

Morphology of the larval mandible and maxilla in the subgenus *Carrollia* Lutz of *Culex* L. (Diptera: Culicidae)

JONATHAN LIRIA^{1,2} & JUAN-CARLOS NAVARRO¹

¹Instituto de Zoología Tropical, Laboratorio de Biología de Vectores, Universidad Central de Venezuela. Apdo. 47058, Caracas 1041-A, Venezuela; email: jnavarro@strix.ciens.ucv.ve

Current address: ²Departamento de Biología, Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo, Estado Carabobo, Venezuela; email: jliria@uc.edu.ve

Abstract

Larval mouthpart morphology has proven to be an additional taxonomic tool at the subgeneric and generic level in mosquitoes (Diptera: Culicidae) of the genus *Anopheles* and some members of the tribe Sabethini. However, little is known about these characters in genus *Culex*. The present work describes the mouthpart morphology of four species belonging to the recognized groups in the subgenus *Carrollia*. Five mandibular characters are diagnostic in the subgenus, four on the mandible and one on the maxilla: posterior dorsal tooth (PDT), mandibular comb (MnC), mandibular spiculate area (MSA), postartis (Poa) and dorsal maxillary suture (DMxS). The maxilla offers two characters that allow the separation of the groups proposed by Valencia (1973) for this taxon: seta (4-Mx), *Cx. bihaicola* and *Cx. rausseoi* (*bihaicola* group) is large (0.127-0.159 mm), little sclerotized, not branched and located posterior beyond the laciniastrum one (LR1), in *Cx. iridescens* and *Cx. urichii* (*iridescens* group) it is very large (0.203-0.229 mm), strongly sclerotized, branched on the apex and medial to the maxillary body (MxBo).

Key words: Culicini, *Carrollia*, mouthparts, morphology, systematics

Introduction

The genus *Culex* L. (Diptera: Culicidae) represents close to 800 named species worldwide. According to the most recent catalogs (Knight & Stone 1977; Ward 1992; Harbach & Peyton 1992; Guimarães 1997) and the recent checklist of genera and subgeneric abbreviations Reinert (2001), 24 subgenera are assigned to the genus, ten in the Old World, 11 restricted to the New World and 3 with worldwide distribution (*Culex*, *Lutzia* Theobald and *Neoculex* Dyar).

The subgenera with a Neotropical distribution are: *Aedinus* Lutz, *Allimanta* Casal & Garcia, *Anoediopora* Dyar, *Belkinomyia* Adames & Galindo, *Phenacomysia* Harbach & Peyton, *Carrollia* Lutz, *Deinocerites* (Theobald), *Melanoconion* Theobald, *Micraedes* Coquillett, *Microculex* Theobald, and *Tinolestes* Coquillett.

The subgenus *Carrollia* Lutz, 1904 is one of the most distinctive subgenera, with a characteristic metallic coloration in the adults. The only systematic revision is that of Valencia (1973), who based his analysis on adult, larval, and pupal characters and proposed two species groups: *bihaicola* (5 spp.) and *iridescens*; the latter containing the *urichii* (2 spp.) and *iridescens* (9 spp.) subgroups. Valencia (1973) also proposed that the larval stages of this subgenus offer excellent phylogenetic characters, but he did not carry out on a cladistic analysis.

Historically, the taxonomy and classification of the family Culicidae has been based on traditional adult and immature morphological characters. Snodgrass (1959) was the first to describe morphology of some mosquito mouthparts, Knight (1971) and Harbach & Knight (1977), reported on the structural diversity of the mandibles of several genera and recorded a variety of maxillary structures and shapes. Later, Harbach & Peyton (1993) recognized the importance of these structures for the identification of sabethine genera. Pérez & Navarro (1995) reported diagnostic characters for three *Anopheles* Meigen subgenera of medical importance, and Liria & Navarro (1999) pointed out that the posterior lobe (MnPL) is an attribute diagnostic for the subgenus *Deinocerites*.

