# グァテマラにおける Simulium ochraceum の分布に関する地質・地形学的研究

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# Morphological observations on the first- and the last-instar larvae of the genus *Gigantodax* (Diptera: Simuliidae)*

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**Abstract:** Using a scanning electron microscope and a phase-contrast light microscope, morphological observations were made on the first- and the last-instar larvae of two species, *Gigantodax aquamarensis* and *G. wrighti*, collected in Guatemala, Central America. Between the first- and the last-instar larvae, no substantial morphological change was observed. The first-instar larvae of the two species are very similar and characterized by the following features. Cephalic fans are well-developed. Anteromedian palatal brush consists of simple hairs. At the ventral base of hypostomial teeth II–IV, a hollowed space is present at each side. Mandibular teeth differentiated as seen in the later-instar larvae. Covering brush and apical brush of mandible are present. These characters of the genus *Gigantodax* are shared by the genus *Simulium* but not by the genus *Prosimulium* sensu Peterson, 1970. This suggests that the genus *Gigantodax* has closer affinities with the genus *Simulium* than with the genus *Prosimulium* sensu Peterson.

After Davies (1960) reported the fanless first-instar larva of the genus *Prosimulium* in Canada and discussed on the phylogentic relation among the primitive forms of simuliids, morphological studies on the first-instar larvae of various genera were carried out by Coscarón and Wygodzinsky (1962), Dumbleton (1964), Davies (1965), Craig (1974, 1975) and Ross and Craig (1979). In the study on morphological comparison of primitive simuliids (Craig, 1974) the close relation among the genera *Prosimulium, Twinnia* and *Gymnopais* was suggested by showing the morphology of the first-instar larvae. The first-instar larvae retain closer morphological similarities than the later-instar larvae. Thus the morphological comparison of the first-instar larvae was shown to be useful and important for phylogenetic studies. However, the detail comparison among the genus *Gigantodax* and other genera at the first-instar larval stage has not been undertaken. In the present study, observations of the first- and last-instar larvae of two species, *Gigantodax aquamarensis* and *G. wrighti* collected in Guatemala, Central America, and comparison with other genera are made.

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## MATERIALS AND METHODS

Larvae of *Gigantodax aquamarensis* (De León) were collected in the Stream Aguas Amargas, Zunil, Quezaltenango on February 3, 1980 and the larvae of *Gigantodax wrighti* Vargas, Martínez and Díaz were collected in the stream Sesenta Vueltas, Encuentros,

Sololá on April 5, 1980. All the larvae were fixed in 3 % formalin and preserved in 70 % alcohol.

The larvae were observed by scanning electron microscopy and phase-contrast light microscopy. Larvae for scanning electron microscopy were dehydrated with a graded series of ethanol and isoamyl acetate, then prepared by critical-point drying. The materials were coated with gold and examined with a model JSM-35, JEOL, scanning electron microscope. The larvae for light microscopy were boiled in 10 % KOH to remove soft tissue. After washing, the larvae were dissected and mounted in gum-chloral solution.

The terminology used here follows that of Craig (1974, 1977).

