

A chaetotaxy for cephalic sensilla in larval Simuliidae (Diptera)

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Abstract: To develop a chaetotaxy for heads of larval Simuliidae (Diptera: Culicomorpha), position of head sensilla relative to other structures, plus sensillar innervation, were used to establish homologies with head sensilla of larval mosquitoes (Culicidae). Cleared whole mounts of first- and later-instar larval simuliids were examined using light microscopy. Histological sections were used to determine innervation, as was in vivo methylene blue staining. First-instar larval simuliids have 18 primary sensillar pairs that allow homologies with sensilla of later-instar larval culicids to be established. These homologies can be tracked in later-instar larval simuliids even though numerous secondary sensilla are present and positions of primary sensilla change. Homologies could not be determined for antennal sensilla of larval simuliids. Innervation of cephalic sensilla from the brain is in agreement with embryological origin of the various head regions. Innervation patterns of cephalic sensilla appear conserved in culicomorphs and it is proposed that, when possible, sensillar innervation be used to establish chaetotaxies.

Résumé : La position des sensilles de la tête des larves de Simuliidae (Diptera : Culicomorpha) par rapport aux autres structures ainsi que l'innervation des sensilles ont servi à établir les homologies avec les sensilles céphaliques des larves de moustiques (Culicidae). Des montages de larves entières et éclaircies de premier stade et des stades subséquents ont été examinés au microscope photonique. Des coupes histologiques et des colorations in vivo au bleu de méthylène ont servi à déterminer l'innervation. Les larves de premier stade de simuliidés possèdent 18 paires de sensilles primaires dont l'homologie peut être établie avec celles des larves de culicidés de stades plus avancés. Ces homologies se retrouvent chez les larves des stades plus avancés de simuliidés, même si de nombreuses sensilles secondaires se sont ajoutées et que les emplacements des sensilles primaires ont changé. Les homologies n'ont pu être établies pour les sensilles des antennes des larves de simuliidés. L'innervation des sensilles céphaliques depuis le cerveau est en accord avec l'origine embryologique des différentes régions de la tête. Les patterns d'innervation des sensilles céphaliques semblent bien conservés chez les culicomorphes et, lorsque c'est possible, l'innervation des sensilles devrait être utilisée pour établir les chétotaxies.

[Traduit par la Rédaction]

Introduction

The arrangement of sensory hairs (chaetotaxy) in larval (Stehr 1987, 1991) and adult insects is of importance in taxonomy, particularly in dipterans (McAlpine 1981). Within the Culicidae, there is a well-established chaetotaxy (Fig. 1) that distinguishes subfamilies and genera (Belkin 1962; Wood et al. 1979; Harbach and Knight 1980), and similarly in the Chironomidae (e.g., Saether 1980; Kowalyk 1985) and Ceratopogonidae (e.g., Lawson 1951; Borkent and Craig 2004).

Although within families there is phylogenetic information to be derived from chaetotaxy (e.g., Craig 1974), the different chaetotaxal systems used for each taxon have, in large part, precluded determining homologies of sensilla between families. Thus, it has not been possible to apply this in higher level phylogenetic analysis. For example, although the cephalic sensilla for larval culicids and chironomids are numbered from the anterior margin of the head capsule, the

numbering systems are not exactly the same. For ceratopogonids, the chaetotaxal system uses letters and numbers.

In simuliids, chaetotaxy is not widely used for taxonomy of the larval stage. Furthermore, larvae of this family are unusual in that, as they progress through instars, the first-instar complement of sensilla (Figs. 2–5) is augmented by additional sensilla, termed secondary by Craig (1974) (s s in Fig. 10). These secondary sensilla, when strongly developed, have, however, been used as diagnostic of species (Craig and Joy 2000). Primary and secondary sensilla can be easily distinguished from one another because the former tend to be longer and to have sockets distinct from those of the latter (Fig. 10).

Primary sensilla of the anterior larval head were used, in part, by Craig (1974) to establish the homologies of the larval labral fan to labral structures in those simuliid genera possessing markedly reduced fans, or lacking them entirely, such as first-instar larval *Helodon*, *Prosimulium*, *Twinnia*, and *Gymnopais* species, and of later instars of the latter two genera. Since first-instar simuliids have a chaetotaxy markedly similar to that of larval culicids (Burgess and Rempel 1966), it was also possible to confirm the homology of simuliid labral fans to the lateral palatal brushes of larval culicids. Fundamental to that study by Craig was the ability to trace the dendritic sheath of a neuron innervating a particular

Received 21 June 2004. Accepted 18 February 2005.
Published on the NRC Research Press Web site at
<http://cjz.nrc.ca> on 30 April 2005.

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sensillum to the serially homologous sensillum developing in the next (pharate) instar, in a manner similar to Belkin (1960).

Intrafamilial chaetotaxies are usually established by determining homologies of larval sensilla of the different taxa under consideration. Commonality in the number of sensilla, their distribution pattern in relation to one another and to other structural landmarks are typically used (Harbach and Knight 1980; Kowalyk 1985). McIver (1982), in a review of the sensilla of mosquitoes, discusses the difficulties in establishing homologies.

When a particular body part such as the labrum is markedly modified (cf. Figs. 3, 4), the relative position of a particular sensillum is often changed. The structure of external cuticular processes of individual sensilla is also problematic, since they are labile in form. A particular sensillum may range from trichoid to markedly pectinate, or branched. Further, most cephalic trichoid sensilla are probably mechanoreceptive (e.g., Craig 1974; Craig and Batz 1982; McIver 1982) and are functionally and ontogenetically (Lees 1942; Heming 2003) homologous to campaniform or dome sensilla. That is, campaniform sensilla have the trichoid portion of their structure reduced to a dome. Difficulty arises when homologous sensilla can be of either form, or of intermediate form, so that they may or may not be included in a chaetotaxy (see below). This structural difference was discussed in detail by Craig and Batz (1982) for antennal sensilla of larval simuliids. This change in structure of cephalic sensilla can be seen for sensilla 8-C and the pair 11-C plus 13-C (cf. left and right, Fig. 2; cf. Figs. 2, 4). Neither Belkin (1962) nor Harbach and Knight (1980) commented about campaniform sensilla, but did recognize a “ring organ”, an obvious campaniform sensillum on culicid mandibles. Further, both works illustrate a campaniform organ between sensilla 11-C and 14-C, and associated with the precoila (thickening of the anterior head capsule involved with the mandibular base), but make no comment. Kowalyk (1985) describes what are probably campaniform sensilla in chironomids, but designated them as sensory pores. Lawson (1951) labeled campaniform sensilla in ceratopogonids. Hence, for a chaetotaxy that relies on strict counting of trichoid sensilla, ignoring campaniform sensilla might result in a lower count of sensilla. Perhaps this explains the difference between larval heads of culicids and simuliids, both of which have 18–19 pairs of cephalic sensilla (Harbach and Knight 1980; and this work), and the 11–12 pairs of cephalic setae of chironomids (Kowalyk 1985). If the four “sensory pores” on the larval head of chironomids are included, the total count of sensillar pairs is closer to that of culicids and simuliids.

There are few studies of sensillar innervation in larval mosquitoes. Christophers (1960) illustrated and listed the general nerves of the head, and Burgess and Rempel (1966) determined the innervation of anterolateral sensilla on the larval head of *Aedes aegypti* (L., 1762). The main thrust of their study, however, was the neurosecretory system. They used Christophers' (1960) terms for sensilla and these differ a little from those now generally accepted (Harbach and Knight 1980) (Fig. 1). Of note is that in larval *A. aegypti*, sensilla 2-C and 3-C are apparently absent. Clements (1992) briefly summarized the embryonic development of the cen-

