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# Ultrastructure of the larval ventromental plates of *Chironomus* balatonicus Devai, Wuelker & Scholl (Diptera: Chironomidae)

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The micro-architecture of larval ventromental plates of *C. balatonicus* Devai, Wuelker & Scholl has been studied by scanning electron microscopy. The plates of this species are shown to have a number of ultrastructural features previously observed amongst European *Chironomus* only in *C. plumosus* L. and the closely related *C. muratensis* Ryser *et al.*, and *C. nudiventris* Ryser *et al.* The taxonomic implications of these findings are discussed.

## INTRODUCTION

Chironomus balatonicus Devai et al. was described as a new species on the basis of chromosomal banding patterns which, together with biochemical genetic differences, distinguish it from the morphologically very similar species C. plumosus L. (Devai et. al., 1983). Larvae of C. balatonicus were assigned by Devai et al. (1983) to the plumosus-type, one of eight categories of Chironomus larvae initially recognized by Lenz (1954) and defined mainly on the condition of tubular appendages of the posterior abdominal segments. In addition to C. plumosus, LINDEBERG & WIEDERHOLM (1979) listed some 11 European species of Chironomus with larvae of the plumosus-type, several of which (e.g. C. dorsalis sensu Keyl & Keyl, C. luridus Strenzke, C. uliginosus Keyl) are karyologically and enzymatically quite distinct from C. plumosus (e.g. MARTIN, 1979, SCHOLL et al., 1980). In contrast, Ryser et al. (1983) described two species, C. muratensis RYSER et al. and C. nudiventris RYSER et al., which are chromosomally and biochemically closely related to C. plumosus, while their larvae belong to different morphological types (Scholl et al., 1980, Ryser et al., 1983). Ryser et al. (1983) suggested that C. plumosus, C. muratensis and C. nudiventris should be included as the only bona fide members of a "plumosus-species group".

A distinction of *C. plumosus*, *C. muratensis* and *C. nudiventris* from other European species of *Chironomus* has been supported by studies of one aspect of larval morphology, the ventromental plates which are structures involved in the processes of silk-spinning (Webb *et al.*, 1981). The ventromental plates of *C. plumosus*, *C. muratensis* and *C. nudiventris* share a number of ultrastructural features which distinguish them from ventromental plates of all the other thirty-one species of European *Chironomus* examined to date, amongst which *C. balatonicus* is not included (Webb 1980, Webb *et al.*, 1981, 1985, 1987; Webb & Scholl, 1985, 1987).

The possibility of including *C. balatonicus* in the *plumosus*-species group sensu RYSER et al. has not been directly addressed. The ultrastructure of the ven-

tromental plates of *C. balatonicus* is clearly of relevance to this question and forms the subject of the present communication.

## MATERIALS AND METHODS

Head capsules from a total of sixteen fourth instar larvae were examined, including twelve specimens used in the original description of *C. balatonicus* by Devai *et al.* (1983). Larvae were identified on the basis of banding patterns of their polytene chromosomes and were all collected from the type locality of *C. balatonicus*, the Keszthely Basin of Lake Balaton, Hungary, by Gy. Devai and W. Wuelker.

Head capsules were fixed and preserved in 70% alcohol and dissected under a binocular microscope with a pair of fine tungsten needles sharpened electrolytically. Whole head capsules and dissected pieces were dehydrated to absolute alcohol, air dried and attached to stubs for examination in the scanning electron microscope (SEM). Specimens on stubs were coated with a thin layer of gold in a Balzers or Dynavac sputter coater and viewed with a Philips 500 or Jeol JSM-T330 SEM.

## **RESULTS**

The ventromental plates of *C. balatonicus* appear as approximately fanshaped structures lying lateral to, and partly behind the toothed mentum (Fig. 1a, b). The range of plate size, as indexed by peripheral length, overlaps size ranges of plates of *C. plumosus*, *C. muratensis* and *C. nudiventris*, although mean size is smaller than in these three species (Tab. 1; Webb *et al.*, 1985).

