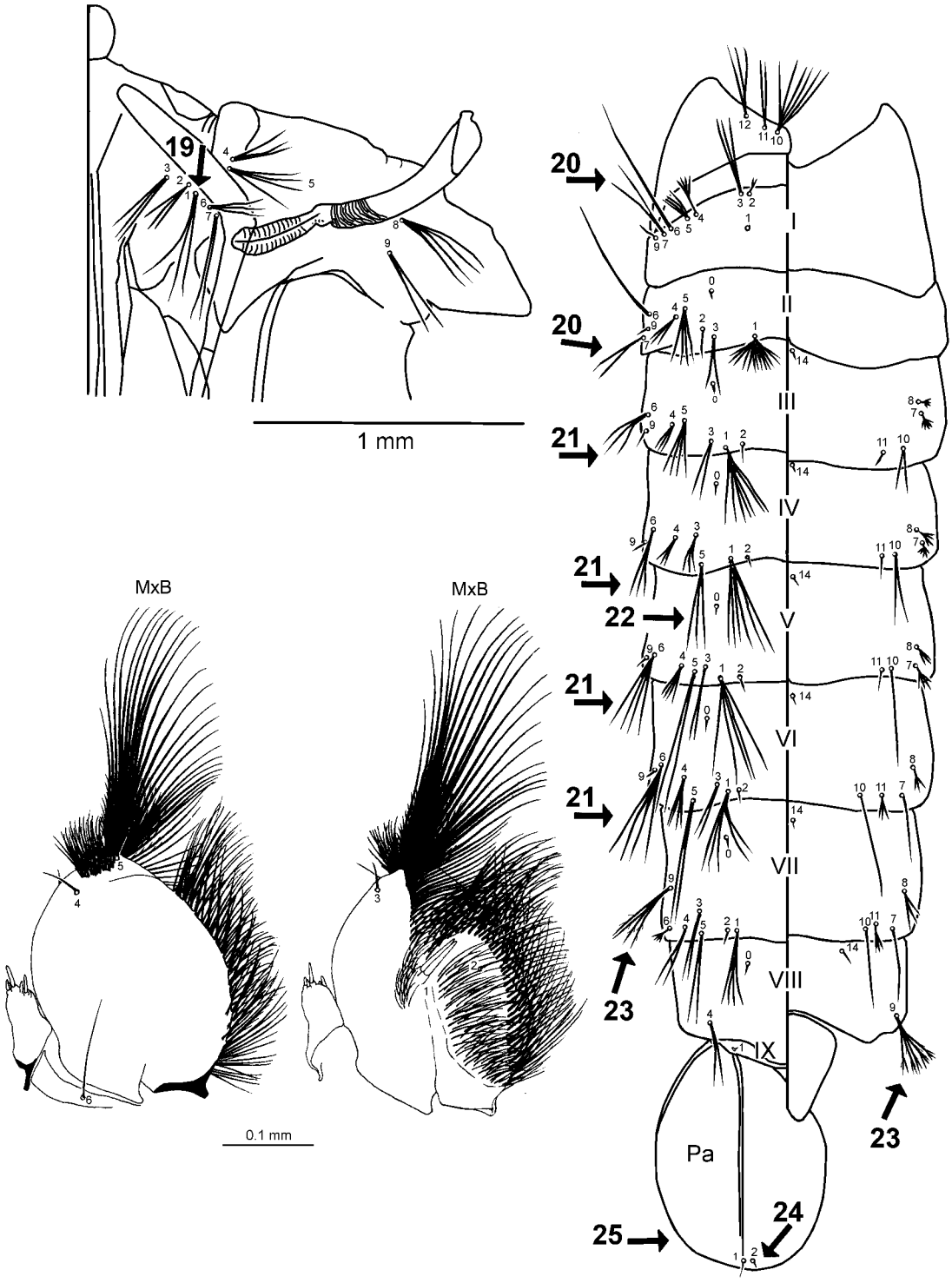

FIGURE 6. Pupa and larval maxilla of *Cx.* (*Cux.) pipiens.*

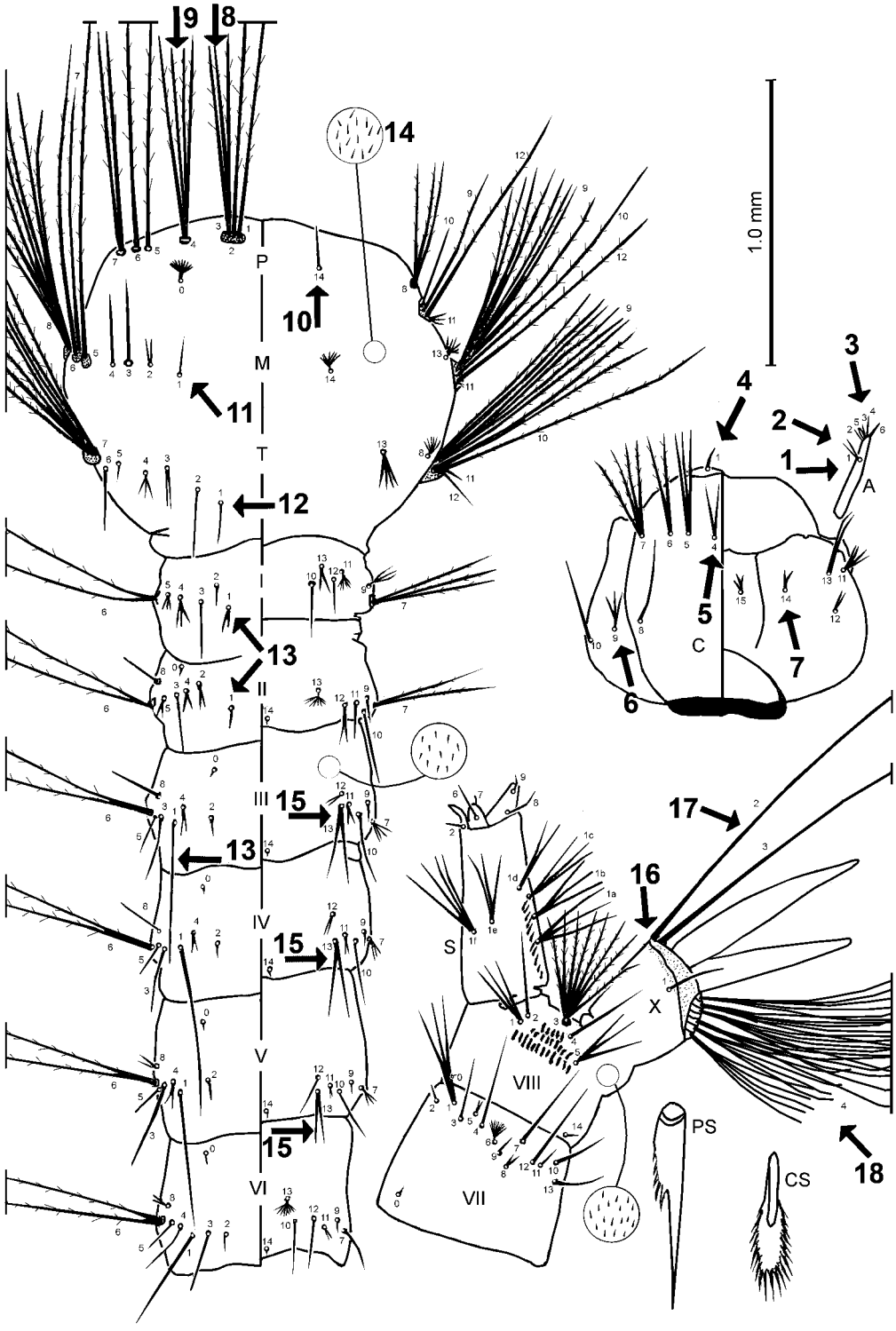

FIGURE 7. Larva of *Cx*. (*Phe.*) *corniger.*

Culex renatoi. 7 M, 5 GM, 10 F, 17 Pe, 13 Le, 10 L as follow: **Argentina**, Buenos Aires, Punta Indio, Balneario Sarandí, 2 M, 2 F, 4 Pe, 2 Le, 04-06-2006, Rossi leg; Entre Ríos Province, Chajarí, 09-24-1989, 1 F, Balseiro leg; idem, Ayuí, 12-19-2003, 1 M, 1 GM, 4 F, 5 Pe, 2 L, 5 Le, Rossi leg; Misiones Province, Santa Ana River at Route 12, 11-16-1999, 4 M, 4 GM, 4 F, 8 Pe, 6 Le, 8 L, Rossi leg.

