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Comparison of selected parts of larval morphology in some species of the genus *Chironomus* (Diptera: Chironomidae) using Scanning Electron Microscope

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ABSTRACT

Comparison of small sized and weakly chitinized structures such as the labrum (labral setae SI-SIII, labral lamella, tormal bar, pecten epipharings) and the maxilla (maxillary palp, lacinial chaeta) is provided in larvae of *Chironomus plumosus, C. balatonicus*, *C. usenicus C. melanotus*, *C. acerbiphilus*, and *C. riparius* using Scanning Electron Microscope (SEM). The general morphological structures of the labrum and the maxilla were similar in all of the *Chironomus* larvae. They differed only in fine details such as labral seta, mainly S I. Seta S I is always plumose on each side and differently figured: elongated in *C. balatonicus*, *C. plumosus*, *C. melanotus* and *C. usenicus*; rounded in *C. acerbiphilus* and *C. riparius*. Some differences were also observed in the shape, length or ratio of lacinial chaetae (LCh I, LCh II). Based on morphological variation in the larvae of some *Chironomus* species, results indicated that the SEM method is apparently beneficial and useful in the analysis of smaller and weakly chitinized morphological structures such as the labrum and the maxilla.

Key words: *Chironomus*; larvae; morphology; labrum; maxilla; SEM.

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INTRODUCTION

The genus *Chironomus* Meigen has the largest number of species within the Chironomidae family (Fauna Europaea, 2017), yet larvae of most species show slight morphological differences. Important taxonomic features for species identification at the larval stage, which are easily visible under a light microscope, are large, strongly chitinized structures: the mentum and the mandible, and additionally important features are the antenna and the lateral and ventral tubules (Pinder and Reiss, 1983; Cranston, 2010; Orentd and Spies, 2012). However, poorly chitinized structures such as the maxilla and the labrum, which are difficult to recognize under light microscopy, receive little attention and are rarely described (Mozley, 1971). The use of Scanning Electron Microscope (SEM) allows a better understanding of these structures (Kownacki *et al*., 2015).

The aim of this study was to describe smaller and weakly chitinized morphological structures such as the labrum and the maxilla of some species of *Chironomus* larvae: *C. plumosus* Linnaeus 1758, *C. balatonicus* Devai, Wuelker & Scholl 1983, *C. usenicus* Loginova & Belyanina 1994, *C. melanotus* Keyl 1961, *C. acerbiphilus* Tokunaga 1939, and *C. riparius* Meigen 1804 using a Scanning Electron Microscope.

METHODS

Larvae of *Chironomus* were collected from different water bodies of Poland: *C. plumosus* – from Lake Gardno, a shallow eutrophic, coastal lake, separated by a sandy spit from the Baltic Sea and located in the Slowinski National Park (northern Poland), *C. balatonicus* – from the Vistula Lagoon (southern part of the Baltic Sea), *C. usenicus* – from the eutrophic Kortowskie Lake located in the city of Olsztyn. *C. melanotus* – from Luknajno Lake, shallow, eutrophic, covered with aquatic vegetation, located in the Masurian Landscape Park (north Poland), *C. acerbiphilus* – from the post-mining, acidotrophic (pH 3) reservoir located in Luk Muzakowa Landscape Park (western Poland). Larvae of *C. riparius* came from a laboratory strain. The larvae of species collected in their natural habitat were previously identified on the basis of band patterns of their salivary gland chromosomes (Jabońska-Barna 2004; Jabłońska-Barna and Michailova 2006; Jabłońska -Barna *et al*., 2012, 2013).

For Scanning Electron Microscope (SEM) the samples were fixed in 2.5% glutaraldehyde GLU in 0.1 phosphate buffered saline PBS by 2 hours, rinsed with PBS 2x10 min and dehydrated in graded alcohols. Finally, it was placed in transitional liquid *i.e.* 100% acetone and transferred to Critical Point Drier, CPD E3000/E3100 Quorum Technologies. Then it was coated with gold using JFC—1100E Ion sputter, Jeol. For coating, the materials were placed on the holder with conductive carbon adhesive tabs, Electron Microscopy Sciences. Morphological characters were observed by means of Scanning Electron Microscope (SEM), JSM-5410 operated at accelerating voltages of 15 kV in the Scanning Microscopy Laboratory of the Jagiellonian University (Kownacki *et al*., 2015). Three larvae of one species were placed on the same slide, and in the case of *C. acerbiphilus* only one larva was used.