In common with other species of *Chironomus*, the inner (dorsal) surface of the plates of *C. balatonicus* bears a series of lamella-like strial ridges which originate posteriorly from an elliptical area of unstriated cuticle occupying the extreme rear section of plates (Fig. 1c, d; Webb *et al.*, 1981, 1987; Webb & Scholl, 1987). This rear section bears some small, irregular folds which are prominent distally (Fig. 1e), an arrangement that has been recorded in several *Chironomus* species (Webb *et al.*, 1985, 1987; Webb & Scholl, 1987). However in specimens of *C. balatonicus* examined the unstriated rear section of plates also has a ridge running just behind its anterior boundary (Fig. 1f), a structure previously ob-

Tab. 1: Quantitative variation in ventromental plates of Chironomus balatonicus.

Plate feature	n	range	x	S.E.
size* (μm)	11	300.0-373.0	327.64	7.45
number of striae	13	75-91	83.85	1.37
total number of inner spines	8	365-428	397.13	7.01
group size of inner spines+	158	3-6	4.73	0.06
total number of outer spines	10	68-95	82.22	2.78

<sup>\*</sup> peripheral length; + over middle section of plate; n, number of observations;  $\bar{x}$ , mean value; S. E., standard error of mean.

served only in members of the *plumosus*-group *sensu* Ryser *et al.* (Webb *et al.*, 1985).

Features of strial organization observed in *C. balatonicus* and shared by other *Chironomus* species are the progressive shallowing and broadening of striae as they run anteriorly and the apparent subdivision through folding of striae occupying the extreme distal and proximal portion of plates (Fig. 2a–f; Webb *et al.*, 1985, 1987; Webb & Scholl, 1987). However striae of *C. balatonicus* appear narrower and are certainly more numerous than those of all other European *Chironomus* examined, apart from species of the *plumosus*-group *sensu* Ryser *et al.* (Webb *et al.*, 1985, 1987; Webb & Scholl, 1985, 1987). The range of strial numbers encountered in *C. balatonicus* overlaps that of *C. plumosus* and mean values of striae in the two species are similar (Tab. 1; Webb *et al.*, 1985). In

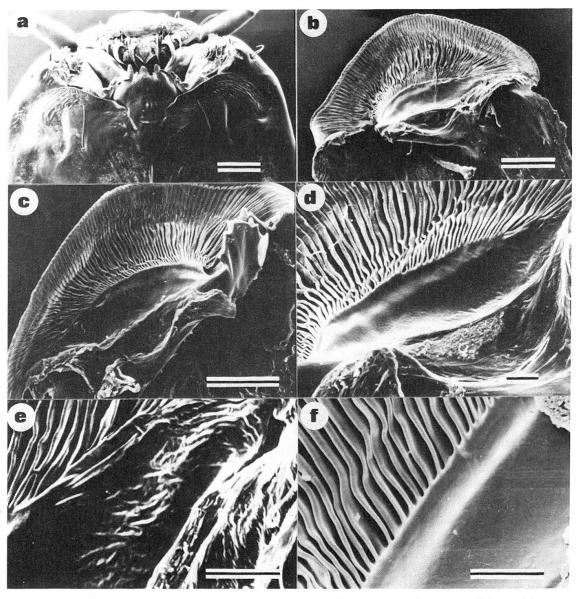


Fig. 1. Views of ventromental plates of *C. balatonicus*. (a) Outer (ventral) surface, (b) and (c) inner (dorsal) surface, (d) rear section of inner surface, (e) rear-lateral section of inner surface, (f) rearmid section of inner surface. –b & c: mentum removed. Scales a  $100 \, \mu \text{m}$ , b & c  $50 \, \mu \text{m}$ , d  $-f \, 10 \, \mu \text{m}$ .

