

SIMILARITIES IN DISTAL ANATOMY OF MAXILLARY AND LABIAL PALPS OF ZOOPHAGES STONEFLY NYMPHS

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Abstract. The maxillary and labial palps of the nymphs of three carnivorous stonefly species - *Perla marginata* (Panzer, 1799), *Dinocras cephalotes* (Curtis, 1827) and *Perlodes microcephalus* (Pictet, 1833) - were studied by means of a SEM (scanning electron microscope). These species have active predator nymphs, feeding on different aquatic invertebrates. The external morphology of their mouthparts is adapted for a carnivorous feeding type, presenting symmetrically elongated mandibles, maxillae and a labium with segmented long palps. The distal parts of the mouthparts of carnivorous stonefly larvae show two different morphological patterns: the mandible and lacinia end with sharp-elongated teeth, while the galea, maxillary and labial palps, glossa and paraglossa end with sensory complexes involved in the prey detection. All mouthparts segments bear different types of sensilla. The maxillary and labial palps present the same types of sensilla: trichoid sensilla, sensilla basiconica (flat-tipped and blunt-tip), sensilla campaniformia and an apical cone complex containing a big apical peg. These sensilla function as mechanoreceptors and chemoreceptors. The diversity of sensilla on the maxillary and labial palps is less than that on the antennae.

Keywords: plecoptera, maxillary palp, labial palp, sensillar complex.

Rezumat. Similități în anatomia distală a palpilor maxilari și labiali la nimfele carnivore de plecoptere. Palpii maxilari și labiali ai nimfelor a trei specii carnivore de plecoptere - *Perla marginata* (Panzer, 1799), *Dinocras cephalotes* (Curtis, 1827) și *Perlodes microcephalus* (Pictet, 1833) - au fost studiate cu ajutorul microscopiei electronice cu baleiaj (SEM). Nimfele acestor specii sunt predatoare active, hrănindu-se cu diferite nevertebrate acvatice. Morfologia externă a pieselor bucale este adaptată tipului de hrănire carnivor, nimfele prezentând mandibule și maxile simetrice, alungite și un labium prevăzut cu palpi segmentați, alungiți. Partea distală a pieselor bucale la nimfele acvatice ale plecopterelelor zoofage prezintă două modele morfologice: mandibula și lacinia se termină cu dinți alungiți și ascuțiți, în timp ce galea, palpii maxilari și labiali, glossa și paraglossa se termină distal cu complexe senzoriale implicate în detectarea prăzii. Toate piesele bucale prezintă diferite tipuri de sensile. Palpii maxilari și labiali poartă trei tipuri de sensile: trichoide, baziconice (cu vârf aplatizat și cu vârf rotunjit), campaniforme și un complex apical conic ce conține o sensilă apicală de dimensiuni mari. Aceste sensile funcționează ca mecano- și chemoreceptori. Diversitatea sensilelor la nivelul palpilor maxilari și labiali este mai redusă decât cea prezentă la nivelul antenelor.

Cuvinte cheie: larve de plecoptere, palp maxilar, palp labial, complex sensilar.

Introduction

The insect mouthparts have been modified in different lineages as insects have exploited particular food resources (Angelini & Kaufman, 2005). The morphology of these ventral appendages has a serial homology to the antennae and legs, the maxillary and labial palps being made up of segments (podomeres) considered homologous to the telopodites of legs. The maxillary and the labial appendages show a similar anatomy correlated with a chewing function in some orders of insects.

Monakov (2003) shows that the predator nymphs (some Perlidae, Perlodidae and Chloroperlidae) have an external morphology of mouthparts adapted for a carnivorous feeding type, presenting symmetrical elongated mandibles, maxillae bearing five-segmented palps and a labium with three-segmented long palps.

In the adults of the insects, the morphology of maxillary palps, e.g. those of the caddisflies (Ljungberg & Hallberg, 1992), associated with the sexual dimorphism of some species, play an important role in building and the evolution of the premating behavior.

The morphology of maxillary and labial palps serves as a criterion for taxonomical classification.

Lucy *et al.* (1990) show that the nymphs of the family Perlidae are among the largest macroinvertebrate predators in running waters, feeding on different invertebrate types and plant matter, with an important effect on the benthic community structure.