The pictures of the labrum and the maxilla from SEM were compared within the *Chironomus* larvae we studied. Special attention was paid to the structures as labral setae S I-S III (mainly S I), labral lamella (LL), tormal bar (TB), pecten epipharingis (PE), maxillary palp (MP), lacinial chaeta (LCh) and plate X (Pl X). Additional information was obtained by calculating the index LCh=LChI/LChII. The information obtained with SEM on the labrum and the maxilla of the *Chironomus* larvae was compared with *Glyptotendipes glaucus* larvae (Kownacki *et al*., 2016).

RESULTS

Labrum. Seta anteriores (S I) of *Chironomus* is always plumose on each side, but its shape differs among the species. It is elongated in *C. balatonicus, C. plumosus*, *C. melanotus* and *C. usenicus*, while rounded in *C. acerbiphilus* and *C. riparius*. Seta posteriors (S II) is usually single, curved, smooth and without bristles, and seta minuscula (S III) is very small, hair-like (Fig. 1).

Labral lamella (LL) is always arched, its upper edge is smooth, the outer edge is rounded variously, the lower edge is plumose and covered with a row of long dagger-like appendices (Fig. 2).

Tormal bar (TB) is below LL and consist of two arched, sclerotized plates. Pecten epipharyngis (PE) consists of 12-15 single teeth of equal size, or declining towards the lateral edges (Fig. 2).

Maxilla. The maxilla is composed of three major parts: the lacinia (La), galea (G) and maxillary palp (MP). The lacinia (Fig. 3) in all species is sharply ended and triangular, and has two lacinial chaetae (LCh): The first lacinial chaeta (LCh I) is dagger-shaped with smooth edges and the second lacinial chaeta (LCh II) is always shorter than LCh I and lanceolate (for example, *C. plumosus*) or triangular (for example, *C. riparius*, *C. balatonicus*) in shape. The upper edge of LCh II is plumose and the lower is smooth (Fig. 3). Antaxial seta (Aa) is single and slightly curved. The value of the index LCh in the *Chironomus* species is the following: *C. plumosus* - 1.53, *C. balatonicus* 1.52, *C. usenicus* 1.67, *C. melanotus* 1.42, C. *acerbiphilus* 1.67, and *C. riparius* 2.12.

Maxillary palp (MP) (Fig. 4). The length of the basal segment is greater than its width. Seta A has a different length, for example, it is longer in *C. melanotus* and *C. usenicus* than in *C. acerbiphilus* and *C. plumosus*.

Plate X (Pl X) in *Chironomus* larvae consists of a group of bristles situated in the corner between the mentum (M), ventromental plate (VmP) and lacinia (La) (Fig. 5).

DISCUSSION

Scanning Electron Microscopy has been relatively rarely used to determine the morphological structure of Chironomidae larvae. Earlier studies indicate that the SEM method is very useful in the description of morphology of larvae and species differentiation (Sublette, 1979; Kownacki *et al*., 2015; Kownacki *et al*., 2016). Our investigation of *Chironomus* larvae shows that closely related species can be identified on the basis of lacinial chaetae. For example, the LCh II is elongated in *C. plumosus*, while triangular in shape in *C. usenicus* and *C. balatonicus*. These species are difficult to distinguish on the basis of the external morphology of larvae under the light microscope.

Maxilla *Chironomus anthracinus* described by Mozley (1971) has the same morphological structure as in the studied *Chironomus* larvae. Lack of such details as plumose upper edge of LCh II in *C. anthracinus* is due to lower magnification used in a camera lucida apparatus. However, in a drawing by Mozley (1971) we can see details on the inner side of the maxilla such as the end of lacinia (La) or chaetulae of palpiger (ChP), which results from the use of a light microscope. The SEM image only allows observation of the outer parts of objects.

Our studies and those made by Sublette (1979) show a similar structure of the maxillary palp in the tribe Chironomini. We found that such a detail as the length of seta a (A) may be useful in the differentiation of *Chironomus* species.