## Observations

*Gigantodax aquamarensis (De León)*

*First-instar larva.* Total body length 0.50–0.63 mm, normal simuliid form. Head: width 0.12 mm, pale brown, weakly sclerotized, dorsal view as Fig. 1. Posterior margin of cephalic apotome conical, apex almost reaching postocciput; egg bruster placed medially, surrounded by brown area, apically darkened. Cervical sclerites not developed. Antenna of one article with distal basiconic sensillum, slightly pigmented; antennal puncture sensillum (Fig. 2) ovoid, moderated size, accompanied seta situated anterodorsally to antennal puncture sensillum. Cephalic fan with 10–12 rays; pectinations of long microtrichia interspersed between 6–8 small microtrichia; stalk present. Mandible (Fig. 3) with three, broad, apically round outer teeth; a slender tooth at base of the first outer tooth; apical tooth broad and prominent; five preapical teeth pointed, of which apical three decreasing their size toward the third tooth, basal two slender, higher than the third tooth; lacking mandibular serrations; apical brush of two rows consisting of 7–12 comblike hairs; covering brush consists of some rays branching distally. Hypostomium (Fig. 4) with 17 teeth at the dorsal edge of distal end; median tooth (I) basally broad, apically rounded, the highest of all teeth; teeth II and III behind the shallow anteromedian hollowed space at each side; teeth V broad basally and tapered distally, prominent; teeth VI–VIII gradually decrease its height toward outside; teeth IX higher and broader than teeth VIII; one hypostomial seta on each side. Anteromedian palatal brush (Fig. 5) of simple hairs. Anal sclerite and circular sclerite not developed. Posterior circlet consisting of three hooks in about 45 rows. Inside of posterior circlet two slender hairs present. Rectal gills of three, simple, finger-like lobes.

*Last-instar larva.* Head, shape typical simuliid form, width 0.75–0.80 mm. Cephalic apotome broad posteriorly, cervical sclerites marged with sclerotized extensions from upper ends of postocciput. Antenna of three articles and a distal basiconic sensillum; dorsal side of the first and second articles, distal article and basiconic sensillum brown. Cephalic fan with 40–44 (ave. 42) rays, pectinations of long microtrichia interspersed between 6–11 small microtrichia. Mandible (Fig. 6) with three outer teeth, of which the first one larger than the other two; some aborted slender teeth which are not visible at lateral view are present at the base of the first outer tooth; apical tooth large, protruded; three preapical teeth, of which the third one slender, higher than the other two; at least three rows of spinous teeth; 10–15 (ave. 12) mandibular serrations; distal row of apical brush comb-like, of basally broad and distally curved, stout hairs, the other rows of sparse, slender, spinous hairs; covering brush of about 8 basally broad and distally pectinated long hairs, pectinations irregularly sized, sulcus delimiting it from the rest of mandible. Hypostomium (Fig. 7) with 17 cone-shaped teeth; anteromedian hollowed spaces at the ventral base of teeth II–III and VI–VIII, teeth I, III, IV and IX connected basally with ventral surface of hypostomium; lateral serrations about 9 teeth, in specimens mounted flatly, tooth I stout, distinctly higher than the other teeth, teeth II as high as or slightly higher than teeth IV, teeth V prominent, teeth VI–VIII decrease its height toward outside, teeth IX larger than teeth VIII. Hairs on dorsal surface of 6th and 7th abdominal segments (Fig. 8) simple, short or long.

*Gigantodax wrighti Vargas, Martínes and Díaz*

*First-instar larva.* Total body length 0.65–0.68 mm, normal simuliid form. Head: width 0.10 m, yellowish brown, moderately sclerotized, dorsal view as Fig. 9. Posterior margin of cephalic apotome conical, apex almost reaching post-occiput; egg bruster placed medially, surrounded by brown area, apically darkened. Cervical sclerites not developed. Antenna of one article with distal basiconic sensillum, brownly pigmented; antennal puncture sensillum (Fig. 10) ovoid, moderate size, accompanied seta situated anterodorsally to antennal puncture sensillum. Cephalic fan with 10–11 rays, pectinations of long microtrichia interspersed between 6–12 small microtrichia; stalk present. Mandible (Fig. 11) with three outer teeth, broad medially and pointed apically, almost same size; a slender tooth present at the base of the first outer tooth; apical tooth slightly larger than outer teeth; five preapical teeth pointed, apical three of them decreasing their size toward the third tooth, basal two slender but longer than the third one; lacking mandibular serrations; apical brush of two rows consisting of 9–12 comb-like hairs; covering brush consists of three rays which branches distally. Hypostomium (Fig. 12) with 17 teeth at the dorsal edge of distal end; median tooth (I) basally broad, distally tapered and pointed, higher than the other teeth; notch between teeth I and II deep; teeth II and III small, behind anteromedian hollowed space at each side; teeth V basally broad and distally tapered and pointed, prominent; teeth VI–VIII small, decreasing its height toward outside; teeth IX higher and broader than teeth VIII; one hypostomial seta on each side. Anteromedian palatal brush (Fig. 13) of simple hairs. Anal sclerite and circular sclerite not developed. Posterior circlet consiting of three hooks in about 35–38 rows. Rectal gills of three simple finger-like lobes.