While these authors found that larval mouthparts provide good morphological characters for alpha taxonomy, Navarro & Liria (2000) also demonstrated that the mouthparts are an excellent and powerful tool to infer evolutionary trends and relationships in the tribe Culicini using cladistic methods. These authors also demonstrated that *Carrollia* is one of the most well supported taxa in the genus. They also proposed the inclusion of *Deinocerites* as a derived taxon (subgenus) in genus *Culex*.

We describe here the mouthpart morphology in four species of the subgenus *Carrollia* belonging to the two species groups proposed by Valencia (1973): *Cx. urichii* (Coquillett) and *Cx. iridescens* Lutz (of the *iridescens* group), and *Cx. rausseoi* Cova Garcia, Sutil & Pulido and *Cx. bihaicola* Dyar & Nuñez-Tovar (of the *bihaicola* group). This is as the first detailed characterization in this subgenus of *Culex*.

Materials and methods

We used fourth instar larvae of *Cx. bihaicola*, *Cx. rausseoi*, *Cx. urichii* and *Cx. iridescens* found in the Mosquito Collection of the Laboratorio de Biología de Vectores, Museo de Biología de la Universidad Central de Venezuela (LBV, after Guimarães 1997) (Table 1).

Ten fourth instar “L₄” larvae of each species were cleared with 10% KOH, dissected, and mounted according to the procedure of Knight (1971), Harbach & Peyton (1993), Pérez & Navarro (1996) with modifications of Liria & Navarro (1999).

The mouthpart structures were observed using a Leitz Wetzlar LABORLUX S, model 513591 light microscope. The maxillary and mandibular nomenclature follow Harbach & Knight (1980) with additions from Navarro & Liria (2000). Generic and subgeneric abbreviations are those Reinert (2001). The morphometric measurements were carried out using the scheme methods of Navarro & Liria (2000).

TABLE 1. List of species examined and data of collection.

Species	Locality	Date	Breeding site
<i>Culex (Carrollia) bihaicola</i>	Loma de Hierro, Aragua State, VZ	Feb'92	<i>Heliconia aurea</i> / palm spathes / artificial containers
<i>Cx. (Car.) rausseoi</i>	Loma de Hierro, Aragua State, VZ	Feb'92	Palm spathes: <i>Euterpe</i> sp.
<i>Cx. (Car.) iridescens</i>	Ranch Río Claro, Zulia State, VZ	Aug'95	Palm spathes: <i>Euterpe</i> sp.
<i>Cx. (Car.) urichii</i>	La Azulita, Mérida State, VZ	Dec'81	<i>Xanthosoma</i> sp (Araceae). / palm spathes

Results

Mandible (Figure 1, table 2)