***Culex* sp. 1**. 7 M, 7 GM, 3 F, 6 Pe, 2 Le, 1 L as follow: **Argentina**, Buenos Aires Province, Punta Indio, Villordo stream, 4 M, 4 GM, 3 F, 6 Pe, 2 Le, 1 L, P. Marino leg; Misiones Province, Santa Ana River and Route 12, 08-13-1999, 3 M, 3 GM, Araki & Pascual legs.

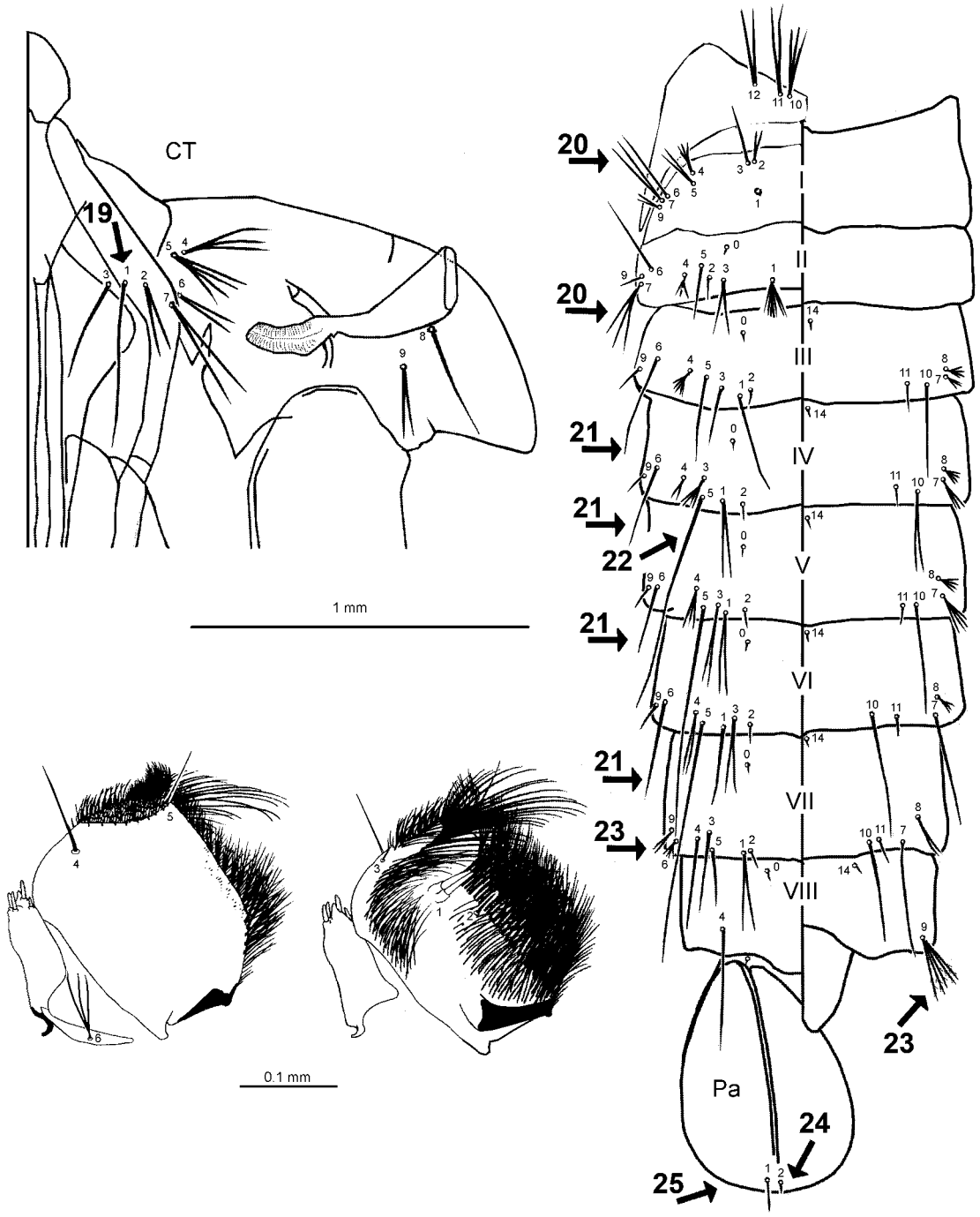

FIGURE 8. Pupa and larval maxilla of *Cx.* (*Phe.*) *corniger.*

***Culex* sp. 2**. 6 M, 6 GM, 8 F, 7 Pe, 7 Le, 2 L as follow: **Argentina**, Corrientes Province, Garapé at Paraná River, 11-23-1999, 3 M, 3 GM, 4 F, 7 Pe, 7 Le, 2 L, Rossi leg; Misiones Province, Posadas, Martires Stream, 08-13-1999, 3 M, 3 GM, 4 F, Araki & Pascual legs.