For example, all stages of *Dinocras cephalotes* and *Perla bipunctata* (Perlidae) are predominantly carnivorous, the main prey being larval mayflies, caddisflies and chironomidae. The same authors show that the cannibalism is common among perlids. Seasonal changes in prey availability, with a shift from carnivory to omnivory, are cited. *Perla marginata*, *Dinocras cephalotes* and *Perlodes microcephalus* are hemimetabolous insects, whose nymphs are carnivorous, strictly aquatic and living under stones in running water.

Fenoglio *et al.* (2005) show that *Perlodes microcephalus* nymphs are the most active of *Dinocras cephalotes*, *Perla bipunctata* and *Isoperla grammatica*, developing the highest attack rate. Stoneflies hunt their prey nocturnally under rocks and, in these conditions, mouthparts sensilla may be involved in detecting and hunting the prey.

Chapman (1998) shows that biting and chewing insects cut off a fragment of food which is pushed back towards the mouth by mandibles, often aided by the maxillae. Before starting to feed, the insect touches first the food with the sensilla present on the tip of its maxillary and labial palps. This behaviour enables the insect "to taste" or "to smell" the structural or produced chemicals of the food, leading it to reject or to accept the food by an exploratory bite following palpation. Most of sensilla on the mouthparts are contact chemoreceptors, but there are mechanoreceptors and olfactory sensilla often present on the palps. Biting and chewing insects have contact chemoreceptors on all the mouthparts except the mandibles. Orthoptera and Blattodea have a large number of sensilla grouped on the top of the maxillary and labial palps. Studying the sensilla of *Supella longipalpa* (Blattellidae), Prakash *et al.* (1995) show that the maximum diversity of gustatory, mechanosensory and olfactory sensilla were found on the most distal segments of maxillary and labial palps.

Gaino & Rebora (2003) show that, in *Baetis rhodani* (Ephemeroptera: Baetidae) nymphs, as well as in other nymphs having an orthognathous head, only the tips of the maxillary and labial palps contact the substrate, correlated with a large number of setae present on the distal tip of palps. Many authors show that the sensilla present on the surface of the mouthparts of water-living insect larvae are involved in the reception of different stimuli from the freshwater environment (temperature variations, water flow characteristics, chemical composition of substrate, prey and water) and these structures function especially as chemoreceptors, mechanoreceptors and olfactory sensilla.

Ljungberg & Hallberg (1992) have described the apical cone complex situated on the tip of maxillary palps of some trichoptera larvae, showing that the apical cone complex consists of a lot of sensilla grouped on a conical extension of the maxillary palp tip. A blunt peg with a flexible socket is situated on the tip of the cone, and along the sides of the cone, there may be 2-3 similar pegs or several smaller pegs situated in depressions. The same authors show that the diversity of sensilla on the palps is considerably less than that on the antennae.

Baets rhodani nymphs, according to Gaino & Rebora (2003), have three-segmented labial palps and two-segmented maxillary palps, bearing a large number of two kinds of chemomechanosensory uniporous basiconic sensilla, similar in their internal structure: flat-tipped sensilla end with an apical flange adhering to the cuticle, and basiconic sensilla without apical flange. These structures are accompanied by mechanosensory sensilla represented by short and long bristles involved in sensing and sweeping the substrate. At the tip of the maxillary palp there are a big mechanosensory

spine, two coeloconic sensilla and a proprioceptor. The authors conclude that the maxillary palps are involved in searching and selecting food, and the labial palps seem to be involved in a mechanical survey of the substrate.

Material and Methods

Nymphs of *Perla marginata*, *Dinocras cephalotes* (Perlidae) and *Perlodes microcephalus* (Perlodidae) were collected from the benthos of the permanent tributaries of Bicaz Reservoir (Eastern Carpathians), using a Surber sampler device. For a scanning electron microscopy (SEM) examination, the head of nymphs was fixed in 70% ethanol and cleaned using an ammonium hydroxide solution, and washed in distilled water. The maxillae and labium were dissected under a OPTIKA stereomicroscope and dehydrated by using ethanol gradients and acetone. As alternative to critical point drying, the detached mouthparts were transferred from 99% ethanol to hexamethyldisilazane (HMDS), air dried, and mounted on aluminum stubs, sputter-coated with silver, examined and photographed with a VEGA TESCAN scanning electron microscope at 30 kV, in the Electron Microscopy Laboratory of the Faculty of Biology, of the “Alexandru Ioan Cuza” University of Iași.