*Last-instar larva.* Head, shape typical simuliid form, width 0.70–0.80 mm. Cephalic apotome broad posteriorly, cervical sclerites merged with sclerotized extensions from up-

Figs. 1–8 *Gigantodax aquamarensis*

1–5: first-instar larva. 1: dorsal view of head capsule. Scale 18.5 μm. 2: lateral view of anterior part of head capsule, showing location of antennal puncture sensillum. Scale 5.0 μm. 3: laterofrontal view of mandible, showing mandibular teeth and apical brush. Scale 2.8 μm. 4: hypostomium, showing hypostomial teeth I–IX and anteromedian hollowed space. Scale 1.9 μm. 5: anteroventral view of palatal region, showing anteromedian palatal brush and setae C-2. Scale 6.3 μm. 6–8: last instar larva. 6: tip of mandible, showing mandibular teeth and apical brush. Scale 20.8 μm. 7: hypostomial teeth. Scale 16.7 μm. 8: hairs on abdominal segment. Scale 16.7 μm.

Figs. 9–16 *Gigantodax wrighti*

9–13: first-instar larva. 9: dorsal view of head capsule. Scale 22.7 μm. 10: lateral view of anterior part of head capsule, showing location of antennal puncture sensillum. Scale 4.2 μm. 11: laterofrontal view of mandible, showing mandibular teeth and apical brush. Scale 6.8 μm. 12: hypostomium, showing hypostomial teeth I–IX and anteromedian hollowed space. Scale 1.7 μm. 13: anteroventral view of palatal region, showing anteromedian palatal brush and setate C-2. Scale 3.8 μm. 14–16: last instar larva. 14: tip of mandible, showing mandibular teeth and apical brush. Scale 15.2 μm. 15: hypostomial teeth. Scale 25.0 μm. 16: hairs on abdominal segment. Scale 6.7 μm.

ABBREVIATIONS USED IN FIGS. 1–16

ab: apical brush, as: accompanied seta, ahs: anteromedian hollowed space, anti: antenna, apb: anteromedian palatal brush, aps: antennal puncture sensillum, at: apical tooth, cb: covering brush, cf: cephalic fan, dab: distal adoral brush, egb: egg bruster, hs: hypostomial setae, mnd: mandible, ms: mandibular serrations, ot: outer teeth, pat: preapical teeth, ps: preapical sensilla, rb: raking bristles, st: spinous teeth, su: sulcus, 2-C: setae, I–IX: hypostomial teeth.