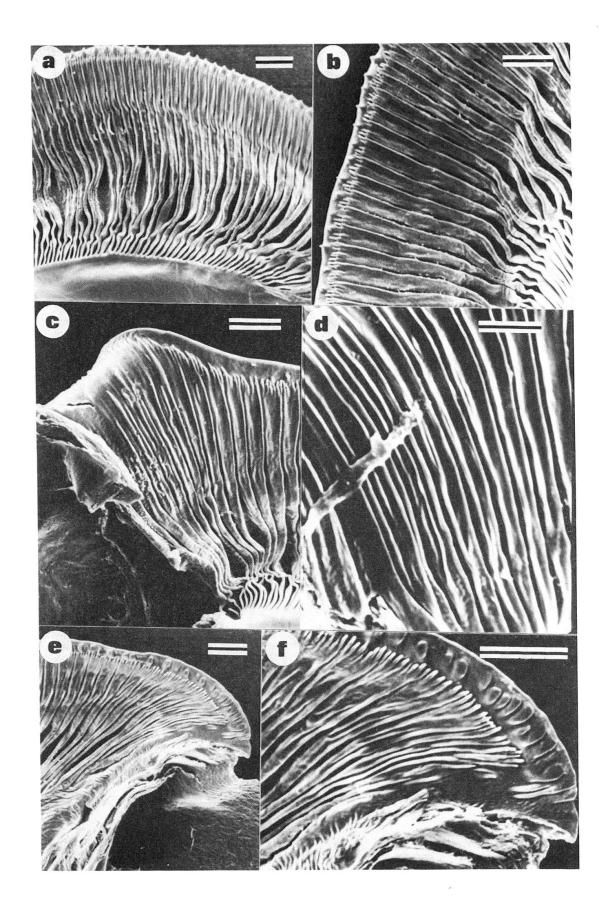


Fig. 2. Views of inner surface of ventromental plates of *C. balatonicus*. (a) and (b) mid section, (c) and (d) proximal section, (e) and (f) distal section. -c & d: mentum removed. Scales all  $10~\mu m$ .

C. balatonicus striae run right up to a series of small, mainly pyriform spines, the inner of two rows of spinous projections found in the anterior section of plates of C. balatonicus (Fig. 3a, b) and all other Chironomus species examined to date (Webb et al., 1981, 1985, 1987; Webb & Scholl, 1987). This type of strial termination, type IIIB in the terminology of Webb et al. (1985), has been found previously only in C. plumosus, C. muratensis and C. nudiventris (Webb, 1980; Webb et al., 1985).

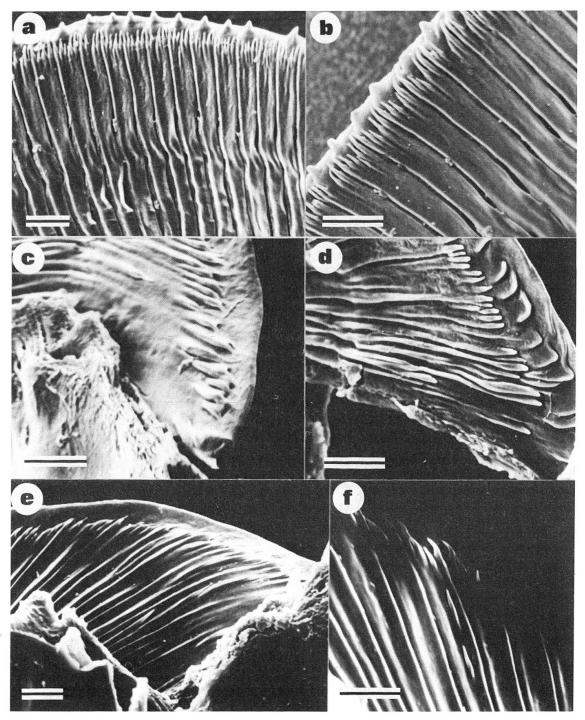


Fig. 3. Views of inner surface of ventromental plates of *C. balatonicus*. (a) and (b) anterior-mid section, (c) extreme anterior-proximal section, (d) extreme anterior-distal section, (e) and (f) anterior-proximal section. -c, e & f: mentum removed. Scales all 5  $\mu$ m.