Results and Discussion

The mouthparts of the stonefly nymphs feeding on animal food correspond to the primitive biting-chewing type: a pair of mandibles armed with strong and sharp teeth, a pair of maxillae (Fig. 1), consisting each on two medial pair of well developed and elongated appendages, lacinia (internal masticatory lobe) and galea (external masticatory lobe), and a pair of five-segmented, elongated, conical maxillary palp, and a labium, bearing two three segmented palps, glossae and paraglossae.

The maxillary palp of carnivorous stonefly nymphs is formed up of five segments, whose dimensions decrease from the base to the distal part of the palp (Fig. 1). These palps are the longer appendices of the mouth, the first one which comes in contact with the substrate or prey, followed by the tip of labial palps.

On the tip of maxillary palps there is a sensory apical cone complex, according to the terminology of Ljungberg & Hallberg (1992), made up of different kinds of sensilla (Figs. 2b; 3b; 4b).

All three analyzed species present on the tip of their maxillary palps a big apical peg, described by Gaino & Rebora (2003) for *Baetis rhodani* nymphs (Ephemeroptera: Baetidae) as “a conical poreless apical peg, innervated by a single neuron ending at the base of the peg”, and by Ljungberg & Hallberg (1992) for caddisflies *Rhyacophila nubila*, *Glossosoma* and *Wormaldia* (Trichoptera) as a mechanosensory/gustatory pit. The same authors show that this apical peg is accompanied by coeloconic and campaniform sensilla. These last two kinds of sensilla are not present at this level on the apical cone complex of the three species of stonefly nymphs.

In the *Perlodes microcephalus* nymphs, the apical cone complex itself is about 45 μm long and wide. The big apical peg is about 23 μm in length and 7 μm in diameter at its base (Fig. 2b).

In the *Dinocras cephalotes* nymphs, the apical cone is 30 μm long and 33 μm in diameter. The apical peg is 20 μm long and 6 μm in diameter at its base (Fig. 3b).

In the *Perla marginata* nymphs, the apical peg is 21 μm long and 6 μm in diameter at its base (Fig. 4b).

In *Perlodes microcephalus* nymph, along the sides of the apical cone of the maxillary palp, there are 8 sensilla basiconica with flat-tip, 5 sensilla chaetica and 2-3 sensilla basiconica with blunt-tip (Fig. 2b). In the *Dinocras cephalotes* nymphs, along the sides of the apical cone, there are 4-5 sensilla basiconica blunt-tip, a few sensilla chaetica

and rare sensilla basiconica with flat-tip (Fig. 3b). In the *Perla marginata* nymphs, the apical cone complex of maxillary palps contains 2 sensilla basiconica blunt-tip, a lot of flat-tipped sensilla basiconica and 2-3 sensilla chaetica (Fig. 4b).

The labium, covered with hair-sensilla, has a symmetrical reduced pair of glossa, a pair of flexed paraglossa and two elongated, three-segmented palps.

The labial palp consists of three elongated segments: I-basal, II-medial and III-distal. On the tip of the labial palps, as well as of the maxillary palps, there is a sensory apical cone complex (Figs. 2a; 3a; 4a). All three analyzed species present on the tip of their labial palps only one big apical peg.

In the *Perlodes microcephalus* nymphs, the apical cone complex of the labial palps contains an apical peg about 20 μm in length and 8 μm in diameter at its base (Fig. 2a). Along the sides of the apical cone of the labial palp, there are 15 flat-tipped sensilla basiconica, 6 sensilla chaetica, 2-3 sensilla basiconica with blunt-tip, 1 long sensillum trichodeum and one peculiar kind of sensillum chaeticum, with a globular tip (Fig. 2b). In *Dinocras cephalotes* nymph, the apical peg of labial palp is 20 μm long and 6 μm in diameter at its base (Fig. 3a). Along the sides of the apical cone, there are a few blunt-tip sensilla basiconica, a few sensilla chaetica and rare sensilla basiconica with flat-tip (Fig. 3a). In the *Perla marginata* nymphs, the apical peg is surrounded by sensilla basiconica blunt-tip, a lot of flat-tipped sensilla basiconica and some sensilla chaetica (Fig. 4a).