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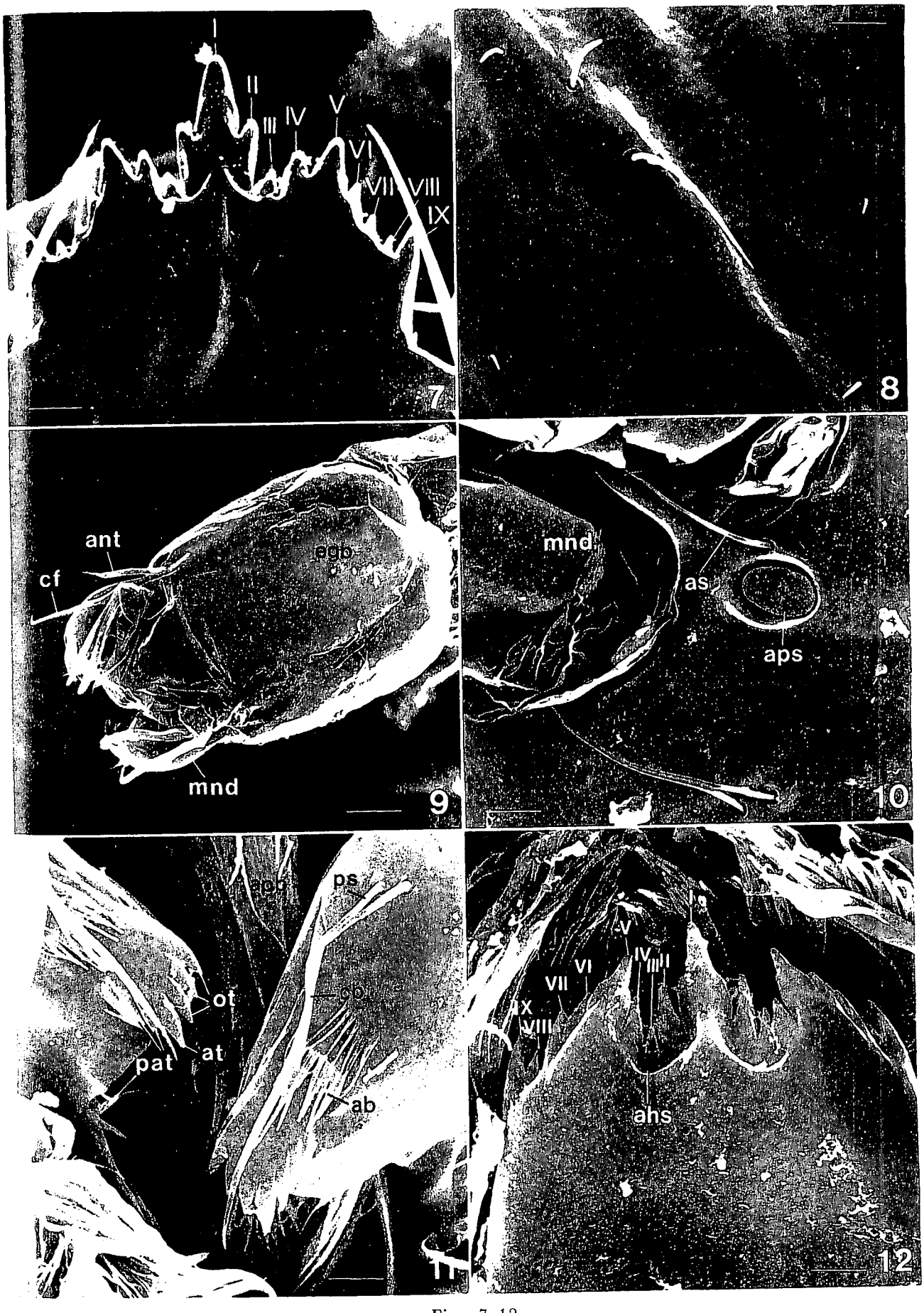
Figs. 1–6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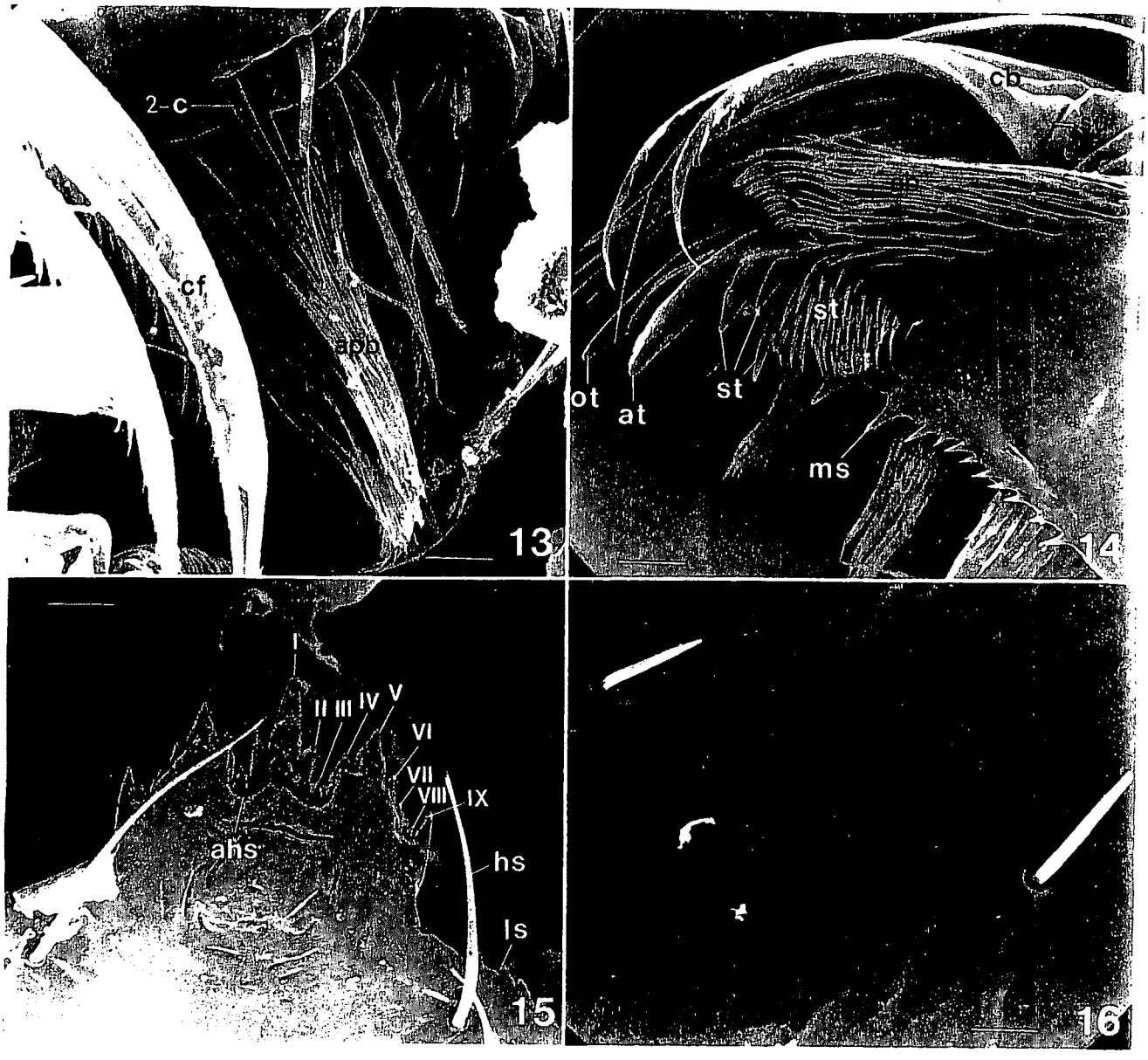
Figs. 7–12