Furrows between strial ridges delimit pyriform spines into groups (Fig. 3a, b). The range and mean number of spines in groups over the mid section of plates (Tab. 1) are very similar to those encountered in *C. plumosus* (Webb *et al.*, 1985). The range and mean value of total number of inner spines in *C. balatonicus* (Tab. 1) are the highest recorded so far in *Chironomus* although information on this

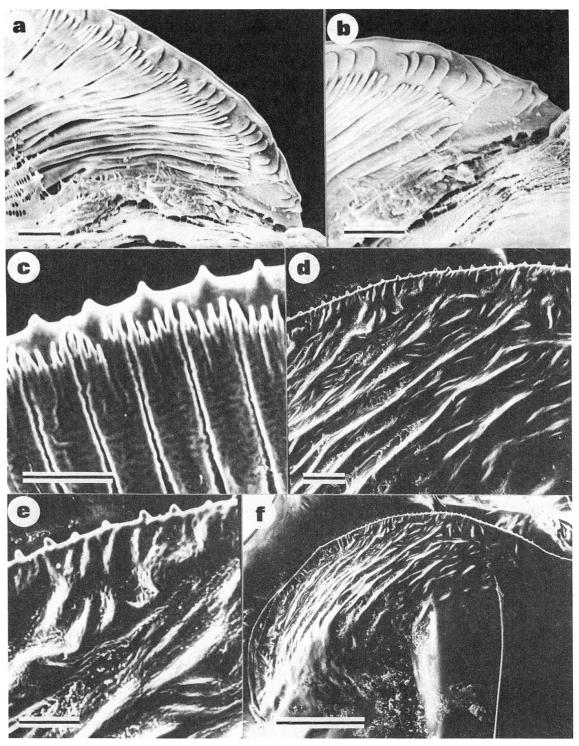


Fig. 4. Views of ventromental plates of *C. balatonicus*. (a) and (b) anterior-distal section of inner surface, (b) anterior-mid section of inner surface, (d) and (e) anterior-mid section of outer surface, (f) outer surface. Scales a, b, c & e 5  $\mu$ m, f 50  $\mu$ m.

character is not available for any member of the *plumosus*-group *sensu* Ryser *et al.* In *C. balatonicus*, as in other *Chironomus* species, spines of the inner series extend around virtually the complete margin of plates, with some differentiation of their morphology in the extreme proximal and distal portions of the series where spines may be somewhat broader, longer or less regular in shape (Fig. 3a–d, 5; Webb *et al.*, 1987; Webb & Scholl, 1987). A small, additional group of pyriform spines situated behind the inner series on the anterior section of proximal striae running under the mentum was observed in *C. balatonicus* (Fig. 3e, f). Similar spines have been found in *C. pallidivittatus* (Edwards), *C. tentans* (Fabricius), *C. montuosus* Ryser *et al.* and *C. storai* Goetghebuer (Webb & Scholl, 1987) but no information is available on their occurrence in members of the *plumosus*-group *sensu* Ryser *et al.* 

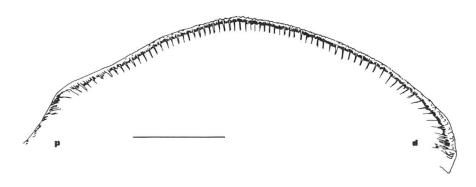


Fig. 5. Tracing of scanning electron micrographs of anterior inner surface of ventromental plate of *C. balatonicus*. Abbreviations: d, distal; p, proximal. Scale  $50 \mu m$ .

In C. balatonicus, as in other Chironomus, spines of the outer series are less numerous than those of the inner row and do not extend as far around the margin proximally (Fig. 3e, 5; Webb et al., 1985, 1987; Webb & Scholl, 1987). The number of spines in the outer series is consistently higher in C. balatonicus than in all previously studied European species of *Chironomus* apart from members of the plumosus-group sensu Ryser et al. with the range and mean number of outer spines in C. balatonicus being similar to those of C. plumosus (Tab. 1, Webb et al., 1985, 1987; Webb & Scholl, 1987). Outer series spines in Chironomus species studied to date are mainly conical in shape although distal members of the series are usually broader and less pointed than the rest (Webb et al, 1987; Webb & SCHOLL, 1987). The situation is similar in C. balatonicus although this species is unusual in that spines of the inner and middle part of the outer row project beyond the anterior margin of the plate and can be seen from the outside of the plate (Fig. 4a-e). Amongst all the species of *Chironomus* examined previously, outer series spines overlapped the anterior margin of the plates of C. plumosus, C. muratensis and C. nudiventris only (Webb et al., 1985, 1987; Webb & Scholl, 1987).