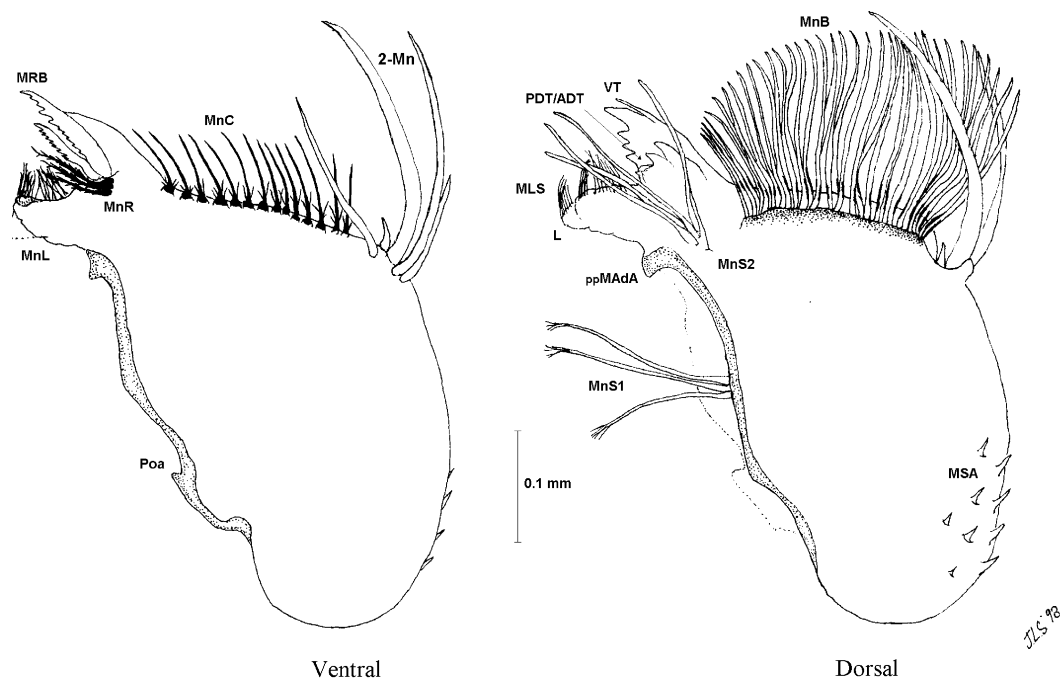


FIGURE 1. Mandibular structures of subgenus *Carrollia*.

TABLE 2. Summary of the mandibular structures of the *Culex* (*Carrollia*) species.

Structure	Bihaicola Group: (<i>Cx. bihaicola</i> and <i>Cx. rausseoi</i>)	Iridesccens Group: (<i>Cx. iridesccens</i> and <i>Cx. urichii</i>)
VT	$VT_0 > VT_1 = VT_2 = VT_3 > VT_4$	Idem
PDT	Simple not serrate, with two accessory teeth on spheric surface.	Idem
ADT	Simple not serrate.	Idem
MnC	Large: One spicule in central position surrounded by short spicules in each insertion	Idem
1-Mn	Absent.	Idem
2-Mn	Several groups of setae (2a, 2b, 2c,...etc.).	Idem
MnS	MnS ₁ and MnS ₂ with same number of filaments.	Idem
MRB	Double serrate	Idem
MSA	Large with abundant spicules.	Idem
MnR	Three or four short pectinated filaments.	Idem
L	Developed.	Idem
MnL	1.5 width of base.	Idem
Poa	U-shaped short.	Idem
ppMAdA	Short: 0.025-0.038 mm	Idem
MnB	Normal.	Idem

Ventral teeth (VT), posterior dorsal teeth (PDT), anterodorsal tooth (ADT), mandibular comb (MnC), mandibular seta number one (2-Mn), seta number two (2-Mn), mandibular sweeper (MnS), mandibular rake blade (MRB), mandibular spiculose area (MSA), mandibular rake (MnR), labula (L), mandibular lobe (MnL), postartis (Poa), posterior projection of the mandibular adductor apodema (ppMAdA), mandibular brush (MnB).

Ventral area: The ventral teeth (VT) with the following distribution of sizes; $VT_0 > VT_1 = VT_2 = VT_3 > VT_4$, mandibular rake blade (MRB) double and serrate. Mandibular rake (MnR) with three to four short pectinate filaments, mandibular comb spicules (MnCS) with large central spicule without branches, surrounded by short ones in each insertion (protuberances). Mandibular seta 1-Mn absent, 2-Mn subdivided into several groups of setae, mandibular lobe (MnL) 1.5 width of base. Postartis (Poa) "U-shaped", short. **Dorsal area:** Mandibular brush (MnB) normal, Anterior dorsal tooth (ADT) simple not serrate, posterior dorsal teeth (PDT) simple not serrate with two accessory teeth located on sphaeric surface. Mandibular sweeper (MnS) MnS₁ and MnS₂ with same number of filaments, labula (L) developed, posterior projection of mandibular adductor apodeme (ppMAdA) short, 0.025-0.038 mm mandibular spiculose area (MSA) large and with abundant spicules.