Figs. 13–16

per ends of postocciput. Antenna of three articles and a distal basiconic sensillum; dorsal side of the first and the second articles, distal article and basiconic sensillum brown. Cephalic fan with 34–37 (ave. 36) rays, pectinations of long microtrichia interspersed between 7–12 small microtrichia. Mandible (Fig. 14) with three outer teeth, of which the first one higher than the other two, some aborted slender teeth which are not visible at lateral view present at the base of outside of the first outer tooth; apical tooth broad, protrudent; three preapical teeth, of which the third one slender, higher than the other two; four rows of spinous teeth; 8–13 (ave. 11) mandibular serrations; distal row of apical brush comb-like, of basally broad and distally curved, stout hairs, the other rows of slender spinous hairs; covering brush of six basally broad and distally pectinated long hairs, pectinations irregularly sized, sulcus delimiting it from the rest of mandible. Hypostomium (Fig. 15) with 17 cone shaped teeth; teeth II–III and VI–VIII are behind anteromedian hollowed spaces, teeth I, IV, V and IX connected with ventral surface of hypostomium at base; lateral serrations of about 10 teeth, each of which bifurcated dorsoventrally; in specimens mounted flatly, tooth I stout, as high as or slightly higher than teeth V, teeth II lower than teeth IV, teeth V prominent, teeth VI–VIII decreasing their height toward outside, teeth IX larger than teeth VI and VIII, as high as