Conclusions

By this comparative analysis of the sensilla of the maxillary and labial palps of three predator stoneflies species, two of them belonging to the Perlidae family and the other one to the Perlodidae family, we observed a similarity between the pattern of distribution, type and number of sensilla of two kinds of palps and between the three analyzed species. We suppose that the maxillary and labial palps have similar functions in prey detection and in the relation established between nymphs and their substrate or aquatic environment. This convergent morphology of chemomechanosensory sensilla may be associated with the common feeding mode more than with the specific characteristics of each species.

The apical cone complex including a big apical blunt peg or a big mechanosensory spine was described by Ljungberg and Hallberg (1992) for some caddisfly larvae and by Gaino and Reborá (2003) for mayfly nymphs. The presence of the apical complex in stonefly nymphs may demonstrate their adaptation to the aquatic environment as well as their predator feeding mode.

The apical cone complex of maxillary palps in *Perlodes microcephalus*, *Dinocras cephalotes* and *Perla marginata* contain a big apical peg surrounded by three other types of hair-sensilla: chaetica, basiconica flat-tipped and basiconica blunt-tipped. The number and the distribution of different kinds of sensilla in the apical cone complex of the maxillary palps are quite similar. The types of sensilla at this level differ from the sensilla described for other aquatic nymphs (some mayflies and caddisflies), excepting the apical cone.

The diversity of sensilla on the maxillary and labial palps is less than that on the antennae. Neagu (2008) identified on the antennae of the same three analyzed species the following types of sensilla: trichodea, basiconica, coniform complexes, sensilla coeloconica and campaniformia. On the tip of maxillary and labial palps, the coniform complexes, sensilla coeloconica and sensilla campaniformia were not identified. Sensilla campaniformia may be present on the distal margin of the first basal segments of the palps. The sensilla basiconica are present with their flat-tipped type, unidentified on the antennae, accompanied by the sensilla chaetica.

Neagu (2008) shows that the number of different types of sensilla on the antennal segments in *Perlodes microcephalus* nymphs is higher. The number and diversity of sensilla in the apical cone complex in *Perlodes microcephalus* nymphs, at the maxillary and especially at labial palps tip level, is highest, may be in relation to the higher attack rate developed by the nymphs of this species.

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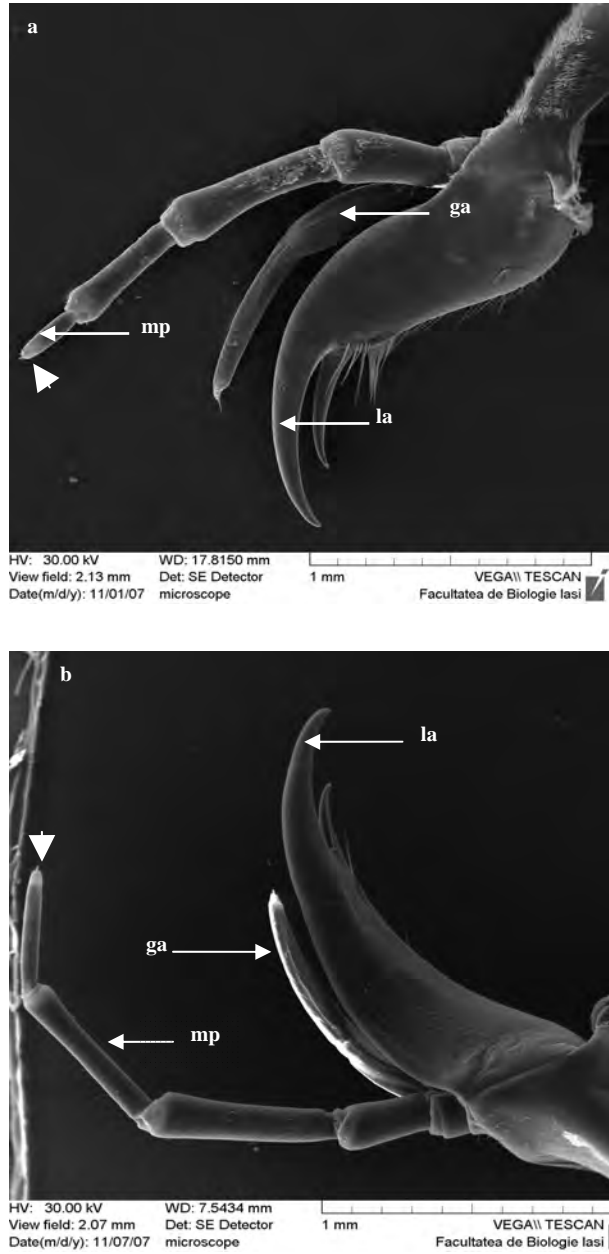


Figure 1. Distal anatomy of the maxillae of carnivorous stonefly nymphs (SEM): a. the maxilla of *Perla marginata*; b. the maxilla of *Perlodes microcephalus*. The arrow heads indicate the position of the sensillar complexes on the tip of maxillary palps. la-lacinia, ga-galea, mp-maxillary palp.