A SEM view of the outside of plates of *C. balatonicus* also reveals the existence of sculpturing on the anterior mid and lateral sections of their outer (ventral) surface (Fig. 4d-f). Similar sculpturing has been found on plates of *C. plumosus*, *C. muratensis* and *C. nudiventris* but none of the other European

species of *Chironomus* which have been investigated previously (Webb *et al.*, 1985, 1987; Webb & Scholl, 1987).

## DISCUSSION

C. balatonicus shares with members of the plumosus-group sensu RYSER et al. several ventromental plate features which are unique amongst European species of Chironomus. These include the high number of striae, pattern of strial termination, size of inner spine groups, number and position of outer spine row and presence of sculpturing on the ventral surface of plates. The ventromental plates of C. balatonicus are clearly very similar to those of the plumosus-group sensu RYSER et al. and in terms of ventromental plate micro-architecture C. balatonicus, C. plumosus, C. muratensis and C. nudiventris form a clearly distinct grouping amongst the European species of Chironomus.

Although ventromental plate ultrastructure has proved of use in the systematics of larval chironomids (Webb & Scholl, 1985, 1987; Webb & Martin, 1987; Webb et al., 1985, 1987) it is not an infallible indicator of taxonomic relationship within Chironomus (Webb et al., 1985). As with other morphological characters, groups of species segregated on the basis of ventromental plate features do not invariably correspond with groupings based on karyology or biochemical genetics (Lindeberg & Wiederholm, 1979; Scholl et al., 1980; Webb & Scholl, 1985; Webb et al., 1985). One reason for this is that ventromental plates are functional features that may be subject to convergent evolution (Webb, 1980; Webb et al., 1981). Within Chironomus, for example, C. striatus Strenzke and C. lacunarius Wuelker & Kloetzli, which are genetically very distinct species with a similar ecological niche, have plates that are ultrastructurally alike (Webb et al., 1985).

While C. balatonicus and C. plumosus are broadly sympatric, C. balatonicus appears to prefer habitats that are poorer in decomposing organic matter (Devai et al., 1983) so may not occupy exactly the same ecological niche. There is also evidence independent of ventromental plate structure for a close relationship between C. balatonicus and members of the plumosus-group sensu Ryser et al. For example, electrophoretic investigations indicate that C. balatonicus has a Nei coefficient of genetic identity (I) with C. plumosus, C. muratensis and C. nudiventris of 0.65 (Deval et al., 1983). Although this is a lower value than the very high identities shared by members of the plumosus-group sensu Ryser et al., where I ranges from 0.81-0.85 (SCHOLL et al., 1980), it exceeds identities recorded between C. plumosus, C. muratensis and C. nudiventris with other Chironomus species that have been examined (Scholl et al., 1980; Wuelker et al., 1981; RYSER et al., 1983). Similarly, although there are karyological differences between C. balatonicus and members of the plumosus-group sensu Ryser et al., particularly in arms A and F, C. balatonicus is still more similar karyologically to C. plumosus, C. muratensis and C. nudiventris than any other European species of Chironomus (Martin, 1979; Wuelker et al., 1981, 1983; Devai et al., 1983; Ryser et al., 1983).

Amongst the European species of *Chironomus*, *C. balatonicus*, *C. plumosus*, *C. muratensis* and *C. nudiventris* are one of the few groups of species that can be associated together in terms of cytogenetics, biochemistry and larval morphology and their inclusion together in a "plumosus-group" does not seem unwarranted.

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