Discussion. The taxonomy of Neotropical mosquitoes of tribe Culicini has changed over the past couple of decades. Harbach and Peyton (1992) introduced *Culex* subgenus *Phenacomyia* for *Cx. corniger* Theobald and two related species, and González Broche and Rodríguez R. (2001) established subgenus *Nicaromyia* for *Cx. nicaroensis* Duret. Navarro and Liria (2000) proposed subgeneric status for genus *Deinocerites* Theobald based on morphological features of larval mouthparts, but this action has not been accepted because it is not supported by the numerous unique characters that distinguish the adults, larvae, and pupae from *Culex* (Har-

bach, 2007). Finally, Tanaka (2003) removed *Lutzia* Theobald from subgeneric status within *Culex* and raised it to its original generic rank. As a result of these changes, 14 subgenera are currently recognized within genus *Culex* in the New World, including: *Aedinus* Lutz, *Allimanta* Casal & García, *Anoedioporpa* Dyar, *Belkinomyia* Adames & Galindo, *Carrollia* Lutz , *Culex*, *Melanoconion* Theobald, *Micraedes* Coquillett, *Microculex* Theobald, *Neoculex* Dyar, *Nicaromyia*, *Phenacomyia*, *Phytotelmatomyia*, and *Tinolestes* Coquillett. Of these subgenera, only *Culex* and *Neoculex* are widely distributed outside of the Neotropical Region. *Culex* is by far the largest subgenus, with 201 species (http://mosquito-taxonomic-inventory.info) distributed throughout the world. The subgenus is divided into two principal groups, the Pipiens and Sitiens Groups (Edwards, 1932) and two smaller groups, the Atriceps and Duttoni Groups (Belkin, 1962 and Harbach, 1988, respectively). The New World species are placed in the Pipiens Group, but are not further classified in subordinate groups as are Old World species of the subgenus. The recognition of subgenus *Phytotelmatomyia* clearly shows that larval characters are more indicative of natural affinities than are the male genitalic characters on which the broad concept of subgenus *Culex* is principally based.

The analysis of the data set of St. John (2007) with the inclusion of character data for *Phytotelmatomyia* (Appendix) produced a very different pattern of relationships in the basal part of the cladogram illustrated in Harbach (2007: Fig. 23) that includes subgenera *Culex* and *Phenacomyia*. As the purpose of the analysis was to test the monophyly of *Phytotelmatomyia*, and a discussion of altered relationships is beyond the scope of the present study, only the segment of the cladogram that shows relationships between species of subgenera *Culex*, *Phenacomyia*, and *Phytotelmatomyia* is dealt with here (Fig. 9). Most importantly, *Phytotelmatomyia* arises from the main stem of the cladogram as a very strongly supported monophyletic group (Bremer support 1.4) interposed between species of subgenera *Culex* and *Phenacomyia*. The monophyly of *Phytotelmatomyia* is supported by a unique combination of seven homoplastic characters (1:0, 22:0, 32:1, 34:2, 39:1, 53:1, 55:1), none of which are listed as diagnostic features in Table 1. Considering all of the data, there is no doubt that *Phytotelmatomyia* is a distinct lineage that appears to have its closest affinities with subgenera *Culex* and *Phenacomyia*.

Fourth-instar larvae of *Phytotelmatomyia* are readily distinguished from the larvae of the other subgenera of genus *Culex*. The larvae and pupae exhibit adaptations to specialized habitats – they survive complete desiccation for hours. The larvae of *Phytotelmatomyia* are capable of moving between leaf axils by sinuous crawling, an uncharacteristic movement of larval mosquitoes that is exhibited elsewhere only in species of *Armigeres* Theobald, *Eretmapodites* Edwards, and *Trichoprosopon* Theobald (Lounibos, 1983). The posture of the larvae while resting on substrate is similar to that of *Cx*. (*Microculex*) *imitator* Theobald, with the siphon downward. This resting posture is not frequently observed among species of subgenus *Culex*. Larval movement is different from the "irregular sustained" movement of *Cx*. (*Cux*.) species and the "sinuous fast" movement of *Cx*. (*Phenacomyia*) *corniger* (Strickman, 1989).