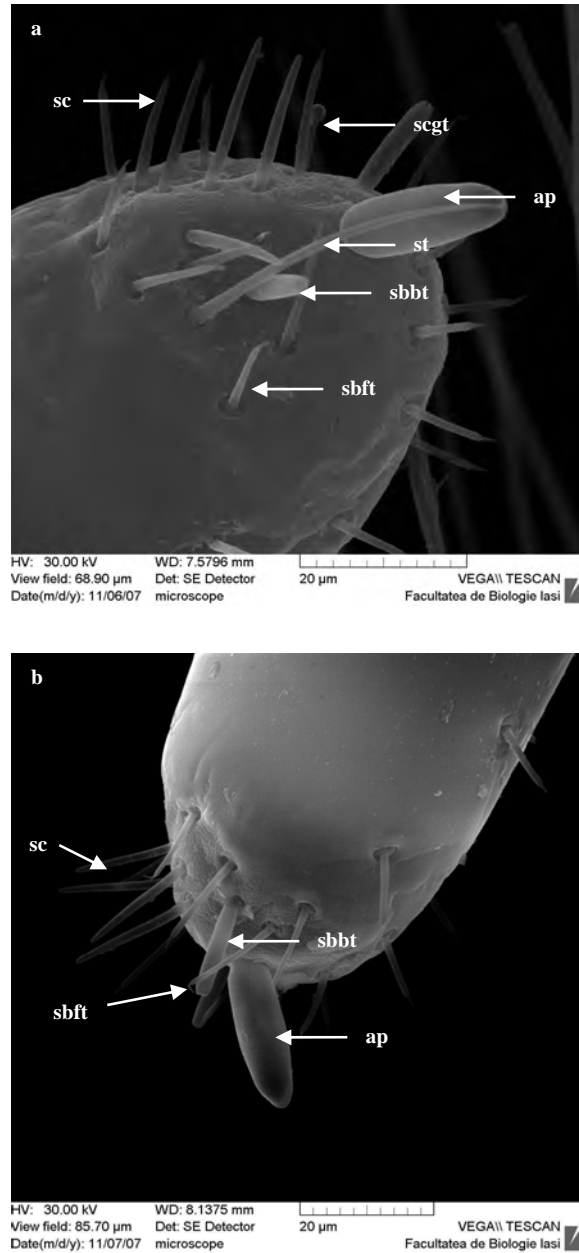


Figure 2. Apical cone complex of labial (a) and maxillary palps (SEM) (b) of the *Perlodes microcephalus* nymphs.
ap – apical peg, sbbt – sensilla basiconica blunt-tipped, sbft – sensilla basiconica flat-tipped, sc – sensilla chaetica, scgt – sensillum chaeticum globular-tipped, st – sensillum trichodeum.