Maxillae (Figs. 2a,b Table 3)

Ventral area: Maxillary brush (MxB) short 0.140-0.178 mm. Seta (4-Mx): *Cx. bihaicola* and *Cx. rausseoi* very large (0.127-0.159 mm), poorly sclerotized unbranched, located beyond laciniarastrium one (LR1); in *Cx. iridescens* and *Cx. urichii* very large (0.203-0.229 mm) strongly sclerotized, branched at apex and medial to maxillary body. Setae of maxillary palpus (8-11-Mx) short 0.013-0.025 mm, maxillary palpus (MPip) not developed. Maxillary body (MxBo) about as long as wide, square shaped, laciniarastrium one (LR1) short in *Cx. rausseoi* and *Cx. bihaicola*, with 9-13 spicules (long in *Cx. urichii* and *Cx. iridescens*) and galeastipal stem (GSS) absent. **Dorsal area:** Seta 1-Mx large (0.064-0.076 mm) thin and posterior, inserted beyond end of dorsal suture (DMxS), 3-Mx present, 7-Mx absent, laciniarastrium two (LR2) and three (LR3) present. Maxillary spiculate area (MSPA) absent; dorsal maxillary suture (DMxS) short with diagonal orientation on maxillary body.

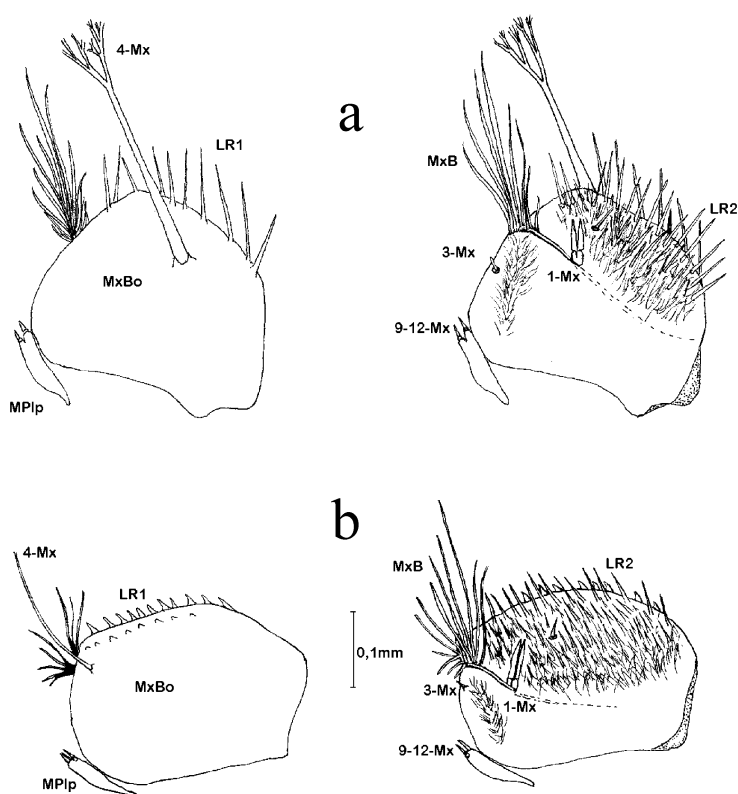


FIGURE 2. Maxillary structures of subgenus *Carrollia*: *urichii* (a) and *bihaicola* (b) groups.

TABLE 3. Summary of the maxillary structures of the *Culex* (*Carrollia*) species.

Structure	Bihaicola Group: (<i>Cx. bihaicola</i> and <i>Cx. rausseoi</i>)	Iridescent Group: (<i>Cx. iridescent</i> and <i>Cx. urichii</i>)
1-Mx	Large (0.064-0.076 mm) thin and inserted beyond end of DMxS.	Idem
3-Mx	Present.	Idem
4-Mx	Very large (0.127-0.159 mm), little sclerotized, not branched and located posterior, beyond laciniastrum one (LR1).	Very large (0.203-0.229 mm), strongly sclerotized, branched on the apex, medial to the maxillary body (MxBo)
7-Mx	Absent.	Idem
8-11-Mx	Short: 0.013-0.025 mm	Idem
MxBo	About as long as wide, square shape.	Idem
MSpA	Absent.	Idem
DMxS	Short with diagonal orientation on MxBo.	Idem
LR1	9 to 13 short spicules.	Large spicules
LR2	Present.	Idem
MPlp	Normal.	Idem
GSS	Absent.	Idem
MxB	Short: 0.140-0.178 mm	Idem

Seta number one (1-Mx), seta number three (3-Mx), seta number four (4-Mx), seta number seven (7-Mx), setae of the maxillary palpus (8-11-Mx), maxillary body (MxBo), maxillary spiculate area (MSPA), dorsal maxillary suture (DMxS), laciniastrum one (LR1), laciniastrum two (LR2), maxillary palp (MPlp), galeastipal stem (GSS), maxillary brush (MxB).