teeth VII. Hairs on dorsal surface of 6th and 7th abdominal segments (Fig. 16) simple and short.

## Discussion

Before the present study the first-instar larva of the genus *Gigantodax* has been reported only on a species, *G. igniculum,* in South America (Coscarón and Wygodzinsky, 1962). The first-instar larva of *G. igniculum* resembles to that of the two species observed in the present study. Critical difference among them is found in antenna. We observed the antenna of one article with a distal basiconic sensillum both in *G. aquamarensis* and *G. wrighti,* while Coscarón and Wygodzinsky found two articles with a distal basiconic sensillum in *G. igniculum.* The two species in the present study are very similar in the first-instar larvae. They can be separated only by the shape of the hypostomial and mandibular teeth. The teeth in *G. aquamarensis* are broad and apically rounded, while in *G. wrighti* those are slender and apically pointed.

Comparing between the first- and the last-instar larvae, no substantial morphological change is seen, but an increase of size and number in many characters is found. In hypostomial teeth the structure is very stable as seen in other genera, *Prosimulium* (Ross and Craig, 1979) and *Austrosimulium* (Dumbleton, 1964). Only in mandible some differences are observed, namely, mandibular serrations are not developed and spinous teeth are not differentiated in shape from preapical teeth in the first-instar larva. Consequently it seems that five preapical teeth are present distally to the smooth inner edge of the mandible. In the later-instar larval stages spinous teeth may be differentiated from basal two of the five teeth.

Although *G. igniculum* was not observed in detail, the first-instar larvae of the three species of the genus *Gigantodax* have some phylogenetically important characters, that is, well-developed cephalic fans and 17 hypostomial teeth. In addition to these, we found other characters in *G. aquamarensis* and *G. wrighti* which were not described in *G. igniculum* by Coscarón and Wygodzinsky (1962) but seemed to be of phylogenetic importance. The two species in the present study have anteromedian palatal brush consisting of simple hairs, anteromedian hollowed space at the ventral base of hypostomial teeth II–III, and apical and covering brushes on mandibles. These characters are also observed in the species belonging to the genus *Simulium* except for 17 hypostomial teeth which are found in the genera *Prosimulium* sensu Peterson, 1970 (same as the subgenus *Prosimulium* sensu Crosskey, 1969) and *Twinnia.* However the character of 17 hypostomial teeth is shared by the three genera, *Prosimulium, Twinnia* and *Gigantodax,* structural differences are seen among them. In the genera *Prosimulium* sensu Peterson and *Twinnia* distal margin of the hypostomium is simple structured with 17 teeth, while in the genus *Gigantodax* the distal margin is double structured with anteromedian hollowed space at the ventral base of the teeth II–III. In all the following species of the genera *Prosimulium* sensu Peterson and *Twinnia, P. susanae, P. magnum, P. travisi, T. biclavata* (Craig, 1974), *P. mixtum/fuscum* (Ross and Craig, 1979), *T. tibblesi* (Davies, 1965), *P. kamui, P. jezonicum, P. yezoense* (Okazawa, unpublished data), which were observed in detail, the first-instar larvae are characterized in reduction of labrum, small number of cephalic fan rays without stalk, anteromedian palatal brush of three pairs of rake-like hairs, and a ventral-most narrow pair with long pectinations. Moreover mandible is lacking apical brush and covering brush and mandibular teeth are not differentiated in the first-instar larvae of *Prosimulium* sensu Peterson.