Clear differences were marked in the structure of the maxilla and the labrum between the genus *Chironomus* and *Glyptotendipes* (Kownacki *et al.,* 2016). The lower edge of labral lamella in *Chironomus* ends with very long dagger-shaped appendices, while in *Glyptotendipes* they are shorter, and blunt at the end. Tormal bar in *Chironomus* is arched and relatively narrow, while in *Glyptotendipes* it is triangular. Pecten epipharyngis in *Chironomus* end with a single row of sharp teeth, while in *Glyptotendipes* they end with a few rows of teeth of different size, rounded at their tips. Chaetae LCh also show significant differences. In *Chironomus,* chaeta LCh II is always shorter than chaeta LCh I, while in *Glyptotendipes* they are of the same length. Another type of structures has lacinia and lacinial chaetae of genus *Dicrotendipes* (Sublette, 1979). That means that the struc-

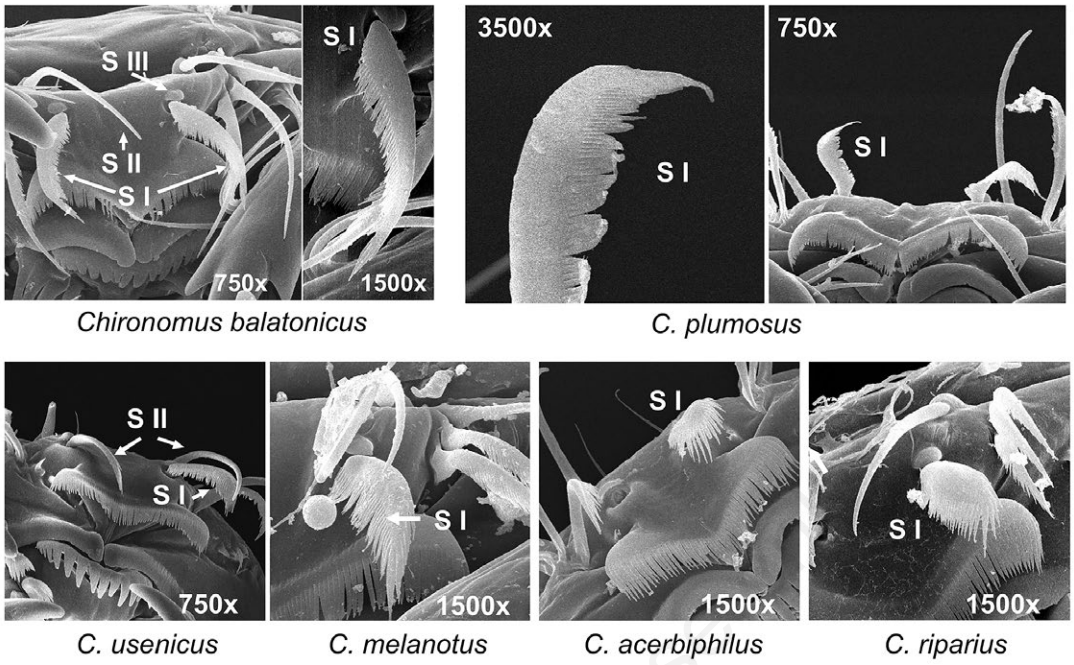

Fig. 1. *Chironomus* larvae – labral setae. S I, seta anteriores; S II, seta posteriors; S III, seta minuscule.