Discussion and conclusions

The following characters represent the synapomorphies previously reported (Navarro & Liria 2000) that support the subgenus *Carrollia* as a strong natural grouping in the genus *Culex* with 99% bootstrap value: posterior dorsal tooth (PDT) simple not serrate with two accessories over spheric surface; mandibular spiculate area (MSA) with large and abundant spicules; mandibular comb (MnC) large with one spicule in a central position surrounded by short spicules at each insertion; maxillary dorsal suture (DMxS) short and diagonal, and postartis (Poa) U-shaped, short.

Valencia (1973), using adult, larval and pupal characters, divided the subgenus *Carrollia* into two groups: *bihaicola* (5 spp.) and *iridescent* (11 spp.). This author described a maxillary seta 5-MP (4-Mx, after Harbach & Knight 1980) whose shape is diagnostic for the *bihaicola* group (shown in Fig. 2b).

The seta 4-Mx allows separation of *Cx. bihaicola* and *Cx. rausseoi* from *Cx. urichii* and *Cx. iridescens*, members of the *bihaicola* and *iridescens* groups, respectively. In *Cx. urichii* and *Cx. iridescens* 4-Mx is very large (0.203-0.229 mm), strongly sclerotized, branched on the apex, and in a medial position on the maxillary body (MxBo). This seta in *Cx. bihaicola* and *Cx. rausseoi* is very large (0.127-0.159 mm), poorly sclerotized, unbranched and located beyond laciniarastrium 1 (LR1). The shape of 4-Mx is very conspicuous in the *iridescens* group and can be seen without dissection. Despite this, Valencia (1973) did not note its taxonomic importance.

In addition, we found the LR1 and the position of 4-Mx are autapomorphic characters (diagnostic) for distinguishing *iridescens* from *bihaicola* species. The *iridescens* group has LR1 with large spicules and the position of 4-Mx is medial to MxBo, while the *bihaicola* group has 9-13 short spicules in LR1 and 4-Mx is located beyond LR1.

The subgenus *Carrollia* seems to be the most ancestral group among Neotropical *Culex* species, probably evolved from subgenus *Culex* and probably represents a sister group to the subgenera *Anoedioparpa*, *Melanoconion*, *Microculex* and *Deinocerites* of *Culex* (Navarro & Liria 2000).

Based on our analysis and the larval key shown in Valencia (1973: 25-26) we add some diagnostic group characters as follow (in bold):

1. Head hair 9-C short, inconspicuous, normal in position at level of 10-C; comb scales apically fringed, more or less spatulated, and arranged in a patch of 2-5 irregular rows. **Laciniarastrium 1 (LR1) of maxilla with 9-13 short spicules and 4-Mx (large, not sclerotized) located beyond LR1** (*bihaicola* group)... 2
- Head hair 9-C large, conspicuous, close to collar; comb scales elongate, spine-like, and arranged in more or less regular row, rarely a few scales displaced into second row. **LR1 with large spicules and the position of 4-Mx (large, strongly sclerotized and branched on the apex) medial to MxBo** (*iridescens* group)... 7

Despite the fact that species of *bihaicola* and *iridiscens* groups can be found in different breeding sites, the four species here studied have the spathes of palms as a common breeding site in different places and biogeographical localities from Venezuela (Table 1). Moreover, the *bihaicola* species group share the breeding sites like palm spathes in the same locality, but are temporary segregated. In addition, *Cx. bihaicola* also breed in other phytothelmata such as *Heliconia* or even artificial containers. The *iridiscens* species group were found in different geographical localities.

More species should be included for a more robust morphological analysis of mouth-parts in the genus *Culex sensu lato*, and searching for additional powerful characters to get an approach to the natural classification and evolutionary trends of *Carrollia*.

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