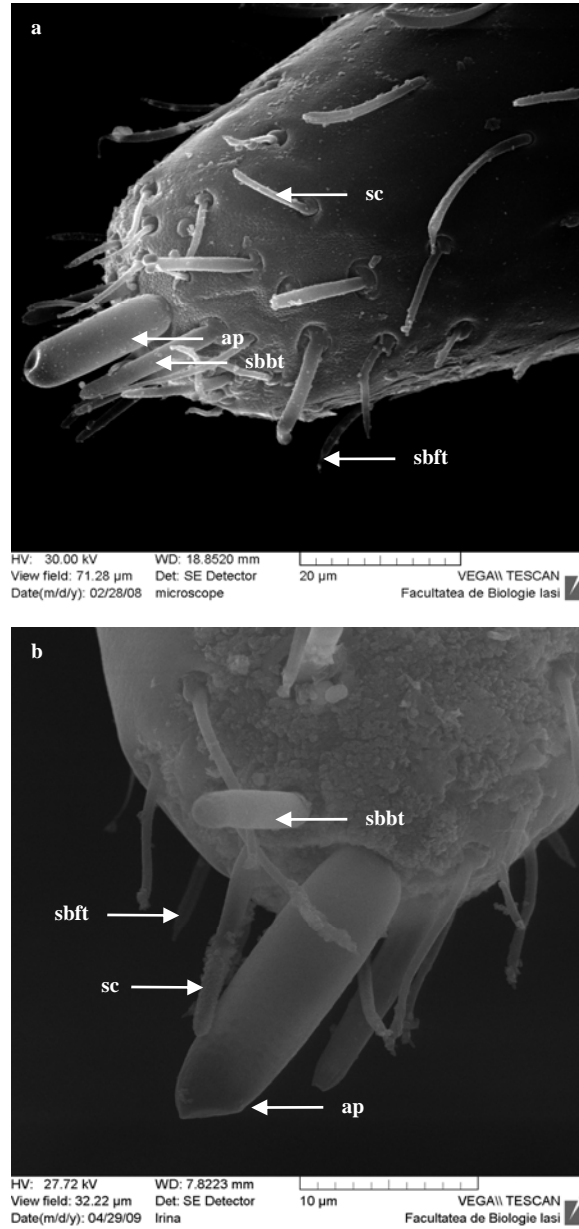


Figure 3. Apical cone complex of labial (a) and maxillary palps (SEM) (b) of the *Dinocras cephalotes* nymphs.
ap – apical peg, sbbt – sensilla basiconica blunt-tipped, sbft – sensilla basiconica flat-tipped, sc – sensilla chaetica.

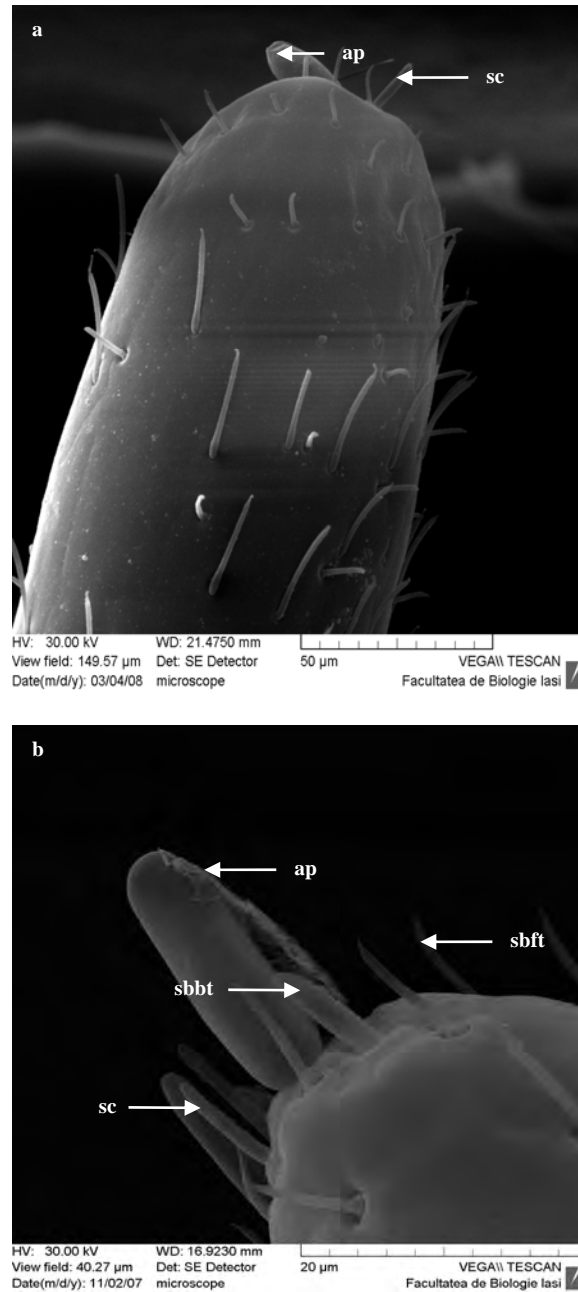


Figure 4. Apical cone complex of labial (a) and maxillary palps (SEM) (b) of the *Perla marginata* nymphs.
ap – apical peg, sbbt – sensilla basiconica blunt-tipped, sbft – sensilla basiconica flat-tipped, sc – sensilla chaetica.