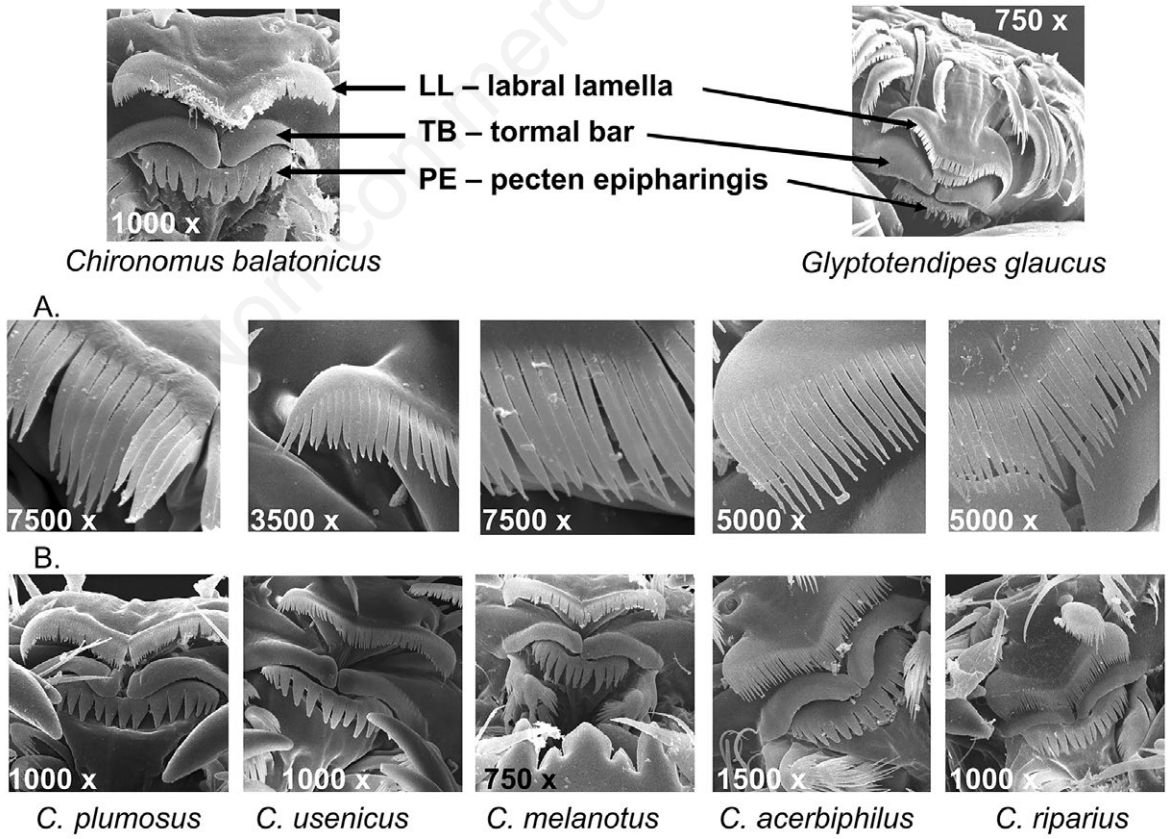

Fig. 2. *Chironomus* larvae – labrum. A) A fragment of the lower edge of labral lamella. B) Labral lamella, tormal bar, and pecten epipharyngis.