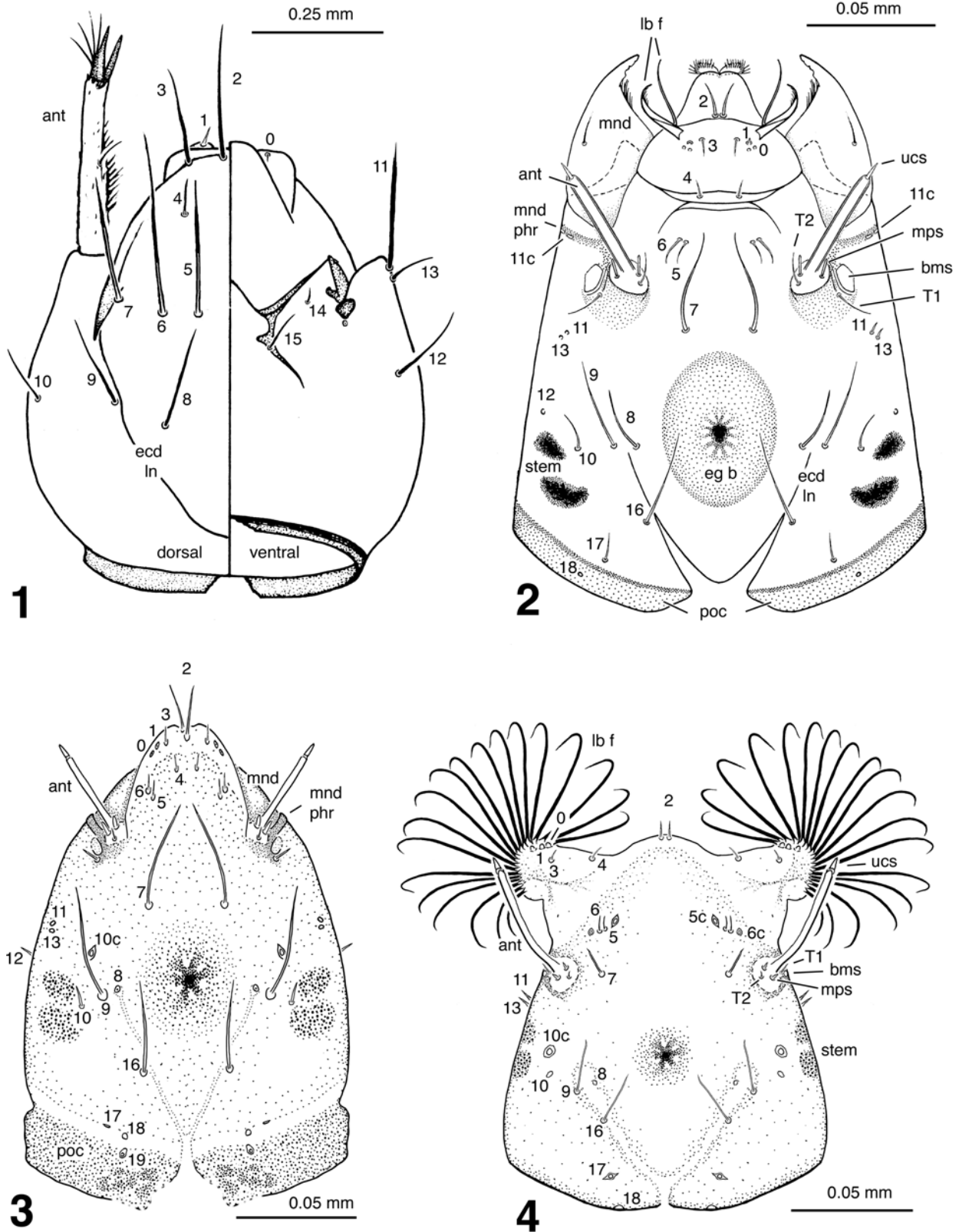
tral nervous system, but not of larval sensilla, and McIver (1982) reviewed the fine structure of culicid sensilla, with emphasis on adults.

The general structure of the central nervous system and innervation of simuliids was investigated by Puri (1925), and by Grenier (1949) and Condon et al. (1976) who emphasized the neurosecretory aspects. Gelbič and Knoz (1972) investigated the cephalic nerves of later-instar larval *Prosimulium*, *Simulium*, and *Twinnia* (the latter genus lacks labral fans in later-instar larvae), but did not determine sensillar innervation. Craig (1974), while using innervation to determine homology of the labral fan of simuliids, emphasized muscle homologies. Craig and Borkent (1980) attempted the determination of homologies of maxillary palpal sensilla at the intra- and inter-family level of lower dipterans; however, while they were able to homologize simuliid sensilla with those of the culicid maxillary lobe, they were unable to do so for the palp, for which they developed their own terminology. Ross and Craig (1979) described the external structure of what they termed an antennal puncture sensillum at the antennal base of *Prosimulium* species, suggesting that it might be homologous to that of larval culicids and chironomids. Craig and Batz (1982) described the fine structure of the simuliid larval antennal sensilla and their innervation. They disagreed with the homology suggested by Ross and Craig (1979) for the “antennal puncture sensillum”, instead interpreting it as a “bacteria-covered multiporous sensillum”. Eight sensilla, innervated with a total of 22 neurons, were identified and an attempt made to determine sensillar homologies not, however, to the chaetotaxy of Harbach and Knight (1980), but rather to that of Zacharuk et al. (1971) and others. Craig and Batz (1982) established that the two trichoid sensilla (their T1 and T2), adjacent to the antennal base, were mechanoreceptors.

Craig (1977) showed that the posterior “serration” of simuliid mandibles was actually a peg-like campaniform sensillum. He attempted to establish homologies of mandibular brushes and other spinous structures with those of culicids, but only a few were supported by their innervation.

Craig (1975) used the culicid notation of Harbach and Knight (1980) for anterolateral sensilla on the head of first-instar and later-instar *Simulium oviceps* Edwards, 1933, but did not establish homologies to more posterior cephalic sensilla. Condon et al. (1976), in a study of the neurosecretory system of larval simuliids, noted the fused corpora allata of Simuliidae to be unlike that of other nematocerans. Okazawa and Nodasaka (1982) used scanning electron microscopy to examine the arrangement of anterior cephalic and antennal sensilla in first-instar larvae of two *Gigantodax* species. The arrangements they described are similar to those shown here. Biggs (1985), in a wide-ranging study of feeding in larval simuliids, used in vivo methylene blue to demonstrate innervation. Although considerable detail was provided for some sensilla and nerves, connections between the two were not often clear. He showed, in general, disposition of larger nerves to be in agreement with this work. Colbo and Okaeme (1988) applied the culicid notation system to sensilla of first-instar larvae of *Cnephia dacotensis* (Dyar and Shannon, 1927); however, they made little comment. Homologies of sensilla were in close concordance to those proposed here.

Fig. 1. Cephalic chaetotaxy of a generalized larval head of an anopheline mosquito. Pectinations on sensilla removed for clarity. Adapted from Harbach and Knight (1980). Dorsal (left) and ventral (right) views. **Fig. 2.** Cephalic chaetotaxy of first-instar larval *Prosimulium* and *Helodon* species. Dorsal view. Adapted from Davies (1960). Left side, *Prosimulium fuscum*; right side, *Helodon susanae*. **Fig. 3.** Cephalic chaetotaxy of a first-instar larval *Twinnia nova*. Dorsal view. **Fig. 4.** Cephalic chaetotaxy of a first-instar larval *Simulium vittatum*. Dorsal view. Number of labral fan rays are not exact. See List of abbreviations after the Reference section for definitions.



My principal goal in this study was to determine homologies of cephalic sensilla of the larval head of simuliids, in spite of marked morphological modification of larval heads within the family. Then, using exterior morphological landmarks, establish homologies of the sensilla to those of larval culicids, again in spite of major differences in overall head structure between the two families. Irrespective of the structural differences between larval heads of simuliids and culicids, it was expected that larval head sensilla of both families would reflect homologous innervation derived from their embryonic ganglion, since it is now apparent that epidermal sense organ precursor cells (SOPs) and their nervous connections show stereotypic conserved development even between orthopterans and higher dipterans — lineages diverging 300 million years ago (Fig. 13.1 in Heming 2003). As such, innervation would provide evidence for homology independent of cuticular components of the head for which positional relationships are labile.

Methods

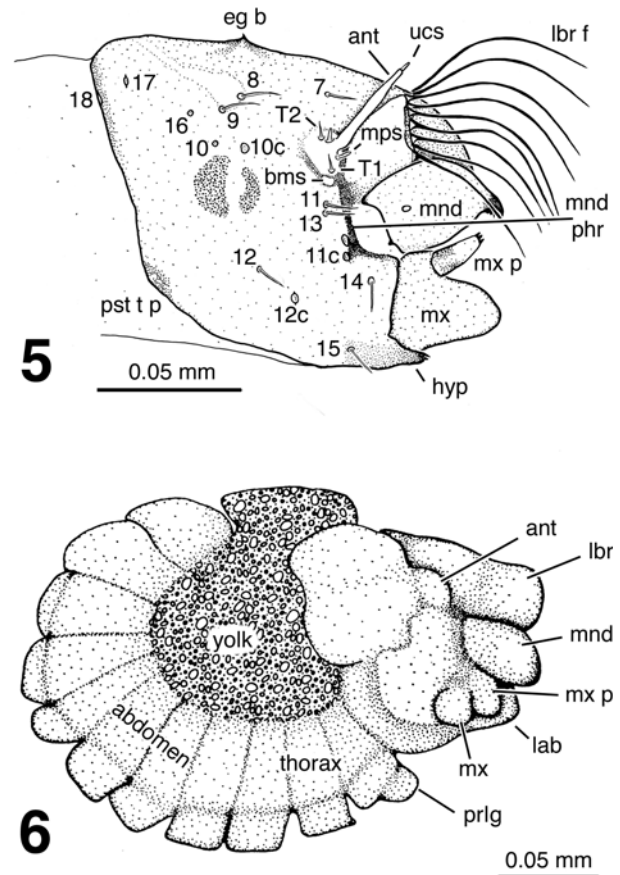
The basic distribution of simuliid cephalic primary sensilla was determined from examinations of whole mounts of first-instar larval simuliids (*Cnephia dacotensis* (Norris Creek, Alberta, Canada); *Crozetia crozetensis* (Womersley, 1937) (Crozet Islands, South Indian Ocean); *Helodon susanae* Peterson, 1970 (Mount Edith Cavell, Alberta, Canada); *Prosimulium magnum* Dyar and Shannon, 1927 (Michigan, USA); *Prosimulium travisi* Stone, 1952 (Nordegg, Alberta, Canada); *Simulium vittatum* Zetterstedt, 1838 (Sturgeon River, Alberta); *Twinnia nova* (Dyar and Shannon, 1927) (Nordegg, Alberta, Canada)), using either light microscopy at high magnification and differential interference contrast or scanning electron microscopy. The literature was also used to confirm the placement of sensilla (e.g., Davies 1960; Craig 1974; Craig and Batz 1982; Okazawa and Nodosaka 1982; Borkent and Wood 1986; Adler et al. 2004). First-instar larvae were the preferred stage, as they lack secondary sensilla.