The adults of *Phytotelmatomyia* resemble members of the Old World Duttoni and Sitiens Groups in having similar markings on the scutum, proboscis, and tarsi. The male genitalia generally resemble those of members of the Pipiens Group (Belkin, 1962; Sirivanakarn, 1976; Harbach, 1988), and Group B of Lane (1953). Seta 1-C is short and thick as it is in species of the Sitiens Group.

The similarities shared with the larvae of other subgenera of *Culex* include: short antenna of nearly uniform diameter and seta 1-A reduced as in *Carrollia* (Valencia, 1973), *Microculex* (Lane and Whitman, 1951), and *Phenacomyia*; seta 4-C multiple as in *Nicaromyia*; seta 3-P multiple branched and short as in *Melanoconion* (Sirivanakarn, 1983) and *Microculex*; Pecten Row Index (PRI, 0.33 – 0.58) larger than in *Cx*. (*Cux*.) (0.31–0.35); PRI and Siphon Index (SI) similar to *Phenacomyia* (0.37– 0.73 and <4, respectively); saddle with long spicules and larval habitat as in *Microculex*; and ventral brush comprised of 4 pairs of setae as in *Microculex* and *Neoculex*.

The principal characteristics that distinguish *Phytotelmatomyia* from the other New World subgenera of *Culex* include, in larvae: seta 1-C short and thick, branched or forked, seta 0-P large and inserted posterior to

5-P, seta 14-P short and multiple branched, setae 1-M,T and 1-I,II strong and branched, and small PRI and SI; in pupae: long setae 1-CT and 5-IV, and presence of paddle marginal serration posteriorly on inner and outer parts.

Culex hepperi and *Cx. castroi* are closely related to one another and to *Cx. renatoi* and *Cx. machadoi*. With regard to the last species, a detailed study and description would seem necessary to determine whether it is a good species or a synonym of *Cx. castroi*. The original descriptions should be consulted for detailed information on these species.