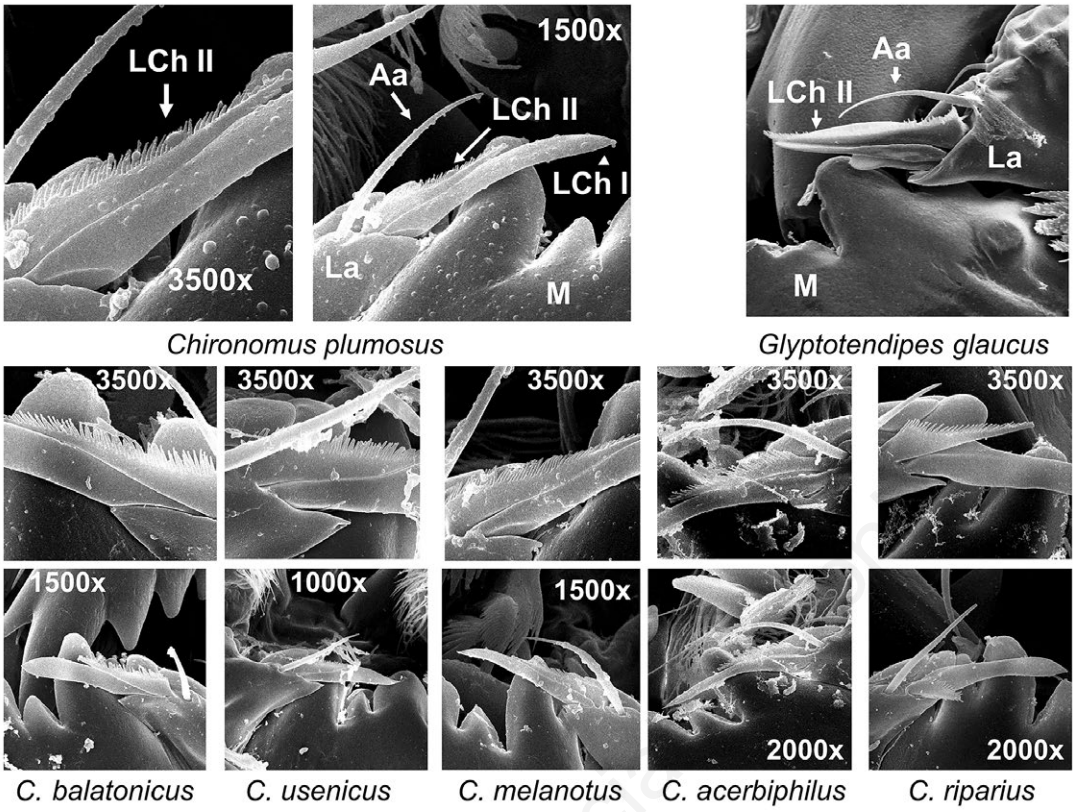

Fig. 3. Chironomus larvae – lacinia. Aa, antaxial seta; La, lacinia; LCh I, lacinial chaeta I; LCh II, lacinial chaeta II; M, mentum.

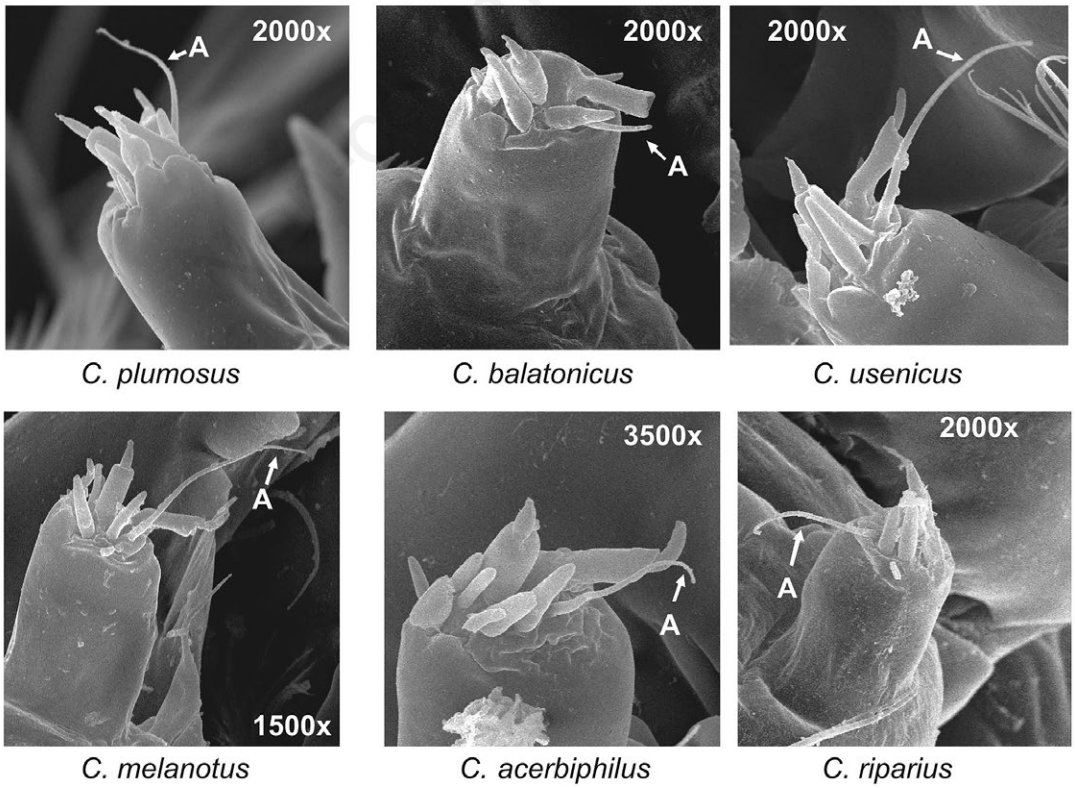

Fig. 4. *Chironomus* larvae – maxillary palp. A, a seta.

ture of the maxilla will be a key element in the differentiation of genus Chironomini. Plate X (Pl X) in *G. glaucus* is in the form of a small plate, on the surface densely covered with tiny spikes (Kownacki *et al*., 2016), while in our studies of *Chironomus* they are groups of bristles.