Whole mounts of last-instar larval heads were examined for a number of simuliid genera, in particular those of later-instar *Parasimulium crosskeyi* Peterson, 1977 (Columbia River Gorge, Oregon, USA), where the antennal sensilla are of importance (see below). For all species, both early- and later-instar larval heads were cleared in hot lactic acid and mounted on microscope slides in Canada Balsam under thickness No. 1 coverslips. At least five specimens were examined for each species.

The segmental composition of the insect head has been contentious (Rempel 1975; Heming 2003). The generally accepted arrangement (the linear model) (Heming 2003) has a nonsegmented acron, followed by six segments: labral, antennal, intercalary, mandibular, maxillary, and labial. Despite recent gene expression evidence for an additional (ocular) cephalic head segment in insects (the “Y” model; Heming 2003) and for ease of comparison with previous studies, I present my results within the linear model (Fig. 6).

To trace the innervation of cephalic sensilla, later-instar larval *C. dacotensis* were stained in vivo with methylene blue following the technique of Burgess and Rempel (1966). Though this technique can produce excellent results (see Burgess and Rempel 1966), these vary markedly. This staining

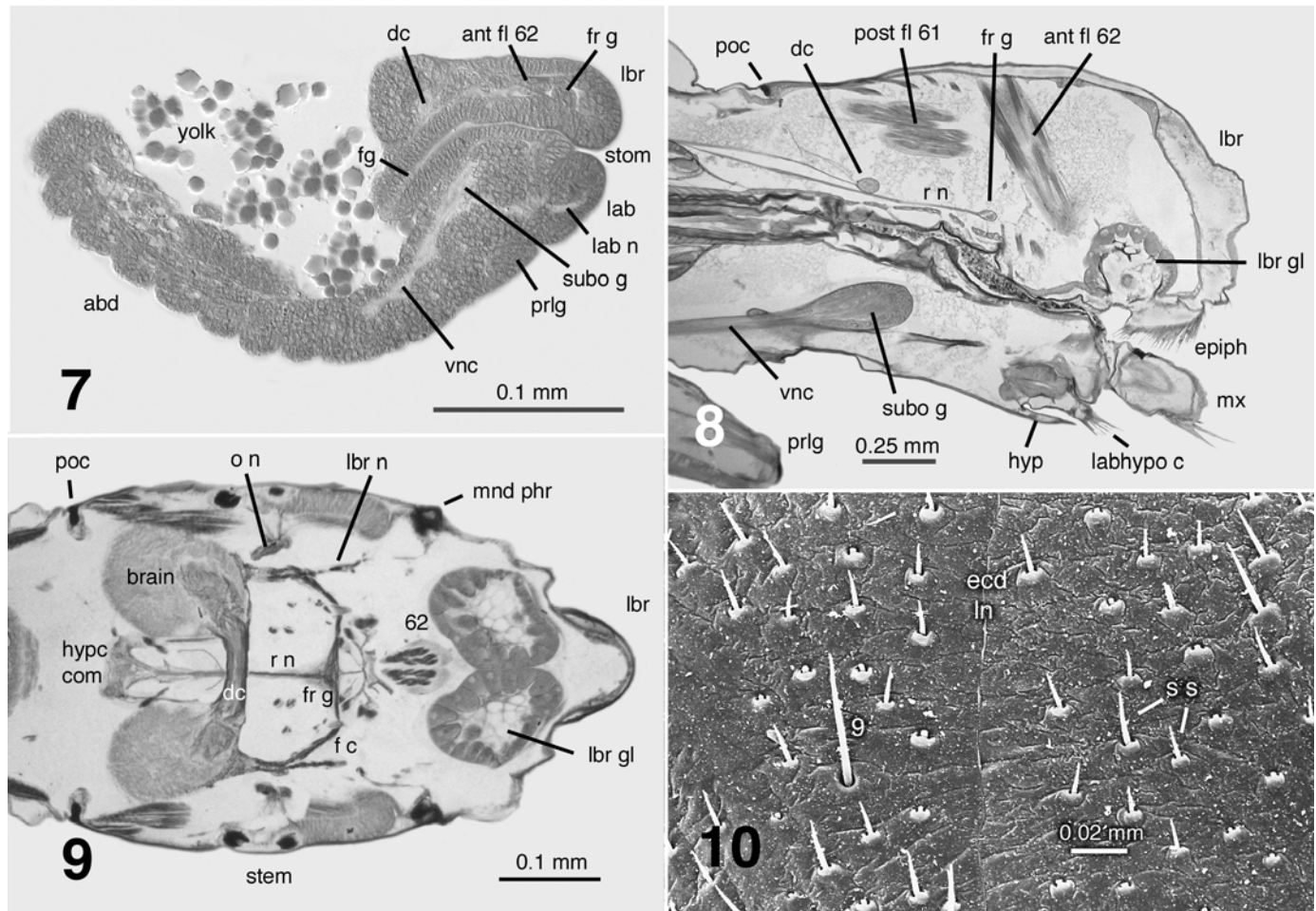
Fig. 5. Cephalic chaetotaxy of a first-instar larval *Cnephia dacotensis* in lateral view. Number of labral fan rays are not exact. **Fig. 6.** Lateral view of a late embryo of *Simulium venustum* prior to dorsal closure and deposition of cephalic capsule cuticle. Adapted from Craig (1969). See List of abbreviations after the Reference section for definitions.



was used to determine the general arrangement of innervation of anterior head sensilla. To determine complete detailed cephalic innervation, larval *C. dacotensis* and *S. vittatum* were sectioned using routine histological techniques (Craig 1974) and sections were stained with Holmes' silver stain (Larson 1960). This technique distinctly stains individual neurons (Fig. 9). Other sections were stained with haematoxylin-eosin (Fig. 7) and aldehyde fuchsin – periodic acid Schiff (Fig. 8) (Humason 1967). Figures illustrating detailed innervation of larval simuliid heads were produced from the silver-stained material by using a drawing tube on a Wild M20, compound research microscope and tracing each nerve, section by section, to produce a stereodiagram. Only three specimens each were available for that.

For a fundamental landmark within the head, the frontal ganglion (fr g) has much to recommend and is taken as such here. In embryos of simuliids (Gambrell 1933; Craig 1969), as in those of other insects (Heming 2003), the frontal ganglion evaginates from the roof of the stomodaeum (Fig. 7). In later instars, the frontal ganglion maintains its relationship to the anterior frontolabral muscle (ant fl 62 in Figs. 7–9), even with the addition of the cibarium (the oral region anterior to the frontal ganglion) and with the marked dilation of the labrum (Craig 1974). In larval mosquitoes, Burgess

Fig. 7. Photomicrograph of a sagittal section of an early embryo of *H. susanae* showing the position of the frontal ganglion (fr g) in relation to the dorsal brain connection (dc) and the stomodaeum (stom) or original mouth. Haematoxylin and eosin stain. **Fig. 8.** Slightly parasagittal section of the head of a last-instar larval *Cnephia dacotensis* showing the position of the frontal ganglion (fr g) in relation to the recurrent nerve (r n) and dorsal brain connective (dc). Aldehyde fuchsin stain. **Fig. 9.** Composite image of eight horizontal sections of the head of a last-instar larval *C. dacotensis* showing the position of the frontal ganglion (fr g), recurrent nerve (r n), and labral nerve (lbr n). Silver stain. **Fig. 10.** *Simulium cataractarum* cephalic apotome cuticle. Scanning electron micrograph of primary sensillum 9-C, ecdysial line (ecd ln), and a plethora of secondary sensilla (s s). See List of abbreviations after the Reference section for other definitions.



and Rempel (1966) showed the labral nerve exiting from the dorsal brain, dividing on either side into the frontal connectives that extend to the frontal ganglion as well as terminally innervating the anterolateral cephalic sensilla, with a separate branch to the epipharyngeal sensilla (Fig. 11). This pattern is the same in simuliids (Fig. 12), although the nerves take a convoluted route in accommodating the large labral glands (lbr gl) and the markedly enlarged labral fan muscles (posterior frontolabral muscle 61; Craig 1974). For an external landmark, the paired epipharyngeal sensilla, which are easily identified on the epipharynx/labrum of many nematoceros larvae (personal observation) and are innervated by a single nerve branching from the labral nerve, are an excellent choice.