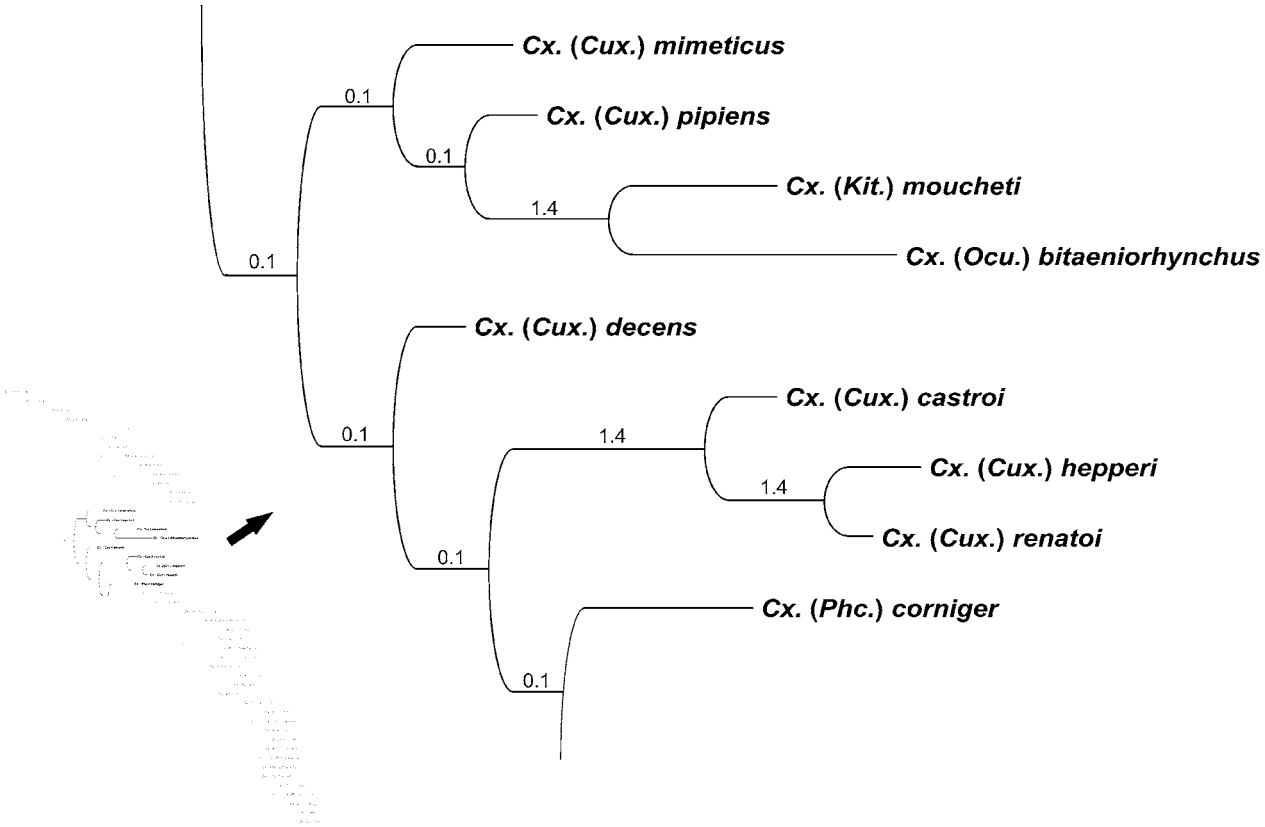

FIGURE 9. Topology of relationships between species of subgenera *Culex*, *Phenacomyia*, and *Phytotelmatomyia* obtained when character data for three species of the last taxon (*Cx. castroi*, *Cx. hepperi*, *Cx. renatoi*) are included in the data set of St. John (2007), and the data are analyzed using implied weights, implemented by PIWE, with the default value of $K = 3$.

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Appendix

Anatomical characters and character states employed in the cladistic analysis of St. John (2007), and character states observed in *Cx. castroi*, *Cx. hepperi* and *Cx. renatoi*. The data matrix containing the character states of the other taxa included in the analysis is available upon request from the second author (r.harbach@nhm.ac.uk).

Life stage	Character and character states	*Cx. castroi*	*Cx. hepperi*	*Cx. renatoi*
Adults (females except where otherwise noted)	1. *Antenna, penultimate flagellomere, length*: same as proximal flagellomeres (0); greater than proximal flagellomeres (1).	0	0	0
	2. *Maxilla, palpomeres* (*males*): five (0); four (1); three, 4th vestigial or absent (2).	0	0	0
	3. *Maxilla, palpomeres 3–5* (*males*): with few setae (0); with numerous setae (1).	1	1	1
	4. *Maxilla/proboscis ratio* (*males*): < 0.7 (0); 0.7-1.0 (1); > 1.0 (2).	2	2	2
	5. *Maxillary palpus, dorsal white scales*: absent (0); present (1).	1	1	0
	6. *Proboscis, pale ring*: absent (0); present (1).	1	1	0
	7. *Vertex, broad scales on central part of orbital line*: absent (0); present (1).	0	0	0
	8. *Vertex, anterior dorsocentral setae*: few (0); numerous (1).	0	0	0
	9. *Scutum, dorsocentral setae*: absent (0); present (1).	1	1	1
	10. *Scutum, acrostichal setae*: absent (0); present (1).	1	1	1
	11. *Scutum, row of acrostichal setae*: incomplete (0); complete line (1).	1	1	1
	12. *Upper proepisternal scales*: absent (0); present (1).	1	1	1
	13. *Upper mesokatepisternal scales*: absent (0); present (1).	1	1	1
	14. *Prealar knob, scales*: absent (0); present (1).	0	0	0
	15. *Postspiracular scales*: absent (0); present (1).	1	1	0
	16. *Upper mesepimeral scales*: absent (0); present (1).	1	1	1
	17. *Lower mesepimeral setae*: absent (0); present (1).	1	1	1
	18. *Hindtibia, length relative to tarsomere 3*: shorter (0); longer (1).	1	1	1
	19. *Pulvilli*: absent (0); present (1).	1	1	1
	20. *Wing, dorsal tertiary fringe scales on proximal half of wing*: absent (0); present (1).	1	1	1
	21. *Wing, dorsal tertiary fringe scales on proximal half of wing* (*males*): absent (0); present (1).	0	0	0
	22. *Anal vein* (*1A*), *termination in relation to junction of mcu and* M_{3+4}: proximal (0); distal (1).	0	0	0
	23. *Terga, apicolateral pale patches*: absent (0); present (1).	0	0	0
	24. *Terga, basolateral pale patches*: absent (0); present (1).	1	1	1
	25. *Terga, basal pale bands*: absent (0); present (1).	1	1	0
	26. *Terga, apical pale bands*: absent (0); present (1).	0	0	0
Male genitalia	27. *Opisthophallus*: absent (0); present (1).	1	1	1
	28. *Opisthophallus, development:* sclerotized (0); membranous (1).	0	0	0
	29. *Aedeagal sclerites*: fused (0); not-fused (1).	0	0	0
	30. *Phallosome, denticles on inner side*: absent (0); present (1).	1	1	1
	31. *Paraproct, basal lateral arm*: absent (0); present (1).	1	1	1
	32. *Gonocoxite, scales*: absent (0); present (1).	1	1	1
	33. *Gonocoxite, subapical lobe*: absent (0); present (1).	1	1	1