CONCLUSIONS

In all the investigated material of *Chironomus* larvae the general morphological structures of both labrum and the maxilla were nearly similar. Only some fine distinctive details can be observed, such like those related to: morphology of labral seta (mainly S I); shape, length or ratio of lacinial chaeta; length of seta a (A) of maxillary palp. Results obtained in this study revealed that the SEM method is useful in the analysis of a smaller and weakly chitinized morphological structures such as the labrum and the maxilla of some species.

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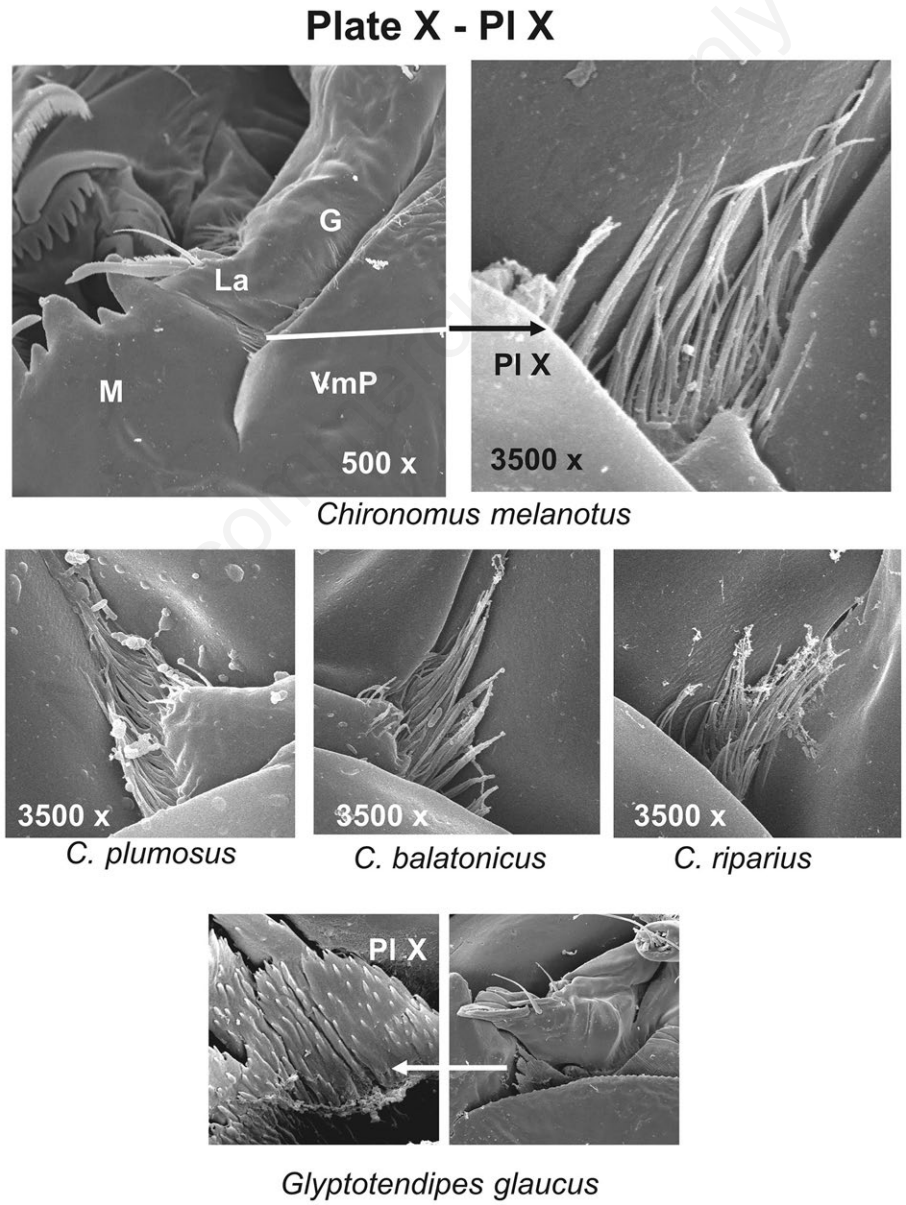

Fig. 5. *Chironomus* larvae – plate X (Pl X). G, galea; La, lacinia; M, mentum; VmP, ventromental plate.

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