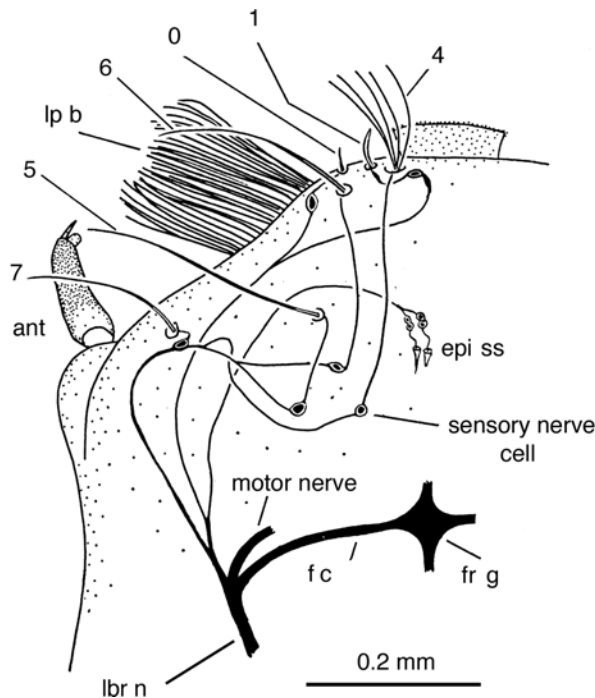
With apparently highly conserved innervation of six anterolateral cephalic sensillar pairs (Burgess and Rempel 1966) and the well-established chaetotaxy for larval heads of culi-

cids (Harbach and Knight 1980), it is not unreasonable to apply that system of chaetotaxy to larval simuliids.

In their detailed compilation and definition of chaetotaxy in mosquitoes, Harbach and Knight (1980) used the notation "seta 3-C" for sensory hairs, with the "C" referring to "cephalic". I follow that notation in part; however, I do not use the term seta for chaetotaxy in simuliids, because some sensilla are not of that form in some instars. Where campaniform sensilla appear to be part of the primary sensillar array, they are defined in relation to the nearest primary trichoid sensillum (e.g., 5c and 6c in Fig. 4). For clarity in the illustrations, sensilla are labeled only by number; however, campaniform sensilla are labeled as above.

Terms for larval head structures of simuliids follow Adler et al. (2004) and those for musculature follow Craig (1974). Where deemed appropriate in the text, the equivalent structural term for culicids is given. For reasons provided by

Fig. 11. Schematic of the innervation of the left dorsal anterolateral cephalic sensilla in a late-instar larval head of *Aedes aegypti*. Adapted from Burgess and Rempel (1966). See List of abbreviations after the Reference section for definitions.



Craig and Batz (1982) and as indicated below, antennal sensilla of simuliids are not formally homologized with those of culicids and are labeled as in their study.

Criteria for homology

Where possible, both morphological landmarks, as defined by Harbach and Knight (1980), and innervation, as defined by Burgess and Rempel (1966), were used to establish homology of larval cephalic sensilla in culicids and simuliids. Innervation in culicids was, however, only available for epipharyngeal and anterolateral sensilla (Fig. 11). For the remaining simuliid sensilla, morphological landmarks from culicids were used (as above), since there was no comparable culicid innervation evidence. Most homologies, however, appear to be definitive (Table 1) with morphological (Harbach and Knight 1980) criteria in agreement for both families; still homologies of a few sensilla are problematic (see below).

Observations

Chaetotaxy

Cephalic sensilla

Epipharyngeal (labropalatal)

The epipharynx in larval simuliids is supported by an inverted T-shaped sclerite, the intertorma (Fig. 13), into which the anterior frontolabral muscle 62 inserts (Figs. 9, 12). The cross piece is toothed, but two pairs of these structures are sensilla (epi ss in Fig. 13) (Figs. 16 and 17 in Craig 1974). In culicids, these sensilla are “3-Lp” and are readily identi-

able, although the intertorma is of variable development in larvae of that family. Two other sensilla in the culicid epipharynx (1-Lp, 2-Lp) are not known for simuliids.

0-C, 1-C

In larval culicids, these sensilla occur anteromedially on the labrum. Occasionally, as in *A. aegypti*, 1-C is spine-like. For simuliids, they are problematic but appear to be homologous to the campaniform sensilla at the base of the labral fan rays (Figs. 2, 4, 12) or on the homologous portion of the labrum (Fig. 3). In most simuliids, three campaniform sensilla can be detected (cf. Figs. 2, 3), with occasionally one of peg-like structure (right side in Fig. 2). As in culicids, 0-C is designated because of its association with 3-C.

2-C

As in culicids, these are a pair of substantial trichoid sensilla medially inserted on the anterior labrum. In simuliids lacking labral fans or where they are reduced as in some first-instar larvae in which the labrum is narrowed (e.g., *Twinnia*, *Crozetia*), these sensilla may be one behind the other (Fig. 3).

3-C

In culicids, these are associated with 0-C. In simuliids with reduced or absent labral fans, they are situated immediately posterior and slightly lateral of 2-C (Figs. 2, 3). In simuliids with labral fans, they are located distally on the fan stem (Fig. 4). Craig (1974, 1975) has 3-C and 4-C reversed.

4-C

As in culicids, in simuliids these sensilla are located posterior of 3-C, when fans are reduced or absent (Figs. 2, 3). In first-instar larvae with fully developed fans, they are inserted anterobasally on the fan stem (Fig. 4); in later-instar larvae more distally (Fig. 12). In later-instar larval *Parasimulium*, both 3-C and 4-C are markedly elongate.

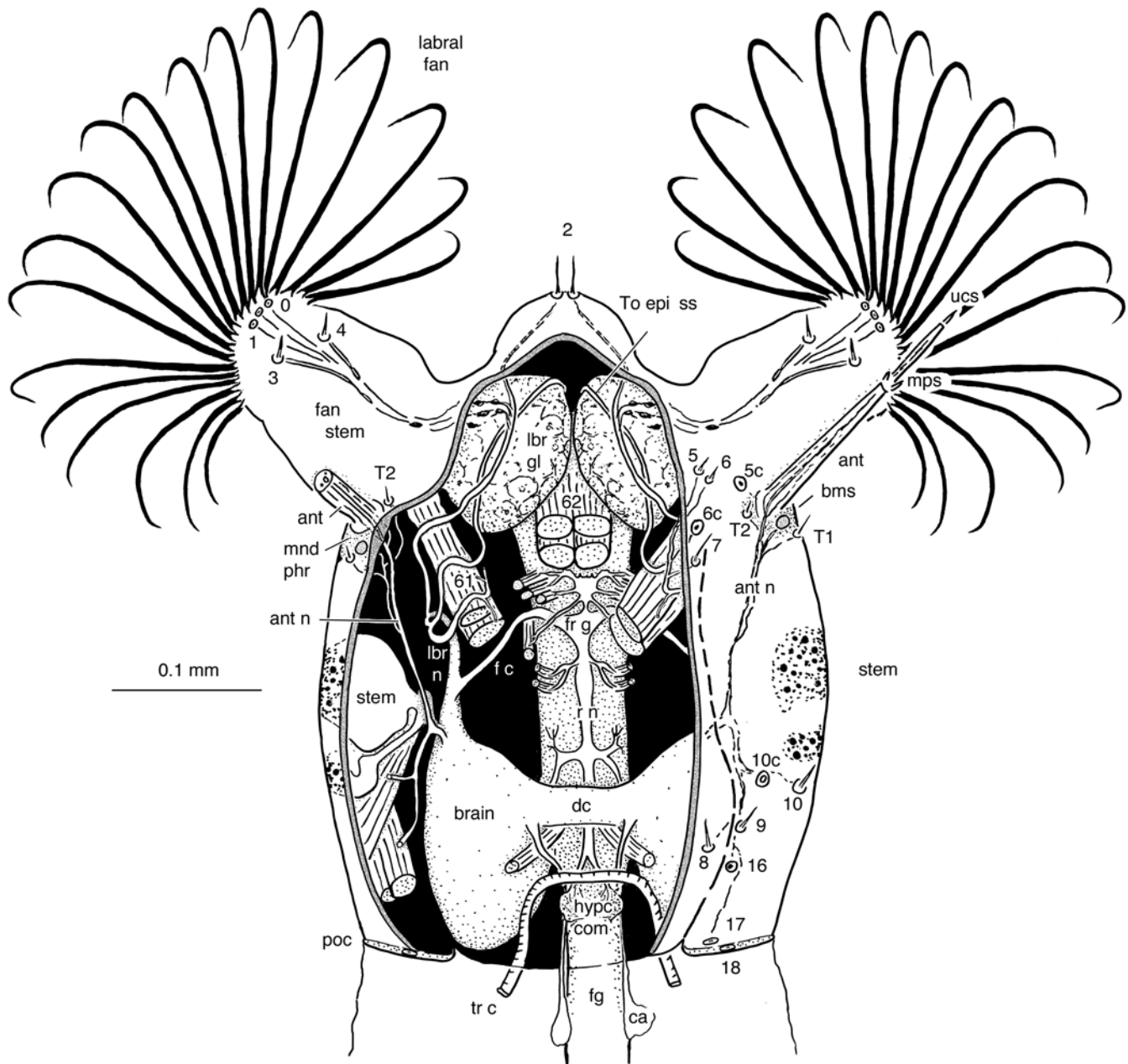
5-C, 6-C

In culicids, these are a pair of sensilla medial of the antennal base. In simuliids, they are a distinct, closely applied pair of sensilla inserted slightly anteromedial of the antennal base. In first-instar larvae, they are sometimes associated with a pair of campaniform sensilla and sometimes so in later-instar larvae. This trichoid pair is easily identified in later-instar larvae (Fig. 12) (also see Adler et al. 2004). In first-instar larval *Helodon*, *Prosimulium*, and *Twinnia* (Figs. 2, 3), there are just the trichoid sensilla. In first-instar larval *Simulium* (Fig. 4), there is an associated pair of campaniform sensilla (5c, 6c), the former being more medial and larger. In later-instar larvae (Adler et al. 2004), with one known exception, 5c is located just medially of the antennal base, while 6c is located more posteriorly nearer 7-C. Occasionally, either one of these campaniform sensilla is absent. In later-instar larval *Parasimulium*, 6c is closely associated with 7-C and 5c is apparently incorporated into the antennal base (see latter).