On the other hand, comparing the genus *Gigantodax* with the genus *Simulium,* they share many important characters above mentioned, though some of them are less developed in *Gigantodax* than *Simulium.* The median hollowed space is completely developed at the ventral base of the teeth I–IV in the first-instar larvae of *S.* (*Simulium*) *japonicum* and *S.* (*Hemicnetha*) *smarti* (Okazawa, in preparation), while in *Gigantodax* tooth I connects with ventral surface of hypostomium at the base. The double structured distal margin of hypostomium is commonly observed in the later-instar larvae of the other genera and subgenera, *Simulium*

(subgenus *Wilhelmia*) (Matsuo, 1979), *Stegopterna, Mayacnephia, Simulium* (subgenera *Eusimulium* and *Psilopelmia*) (Okazawa, unpublished data). We consider that the genera *Austrosimulium* (Dumbleton, 1964; Crosby, 1974), *Cnesia, Araucnephia, Araucnephioides, Paraustrosimulium* (Wygodzinsky and Coscarón, 1973) and many species of *Cnephia* (Rubzow, 1959–1964) might have the double structured distal margin of hypostomium from their illustrations. In the first-instar larvae of *S.* (*S.*) *japonicum* and *S.* (*H.*) *smarti*, cephalic fans are well-developed, anteromedian palatal brush consists of simple hairs, mandibular teeth are differentiated as in later-instar larvae, even spinous teeth can be distinguished from preapical teeth. Mandibular serrations are not developed as in other genera.

*Gigantodax* species have been thought as one of the primitive forms of simuliid. Crosskey (1969) and Wygodzinsky and Coscarón (1973) treated *Gigantodax* to be a full genus and placed it in the tribe Prosimuliini. Rubtsov (1974) placed the genus *Gigantodax* in the subfamily Prosimuliinae which contained more restricted genera than the tribe Prosimuliini sensu Crosskey, 1969. He recognized close affinities between the genera *Gigantodax* and *Helodon* (synonym of the subgenus *Prosimulium* sensu Crosskey, 1969, subgenus of *Prosimulium* sensu Peterson, 1970) by some pupal and adult characters.

Contrally to the pupal and adult characters, the first-instar larvae of the *Gigantodax* suggest closer affinities with *Simulium* than with *Prosimulium* sensu Peterson, 1970, however, some characters, *e.g.*, anteromedian hollowed space and spinous teeth of mandible, show the intermediate feature between the two genera.

Thus the morphological comparison of first-instar larvae revealed another aspect of phylogenetic relationship among the genera of simuliid. For understanding it, further studies may be necessary.

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## 摘　要

*Gigantodax* 属ブユ，1令および終令の形態

中央アメリカ，グァテマラ共和国で採集された *Gigantodax* 属の2種のブユ *G. aquamarensis* および *G. wrighti* の1令幼虫と終令幼虫の形態を走査電子顕微鏡および位相差顕微鏡を使って調べた．1令幼虫の基本的な形態は終令と同じである．この2種の1令幼虫は形態的に大変似ており，系統学上重要と考えられる形質を共有している．すなわち，

1. 柄をもった cephalic fans が発達する．
2. anteromedian palatal brush は単純毛から成る．
3. hypostomium の II-IV 歯の基にくぼみがある．
4. mandible の歯はほぼ2令以後に見られるように分化している．
5. mandible は covering brush と apical brush をもつ．

これらの特徴は *Simulium* 属と共通であり，*Prosimulium* sensu Peterson, 1970 とは共通でない．以上の結果より，1令幼虫では，*Gigantodax* 属は *Prosimulium* sensu Peterson, 1970 よりも *Simulium* 属に形態上より近いと結論できる．