	34. *Gonocoxite, position of subapical lobe*: proximal to mid-length (0); distal to mid-length but not near apex (1); subapical (2).	2	2	2
	35. *Subapical lobe of gonocoxite, development*: short (0); long (1).	1	1	1
	36. *Subapical lobe of gonocoxite, development*: undivided (0); bilobed (1).	0	0	0
Pupae	37. *Trumpet, meatal cleft*: absent (0); present (1).	0	0	0
	38. *Seta 1-II, position*: closer to each other than to seta 3-II (0); closer to seta 3-II than to each other (1).	1	1	1
	39. *Seta 2-II, position*: closer to each other than to seta 3 (0); closer to seta 3 than to each other (1).	1	1	1
	40. *Seta 5-V, development*: single or double (0); multiple-branched (1).	0	0	0
	41. *Seta 5-V, length relative to tergum*: shorter (0); longer (1).	0	1	0
	42. *Seta 6-I, length relative to seta 7-I*: longer (0); equal to or shorter (1).	1	1	1
	43. *Seta 1-Pa*: absent (0); present (1).	1	1	1
	44. *Seta 2-Pa*: absent (0); present (1).	1	1	1
Larvae	45. *Seta 1-A, insertion*: near apex of antenna (0); near mid-length of antenna (1).	1	1	1
	46. *Median labral plate*: fused with dorsal apotome (0); distinct from dorsal apotome (1).	1	1	1
	47. *Seta 3-C*: absent (0); present (1).	1	1	1
	48. *Seta 4-C, insertion*: closer to seta 5-C than to each other (0); closer to each other than seta 5-C (1).	1	1	0
	49. *Hypostomal suture*: not extended caudad of posterior tentorial pit (PTP) (0); extended caudad of PTP (1).	1	1	1
	50. *Seta 9-C, insertion relative to seta 8-C*: anterior (0); posterior (1); on same level (2).	0	0	0
	51. *Setae 3-P, length relative to setae 1,2-P*: shorter (0); equal or longer (1).	0	0	0
	52. *Seta 8-P, development*: single (0); double (1); multiple-branched (2).	1	2	2
	53. *Seta 14-M, development:* weak (0); strong (1).	1	1	1
	54. *Setae 1,2,3-I, relative positions:* points of insertions more or less form an equilateral triangle (0); points of insertions form a narrow scalene triangle, almost inserted in a longitudinal line (1).	0	0	0
	55. *Seta 7-VI, development*: single (0); multiple-branched (1).	1	1	1
	56. *Comb scales, number*: < 10 (0); > 10 (1).	1	1	1
	57. *Seta 4-VIII, insertion*: in line with middle of segment X (0); between base of siphon and dorsal margin of segment X (1).	1	1	1
	58. *Pecten*: absent (0); present (1).	1	1	1
	59. *Pecten spines, development*: without denticles (0); with one or more stout denticles (1); with slender, hair-like denticles (2).	0	1	1
	60. *Siphon, dorsolateral setae*: absent (0); present (1).	0	0	0
	61. *Siphon, ventrolateral setae, arrangement*: in continuous single row (0); single row with one or more setae laterally displaced (1).	1	1	1
	62. *Saddle*: incomplete (0); complete (1).	1	1	1
	63. *Segment X, spicules on posterior area*: absent (0); present (1).	1	1	1
	64. *Saddle, spicules on posterior margin*: absent (0); present (1).	1	1	1