7-C

In culicids, this sensillum is the most lateral of any on the frontoclypeal apotome, immediately adjacent to the antennal base and medial of the anterior extension of the ecdysial line

Fig. 12. Dorsal view of the generalized innervation of cephalic sensilla in a late-instar larval head of *S. vittatum*. See List of abbreviations after the Reference section for definitions.



(ecd ln) (Fig. 1). In first-instar larval *Helodon*, *Prosimulium*, and *Twinnia*, 7-C is located posteromedial of the antennal base and the ecdysial line is not extended as far anteriorly. In first-instar *Simulium* larvae, the sensillum is situated medial of the antennal base (Fig. 4) and in later-instar larvae is located posteromedial of the antennal base and, as in culicids, medial of the anterior extension of the ecdysial line (Fig. 12).

8-C

In culicids, these sensilla are usually the most posterior of those on the frontoclypeal apotome and are medial of the ecdysial line. In first-instar simuliid larvae, 8-C forms a pair with 9-C with the anterior end of the ecdysial line extended between them. In later-instar larvae, the pair (8-C and 9-C)

are well posterior (Fig. 12). In first-instar *Simulium* larvae and later-instar larvae of that and other genera, 8-C may occur as campaniform sensilla (Fig. 4).

9-C

As in culicids, in simuliids these sensilla are lateral of 8-C and the ecdysial line. They occur occasionally as campaniform sensilla (Adler et al. 2004).

10-C

In culicids, these sensilla are lateral to lateroventral of 9-C and are associated with the stemmata (larval eyes). In first-instar larval *Helodon*, *Prosimulium*, and *Twinnia*, they are directly lateral of 9-C and dorsal of the stemmata (Figs. 2, 3). In later-instar larvae, they are posterodorsal of the stem-

Table 1. Summary of larval head chaetotaxy for Simuliidae and Culicidae, with sensillar form, position, and innervation where known.

Designation	Form			Position		Innervation			Homology confidence
	Simuliidae	Culicidae	Simuliidae	Simuliidae	Culicidae	Simuliidae	Culicidae	Culicidae	
Epipharynx	Cone	Cone	Epipharynx	Epipharynx	Epipharynx	Medial labral nerve	Medial labral nerve	Medial labral nerve	****
0-C	Campaniform	Spine/trichoid	Lateral on labrum	Lateral on labrum	Anterolateral on labrum	Lateral labral nerve	Lateral labral nerve	Lateral labral nerve	*
1-C	Campaniform/peg	Spine/trichoid	Lateral on labrum	Lateral on labrum	Anterolateral on labrum	Lateral labral nerve	Lateral labral nerve	Medial labral nerve	*
2-C	Trichoid	Trichoid	Anteromedial on labrum	Anteromedial on labrum	Anteromedial on labrum	Lateral labral nerve	Unknown	Unknown	****
3-C	Trichoid	Trichoid	Medial on labrum	Medial on labrum	Medial on labrum	Lateral labral nerve	Unknown	Unknown	***
4-C	Trichoid	Trichoid	Posterior of 3-C	Posterior of 3-C	Posterior of 3-C	Lateral labral nerve	Lateral labral nerve	Lateral labral nerve	***
5-C	Trichoid	Trichoid	Posterior of 4-C	Posterior of 4-C	Posterior of 4-C	Medial labral nerve	Lateral labral nerve	Lateral labral nerve	****
5c	Campaniform	na	Adjacent to 5-C/antenna	na	na	Unknown	na	na	na
6-C	Trichoid	Trichoid	Lateral to 5-C	Lateral to 5-C	Ventral of 5-C	Medial labral nerve	Medial labral nerve	Lateral labral nerve	****
6c	Campaniform	na	Adjacent to 6-C	na	na	Medial labral nerve	na	na	na
7-C	Trichoid	Trichoid	Adjacent antennal base	Adjacent antennal base	Adjacent antennal base	Medial labral nerve	Lateral labral nerve	Lateral labral nerve	***
8-C	Trichoid/campaniform	Trichoid	Medial of ecdysial line	Medial of ecdysial line	Medial of ecdysial line	Posterior antennal nerve	Unknown	Unknown	***
9-C	Trichoid/campaniform	Trichoid	Lateral of ecdysial line	Lateral of ecdysial line	Lateral of ecdysial line	Posterior antennal nerve	Unknown	Unknown	***
10-C	Trichoid/campaniform	Trichoid	Dorsal of stemmata	Dorsal of stemmata	Near stemmata	Posterior antennal nerve	Unknown	Unknown	**
10c	Campaniform	na	Posterodorsal of stemmata	na	na	Posterior antennal nerve	Unknown	Unknown	na
11-C	Trichoid/campaniform	Trichoid	Posterior of antenna	Posterior of antenna	Ventroposterior of antenna	Mandibular nerve	Unknown	Unknown	**
11c	Campaniform	Campaniform	Embedded in mandible phragma	Embedded in mandible phragma	Near mandibular precoila	Mandibular nerve	Unknown	Unknown	***
12-C	Trichoid	Trichoid	Ventrolateral, posterior of maxilla	Ventrolateral, posterior of maxilla	Ventrolateral, posterior of mandible/maxilla	Posterior maxilla nerve	Unknown	Unknown	**
12c	Campaniform	na	Adjacent to 12-C	na	na	Posterior maxilla nerve	Unknown	Unknown	na
13-C	Trichoid/campaniform	Trichoid	Lateral, adjacent to 11-C	Lateral, adjacent to 11-C	Lateral, associated with 11-C	Mandibular nerve	Unknown	Unknown	**
14-C	Trichoid	Trichoid	Adjacent maxillary base	Adjacent maxillary base	Near maxillary base	Anterior maxilla nerve	Unknown	Unknown	***
14c	Campaniform	na	Adjacent to 14-C	na	na	Anterior maxilla nerve	Unknown	Unknown	na
15-C	Trichoid	Trichoid	Posterolateral of hypostoma	Posterolateral of hypostoma	Posterolateral of hypostoma	Labial nerve	Unknown	Unknown	****
16-C	Trichoid	Trichoid	Near postoccipt/ecdysial line	Near postoccipt/ecdysial line	Lateral, near postoccipt	Posterior antennal nerve	Unknown	Unknown	***
17-C	Campaniform/trichoid	Trichoid	Dorsal near postoccipt	Dorsal near postoccipt	Near postoccipt	Posterior antennal nerve	Unknown	Unknown	***
18-C	Campaniform	Trichoid	Dorsal near postoccipt	Dorsal near postoccipt	Ventrolateral of postoccipt	Posterior antennal nerve?	Unknown	Unknown	**
19-C	Campaniform/absent	Trichoid/absent	Embedded in postoccipt/absent	Embedded in postoccipt/absent	Ventrolateral of postoccipt	Posterior antennal nerve?	Unknown	Unknown	**

Note: For homology confidence, **** is high, * is low, and na is not applicable.

ably the homolog of a single campaniform sensillum between 11-C and 14-C in culicids and associated with what is termed the precoila; illustrated (Fig. 1), albeit not discussed by either Harbach and Knight (1980) or Belkin (1962).

12-C

In culicids, these sensilla are posterior and ventral of 11-C and 13-C, and ventral of the stemmata. They are similarly located in simuliids (Fig. 5), although in first-instar larval *Helodon* and *Prosimulium* (Fig. 2), they are expressed as campaniform sensilla. In first- and later-instar larval *Simulium*, they are well ventral of the stemmata and are associated with accessory campaniform sensilla (12c in Figs. 5, 13).

13-C

Situated as in culicids and in close association with 11-C (Figs. 2, 5).

14-C

As in culicids, in simuliids these sensilla are situated immediately posterior to the maxillary bases (Fig. 5). In later-instar larval simuliids, they are associated with an accessory campaniform sensillum (14c, Fig. 13). At this position in later-instar larval *Parasimulium*, there is only a single campaniform sensillum.

15-C

In culicids, they are the only sensilla of the labiogular region. In simuliids, they are lateroposterior of the hypostomal region (hyp) (Fig. 5). There is only one in first- and later-instar larval *Parasimulium*, but they are multiple in later-instar larvae of other genera, with the anterior-most sensillum primary (Fig. 13). The numbers of secondary sensilla 15-C are markedly variable (Adler et al. 2004).

16-C

In some culicids, these sensilla are one of a pair of small sensilla, markedly lateral and close to the postociput (poc) (not present in *Anopheles*; Fig. 1), the other of the pair being 17-C. In first-instar larval *Helodon*, *Prosimulium*, and *Twinnia*, they are substantial sensilla situated dorsal and well posterior, and lateral of the ecdysial line (Figs. 2, 3). In first-instar larval *Simulium*, they are trichoid (Fig. 4), but are campaniform in later instars (Fig. 12).

17-C

In culicids, these sensilla are as in 16-C, but are closer to the postociput (again, not present in *Anopheles*; Fig. 1). In simuliids, 17-C are immediately anterior to the postociput and in last-instar larvae are associated with the posterior end of the ecdysial lines (Fig. 12). They are trichoid in first-instar larval *Helodon* and *Prosimulium* (Fig. 2), but are campaniform elsewhere (Adler et al. 2004).

18-C

In culicids, these are small, often-obscured sensilla ventrolaterally at the postociput, and generally absent. In simuliids, they are more dorsal and normally embedded within the postoccipital sclerite (Figs. 2–5, 12).

19-C

In culicids, these sensilla, when present, are located more ventrally than 18-C. In simuliids, they are apparently only present in the markedly expanded postoccipital sclerite of

first-instar larval *Twinnia* (Fig. 3), where they are associated with 18-C.

Antennal sensilla

In agreement with those of culicids, larval antennae of simuliids possess six external sensilla. Eight, however, are known from fine structural examination for both simuliids and culicids (Craig and Batz 1982; McIver 1982). Craig and Batz (1982) attempted to establish homologies of antennal sensilla of larval simuliids to those of larval culicids, but failed because of marked structural differences between those of the two families. The same reasoning applies here and no interfamilial chaetotaxy is established. Terms below are those of Craig and Batz (1982).

ucs

A uniporous cone-shaped sensillum, distal on the apical antennal article in all instar larvae (Figs. 2–5).

mps

These are two multiporous cone-shaped sensilla at the base of the single antennal article in first-instar larvae. In second-instar larvae, with addition of a second antennal article, they are situated on the membrane between distal and proximal articles (Fig. 12). In later-instar larvae, they maintain the same relationship, albeit the proximal article is longer and often divided into two articles. Antennae of first-instar larval *Parasimulium* are as in other simuliids; however, in later-instar larvae the distal article becomes markedly elongate (Adler et al. 2004), while the basal article is broadly cone-shaped. There the mps are more spatulate (see below in the Discussion).

T1, T2

These are two minute trichoid sensilla adjacent to the antennal base. T2 is medial of the antennal base and more so in later-instar larvae. T1 is usually more posteroventral of the antennal base and is always associated intimately with a bacteria-covered multiporous sensillum (bms; see below). In later-instar larval *Parasimulium*, T1 is located basally on the cone-shaped basal article, just dorsal of the bms, and T2 is located dorsally on the base of the cone.

bms

In first-instar larvae, these are relatively large multiporous sensilla, embedded in sclerotized cuticle ventral of the antennal base (Figs. 2, 3) and normally covered with bacteria (Craig and Batz 1982). In first- and later-instar larval *Simulium*, the sensilla are relatively smaller and insignificant (cf. Figs. 2, 4, 12). They are always associated with T1.

Innervation

As in many larval dipterans, the brain lies well to the back of the head and indeed protrudes slightly into the prothorax (Figs. 9, 12, 13). The most dorsal nerve to emerge from the brain is the antennal, which almost immediately branches anteriorly and posteriorly (ant n in Fig. 12). The anterior branch extends through the antennal rudiment of the next instar and branches half way along its length. One branch, more substantial, innervates the three sensilla on the shaft of the antenna, with a thicker dendritic sheath extending to the apical sensillum (ucs), with those for the other two sensilla (mps) less robust. The second branch innervates the three

basal sensilla located on adjacent head capsule cuticle; namely the bacteria-covered multiporous sensillum (bms) and the two trichoid sensilla (T1, T2). Puri (1925) thought that this branch ended at the cuticle. The posterior branch of the antennal nerve branches again, with its anterior branch innervating sensilla 8-C, 9-C, 10-C, 16-C, and 17-C (Fig. 12). Innervation of 18-C adjacent to the postoccipital sclerite could not be determined, but I predict that that sensillum in *Simulium*, plus 19-C in *Twinnia* (Fig. 3), will be innervated by the posterior branch of the antennal nerve.

Immediately ventral of the antennal nerve is the optic nerve (o n) that innervates the stemmata (stem). A substantial branch of that nerve extends to the anterior stemma and a smaller one to the posterior stemma (Figs. 9, 12). There appears to be no other branching of the optic nerve.

Ventral to the optic nerve is a substantial labrofrontal nerve that extends forward to branch into a frontal connective (f c) to the frontal ganglion (fr g) and into a labral nerve (lbr n) (Figs. 9, 12). The labral nerve takes a convoluted path dorsally to arch over the dorsal fascicle of the labrofrontal muscle (61) that adducts the labral fan and branches into three. The anterior branch, now immediately under the head cuticle, extends anteriorly over the large labral gland (lbr gl) and branches twice, one branch innervating sensillum 2-C and the other branch sensilla on the labral fan, namely 0-C, 1-C, 3-C, and 4-C. In methylene blue preparations of the labral fan stem, it is possible to ascertain that secondary sensilla on the stem are innervated from this same branch of the labral nerve. Elsewhere, it is not possible to determine the innervation of secondary sensilla.

The middle branch of the labral nerve curves over the labral fan adductor muscle, extends ventrally, then dorsally again following closely the dorsal surface of the large labral gland, and farther extends anteroventrally to the epipharyngeal sensilla (Figs. 12, 13). The third, posterior-most branch of the labral nerve extends over the fan adductor muscle (61), first dorsally, then slightly ventrally, branches into three of which all extend dorsally. One branch innervates sensilla 5-C, 6-C, and campaniform sensillum 6c, and perhaps 5c. The second branch innervates sensillum 7-C, and the third branch is a motor nerve for the labrofrontal muscle 61.

The frontal ganglion, plus a frontal nerve that extends to a subsidiary ganglion anterior to the frontal ganglion, innervate various muscles inserted into the dorsal surface of the foregut (Figs. 9, 12). Similarly, the recurrent nerve (r n) that passes posteriorly along the dorsal surface of the foregut and then under the dorsal connective (dc) of the brain and more posteriorly under a dorsal connective (commissure) of the tracheal system innervates other gut muscles (Figs. 9, 12). Immediately posterior of the dorsal connective of the brain and the tracheal commissure, the recurrent nerve extends into the ganglionic hypocerebral complex (hycp com) (Burgess and Rempel 1966), with nerves extending posterolaterally along the gut wall to the corpora allata.

Ventrally, the suboesophageal ganglion (subo g), a fusion of the mandibular, maxillary, and labial ganglia, is connected by the suboesophageal connectives to the brain (Fig 13). The labial nerve exits the suboesophageal ganglion close to the fused maxillary and mandibular nerves and extends well forward before extending a fine branch to innervate the ventral

wall of the foregut. Two substantial branches extend anteriorly and posteriorly, respectively. The anterior branch further divides into three, with the middle branch anastomosing across the hypostoma and innervating the gut wall, the posterior branch innervates the multiple sensilla 15-C, and the anterior branch extends to multiply-branch again to innervate sensilla of the labiohypopharyngeal complex (original labial palpi). It was not possible to determine the destination of the substantial posterior branch of the labial nerve, but I suspect that it is a motor nerve to muscles in that region.

The maxillary and mandibular nerves are fused on their exit from the suboesophageal ganglion, although internally their respective neurons are distinct. The fused nerve soon branches into maxillary and mandibular nerves. The former extends laterally and beneath a maxillary muscle and branches into three: (1) a substantial nerve extends directly into the maxillary lobe; (2) second innervates the maxillary palpus and sensillum 14-C and the associated campaniform sensillum, 14c; and (3) third is a substantial posterior branch that innervates sensillum 12-C and its associated campaniform sensillum, 12c. A further small branch innervates the maxillary muscle.

The mandibular nerve extends dorsally over the maxillary muscle (Fig. 13) and branches to innervate mandibular sensilla and muscles (not shown). Another branch innervates sensilla 11-C, 13-C, with a separate fine branch to the campaniform sensilla (11c) embedded in the mandibular phragma (left side of Fig. 13).

Nerves to the secondary sensilla of later-instar larvae form a network under the cephalic epidermal cells, but it was not possible to determine where the nerves originated, except for those of the labral fan stem (see above). It would not be unreasonable to expect that these sensilla are innervated from the main nerves associated with their region of the head.

Discussion

Cephalic sensilla

Most cephalic sensilla of larval heads of simuliids can be homologized with confidence to Harbach and Knight's (1980) scheme for culicids. Major modifications of the head capsule in simuliids to accommodate the large labral glands and the labral fans cause minor problems in homologizing sensilla 0-C, 1-C, 3-C, and 4-C, because positional relationships are markedly changed. But, the fact that the complex of sensilla is correctly identified is confirmed by the common pattern of innervation with culicids (compare Figs. 11, 12). Also problematic are sensilla that are either trichoid or campaniform in some instar larvae, but not others, and similarly between larvae of some genera. That most sensilla are trichoid in first-instar larval *Prosimulium* and *Helodon*, basal taxa of simuliids, suggests, however, that the trichoid form is ancestral. Extra campaniform sensilla (e.g., 5c, 6c) occur in many simuliids (e.g., Figs. 4, 12) and appear to originate de novo, that is, have no counterpart in the previous instar larvae.

Irrespective of major modifications to the head capsule of larval simuliids, the innervation of sensilla by the labral nerve follows a pattern similar to that observed in culicids (Figs. 11, 12) (Burgess and Rempel 1966). A single branch

of that nerve innervates the epipharyngeal sensilla (Figs. 11, 12, 13), and hence those sensilla are suggested as a landmark for evaluating chaetotaxy comparatively. Another branch of the labral nerve innervates sensilla 5-C, 6-C, and 7-C in culicids and simuliids (plus, in simuliids, campaniform sensillum 6c and perhaps 5c). Innervation of sensilla 0-C, 1-C, 2-C, 3-C, and 4-C, all from a single branch in simuliids, differs slightly from that in *A. aegypti*, where 2-C and 3-C are absent (Fig. 11) (Burgess and Rempel 1966; Harbach and Knight 1980). This similarity in organization of nerves strongly indicates that innervation can be used with confidence to homologize the anterolateral cephalic sensilla (of labral origin) in larvae of other nematoceros families. The cephalic sensory system thus appears to be evolutionarily conserved as noted by Heming (2003). For other studies on nematoceros dipterans, it is suggested that in vivo methylene blue staining be employed. Methylene blue appears to stain the frontal ganglion, and labral and antennal nerves preferentially, and although variable in result, would allow at least anterolateral cephalic sensilla to be homologized with confidence.

Where possible, use of innervation to assist in establishing sensillar homology is preferable to using the positional relationships of sensilla, for example, to head sutures. It is well established that such structures as the frontoclypeal and ecdysial lines are functional (e.g., Hinton 1962) and bear little if any relationship to the original embryonic segmentation of the head (Riley 1904; Craig 1969; Heming 2003). Indeed, in simuliids there is little evidence of any sutures other than ecdysial lines, albeit in that family some sensilla maintain close relationship to that.

The designation as a tentorial pit of the large campaniform sensillum, 10c, located above the stemmata, by McAlpine (1981) and Adler et al. (2004) is clearly incorrect. At high magnification the structure shows characteristics of a campaniform sensillum and furthermore is innervated by the posterior branch of the antennal nerve (Fig. 12).

First-instar larvae are suggested as the starting point for analysis of larval chaetotaxy. In simuliids, the sensilla appear to be less modified in first-instar larvae, in both structure and position, than in subsequent instar larvae. There is less duplication of sensilla (e.g., 15-C in Fig. 13), and homologies of trichoid sensilla, campaniform in later-instar larvae, can be determined between instars (e.g., 12-C in Figs. 2, 3).

While this chaetotaxy deals with the primary complement of cephalic sensilla in larval simuliids, it is possible that the secondary cephalic sensilla, variable in number, size, and position across simuliid genera (Adler et al. 2004), are of possible phylogenetic significance. In last-instar larval *Parasimulium*, such sensilla, while present anteriorly on the head, are sufficiently fine and sparse that they are not illustrated in Adler et al. (2004). *Parasimulium* is one of the basal sister taxa to all other Simuliidae.

Antenna

Six sensilla are associated with the larval antenna in Simuliidae, the same number as is known for Culicidae and Dixidae (Belkin 1960; Harbach and Knight 1980) and apparently in the ceratopogonid *Austroconops* Wirth and Lee, 1958 (Borkent and Craig 2004). The single sensillum at the apex of the antenna was originally assumed by simuliid taxono-

mists to be the third antennal article in later-instar larvae, but was shown to be a sensillum (ucs) by Craig (1975). In first-instar larval simuliids, the antenna consists of a single elongate article with two multiporous sensilla (mps) closely associated to its base. In second-instar larvae, a basal article is added to the antenna and two sensilla (mps) are then located at the junction of the two articles (Craig 1975). The fine structure of the basal "ring organ" was investigated by Craig and Batz (1982) and was shown to be a bacteria-covered multiporous sensillum (bms), of probable chemoreceptive function, and closely associated with two trichoid sensilla (T1, T2). Although the bms and the two trichoid sensilla are on the head capsule cuticle at the base of the antenna, they are antennal sensilla because of their innervation by a branch of the antennal nerve (Fig. 12) — in keeping with the origin of that region of the head from the embryonic antennal segment (cf. Figs. 5, 6). That two sensilla originally at the base of the antenna on the first-instar larva, become relocated more distally on the antennal shaft, is a good example of the plasticity of the cephalic epidermis and the cuticle it deposits, and reiterates the problem of using positional information to establish sensillar homologies. This problem is further exemplified by the development of the antenna in larval *Parasimulium* (see below).

It is still not possible to establish strict homology between the sensilla of culicid and simuliid antennae, other than those suggested by Craig and Batz (1982). Perhaps the bms is the "ring organ" (RO) of chironomid antennae, or more likely the "blade" (Bl), that is apparently multiporous (Saether 1980). In larval *Austroconops*, a markedly enlarged sensillum on the antenna, also termed the blade by Borkent and Craig (2004; their Fig. 4D), is multiporous and perhaps the homolog to that in culicids. For innervation, however, an expectation might be that three of the six antennal sensilla in larval culicid would be innervated from a separate branch of the antennal nerve and the others from a second branch, as in simuliids. Zacharuk et al. (1971) showed that there were six dendrites within the antenna of *A. aegypti*; their arrangement, however, is not similar to that in simuliids. Perhaps resolution of this problem will require examination of antennal sensilla in first-instar larvae of taxa basal within the Culicomorpha, namely those of Dixidae and Thaumaleidae (Oosterbroek and Courtney 1995; Courtney et al. 2000). The antennae of larval *Parasimulium*, however, may hold part of the key to resolving homologies. In the first-instar larva, the antennal sensilla are the same as all other simuliids (Figs. 2–5). In later-instar larvae, however, unlike others, the distal article becomes markedly elongated, rather than adding extended basal articles. In larval *Parasimulium*, the basal article is a broad, shallow-cone structure (Currie 1988; Adler et al. 2004), and importantly has incorporated the sensilla T1, T2, and surprisingly, what appears to be campaniform sensillum 5c into the cone. The trichoid sensilla are probably homologous to those on the shaft of culicid antennae (e.g., Fig. 1), and 5c is probably homologous to the ring organ on antennae of chironomids. Further, the two mcs are more spatulate than cone-shaped, as in other simuliids, and bear a marked resemblance to Lauterborn organs of chironomid antennae (Saether 1980; Kowalyk 1985). Last-instar larval *Parasimulium* are not apparently known (Adler et al. 2004), but when described may provide other information regarding

homologies of the antennal sensilla of simuliids and other culicimorphs.

A study complementary to that involving larval *Parasimulium* would usefully include the antenna of Thaumaleidae larvae, where antennae lack a shaft and sensilla are on a ring of clear, membranous cuticle. A ring organ is known, as are at least three cone-shaped sensilla (Sinclair 2000; Courtney et al. 2000).

Acknowledgements

Thanks to J. Scott for assistance with histology, and B.S. Heming and J. Ogawa for detailed constructive comments. Financial support for the study was from the Natural Sciences and Engineering Research Council of Canada.

References

- Adler, P.H., Currie, D.C., and Wood, D.M. 2004. The black flies (Simuliidae) of North America. Cornell University Press, Ithaca.
- Belkin, J.N. 1960. Innervation as a criterion of homology of the elements of larval and pupal chaetotaxy of mosquitoes. *Proc. Entomol. Soc. Wash.* **62**: 197.
- Belkin, J.N. 1962. The mosquitoes of the South Pacific (Diptera, Culicidae). Vol. 1. University of California Press, Berkeley.
- Biggs, J. 1985. A study of the filter-feeding behaviour of *Simulium* larvae (Diptera: Simuliidae). Ph.D. thesis, Royal Holloway and Bedford New College, University of London, London.
- Borkent, A., and Craig, D.A. 2004. *Austroconops* Wirth and Lee, a Lower Cretaceous genus of biting midges yet living in Western Australia: a new species, first description of the immatures and discussion of their biology and phylogeny (Diptera: Ceratopogonidae). *Am. Mus. Novit.* **3449**: 1–67.
- Borkent, A., and Wood, D.M. 1986. The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). *Proc. Entomol. Soc. Wash.* **88**: 286–296.
- Burgess, L., and Rempel, J.G. 1966. The stomodaeal nervous system, the neurosecretory system, and the gland complex in *Aedes aegypti* (L.) (Diptera: Culicidae). *Can. J. Zool.* **44**: 731–765.
- Christophers, S.R. 1960. *Aedes aegypti* (L.) the yellow fever mosquito: its life history, bionomics and structure. Cambridge University Press, Cambridge.
- Clements, A.N. 1992. The biology of mosquitoes: development, nutrition and reproduction. Vol. 1. Chapman and Hall, London.
- Colbo, M.H., and Okaeme, A.N. 1988. The larval instars of *Cnephia ornithophilia* (Diptera: Simuliidae), a black fly with a variable molting pattern. *Can. J. Zool.* **66**: 2084–2089.
- Condon, W.J., Gordon, R., and Bailey, C.H. 1976. Morphology of the neuroendocrine system of two larval blackflies, *Prosimulium mixtum/fuscum* and *Simulium venustum*. *Can. J. Zool.* **54**: 1579–1584.
- Courtney, G.W., Sinclair, B.J., and Meier, R. 2000. Morphology and terminology of Diptera larvae. In *Contributions to a manual of Palearctic Diptera*. Vol. I. Edited by L. Papp and B. Darvas. Sciences Herald, 87–161, Budapest.
- Craig, D.A. 1969. The embryogenesis of the larval head of *Simulium venustum* Say (Diptera: Nematocera). *Can. J. Zool.* **47**: 495–503.
- Craig, D.A. 1974. The labrum and cephalic fans of larval Simuliidae (Diptera: Nematocera). *Can. J. Zool.* **52**: 133–159.
- Craig, D.A. 1975. The larvae of Tahitian Simuliidae (Diptera: Nematocera). *J. Med. Entomol.* **12**: 463–476.
- Craig, D.A. 1977. Mouthparts and feeding behaviour of Tahitian larval Simuliidae (Diptera: Nematocera). *Quaest. Entomol.* **13**: 195–218.
- Craig, D.A., and Batz, H. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae. *Can. J. Zool.* **60**: 696–711.
- Craig, D.A., and Borkent, A. 1980. Intra- and inter-familial homologies of maxillary palpal sensilla of larval Simuliidae (Diptera: Culicomorpha). *Can. J. Zool.* **58**: 2264–2279.
- Craig, D.A., and Joy, D.A. 2000. New species and redescrptions in the central-western Pacific subgenus *Inseliellum* (Diptera: Simuliidae). *Ann. Entomol. Soc. Am.* **93**: 1236–1262.
- Currie, D.C. 1988. Morphology and systematics of primitive Simuliidae (Diptera: Culicomorpha). Ph.D. thesis, University of Alberta, Edmonton.
- Davies, L. 1960. The first-instar larva of a species of *Prosimulium* (Diptera: Simuliidae). *Can. Entomol.* **92**: 81–84.
- Gambrell, F.L. 1933. The embryology of the black fly, *Simulium pictipes* Hagen. *Ann. Entomol. Soc. Am.* **26**: 641–671.
- Gelbič, I., and Knoz, J. 1972. Differences in the structure of the labrofrontal nerve in larvae of various groups of the family Simuliidae (Diptera, Nematocera). *Acta Entomol. Bohemoslov.* **69**: 305–311.
- Grenier, P. 1949. Contribution à l'étude biologique des Simuliides de France. *Physiol. Comp. Oecol.* **1**: 168–330. (1948)
- Harbach, R.E., and Knight, K.L. 1980. Taxonomists' glossary of mosquito anatomy. Plexus Publishing, Inc., Marlton, N.J.
- Heming, B.S. 2003. Insect development and evolution. Cornell University Press, Ithaca, N.Y.
- Hinton, H.E. 1962. The ventral ecdysial lines of the head of endopterygote larvae. *Trans. R. Entomol. Soc. Lond.* **115**: 38–61.
- Humason, G.L. 1967. Animal tissue techniques. 2nd ed. W.H. Freeman and Co., San Francisco.
- Kowalyk, H.E. 1985. The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determinations. *Can. Entomol.* **117**: 67–106.
- Larson, J.R. 1960. The use of Holmes' silver stain on insect nerve tissue. *Stain Technol.* **35**: 223–224.
- Lawson, J.W.H. 1951. The anatomy and morphology of the early stages of *Culicoides nubeculosus* Meigen (Diptera: Ceratopogonidae = Heleidae). *Trans. R. Entomol. Soc. Lond.* **102**: 511–574.
- Lees, A.D. 1942. Homology of the campaniform organs on the wing of *Drosophila*. *Nature (Lond.)*, **150**: 375.
- McAlpine, J.F. (Editor). 1981. Manual of Nearctic Diptera. Vol. 1. Monograph 27. Research Branch, Agriculture Canada, Ottawa.
- McIver, S.B. 1982. Sensilla of mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* **19**: 489–535.
- Okazawa, T., and Nodasaka, Y. 1982. Morphological observations on the first- and last-instar larvae of the genus *Gigantodax* (Diptera: Simuliidae). *Jpn. J. Sanit. Zool.* **33**: 95–103.
- Oosterbroek, P., and Courtney, G. 1995. Phylogeny of the nematocerous families of Diptera (Insecta). *Zool. J. Linn. Soc.* **115**: 267–311.
- Puri, I.M. 1925. On the life-history and structure of the early stages of Simuliidae (Diptera, Nematocera). Part I. Parasitology, **17**: 295–334.
- Rempel, J.G. 1975. The evolution of the insect head: the endless dispute. *Quaest. Entomol.* **11**: 7–25.
- Riley, W.A. 1904. The embryological development of the skeleton of the head of *Blatta*. *Am. Nat.* **38**: 777–810.
- Ross, D.H., and Craig, D.A. 1979. The seven larval instars of *Prosimulium mixtum* Syme and Davies and *P. fuscum* Syme and Davies (Diptera: Simuliidae). *Can. J. Zool.* **57**: 290–300.

- Saether, O. 1980. Glossary of chironomid morphology terminology (Diptera: Chironomidae). *Entomol. Scand. Suppl.* **14**: 1–51.
- Sinclair, B. 2000. Immature stages of Australian *Austrothaumalea* Tonnoir and *Niphta* Theischinger (Diptera: Thaumaleidae). *Aust. J. Entomol.* **39**: 171–176.
- Stehr, F.W. (Editor). 1987. Immature insects. Vol. 1. Kendal/Hunt, Dubuque, Iowa.
- Stehr, F.W. (Editor). 1991. Immature insects. Vol. 2. Kendal/Hunt, Dubuque, Iowa.
- Wood, D.M., Dang, P.T., and Ellis, R.A. 1979. The insects and arachnids of Canada. Part 6. The mosquitoes of Canada (Diptera: Culicidae). *Can. Dept. Agric. Publ.* **1686**: 1–390.
- Zacharuk, R.Y., Yin, L.R-S., and Blue, S.G. 1971. Fine structure of the antenna and its sensory cone in larvae of *Aedes aegypti* (L.). *J. Morphol.* **135**: 273–298.

List of abbreviations

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|-----------|--|------------|-----------------------------------|
| abd | abdomen | lab | labium |
| ant | antenna | lab n | labial nerve |
| ant fl 62 | anterior frontolabral muscle 62 | labhypo c | labiohypopharyngeal complex |
| ant n | antennal nerve | lab p | labial palpus |
| bms | bacteria-covered multiporous sensillum | lbr | labrum |
| ca | corpra allata | lbr f | labral fan |
| dc | dorsal connective of brain | lbr gl | labral gland |
| ecd ln | ecdysial line | lbr n | labral nerve |
| eg b | egg burster | lp b | labropalatal brush |
| epiph | epipharynx | mnd | mandible |
| epi ss | epipharyngeal sensilla | mnd n | mandibular nerve |
| f c | frontal connective | mnd phr | mandibular phragma |
| fg | foregut | mps | multiporous cone-shaped sensillum |
| fr g | frontal ganglion | mx | maxilla |
| hyp | hypostoma | mx n | maxillary nerve |
| hycp com | hypocerebral complex | mx p | maxillary palpus |
| | | mx+mnd n | maxillary plus mandibular nerve |
| | | o n | optic nerve |
| | | poc | postocciput |
| | | post fl 61 | posterior frontolabral muscle 61 |
| | | prlg | proleg |
| | | pst t p | posterior tentorial pit |
| | | r n | recurrent nerve |
| | | s s | secondary sensilla |
| | | stem | stemmata |
| | | stom | stomodaeum |
| | | subo g | suboesophageal ganglion |
| | | T1, T2 | trichoid sensilla |
| | | tr c | tracheal commissure |
| | | vnc | ventral nerve cord |
| | | ucs | uniporous cone-shaped sensillum |
| | | 1–19 | primary cephalic sensilla |
| | | 61 | posterior frontolabral muscle |
| | | 62 | anterior frontolabral muscle |